Toxicological Effects of Alpha Lipoic Acid in Streptozotocin-Induced Diabetes in Wistar Rats

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ABSTRACT

Alpha lipoic acid (ALA), through its antioxidant activity plays an important role in preventing the progression of diabetes mellitus without recourse to its safety or toxicity risk. The aim of the study was to investigate the toxicological effects of ALA in streptozotocin-induced diabetes in Wistar rats.A total of twenty (20) Wistar rats weighing between 150 and 250 g which included both normal and diabetic animals were randomlydivided into four groups (1-4) of five animals each. Animals in group 1 which included normal control animals were administered 1 ml of distilled water. Animals in groups 2-4 which were induced with diabetes using diabetogenic dose of streptozotocin (60 mg/kg body weight) were also treated with distilled water, 100 mg/kg body weight of ALA and 2 mg/kg body weight of glibenclamide (reference antidiabetic drug) respectively. The treatments were given orally once daily for nine days. Compared with the diabetic control, while treatment with 100 mg/kg body weight of ALA did not significantly ($P > 0.05$) affect the concentrations of high density lipoprotein cholesterol (HDLC) and low density lipoprotein cholesterol (LDLC), the administration of 100 mg/kg body weight of ALA significantly ($P < 0.05$) decreased the concentrations of total cholesterol and triglyceride in the serum of the diabetic animals. The administration of 2 mg/kg body weight of glibenclamide (GLB) did not significantly ($P > 0.05$) alter the concentrations of serum total cholesterol and HDLC when compared with the normal control. However, when compared with the normal control animals, the administration of 2 mg/kg body weight of GLB significantly ($P < 0.05$) reduced the concentration of triglyceride and significantly ($P < 0.05$) increased the concentration of LDLC in the serum of the diabetic animals. When compared with the normal and diabetic controls, administration of ALA did not significantly ($P > 0.05$) affect the levels of total bilirubin, albumin, globulin and albumin/globulin ratio in the serum of the diabetic animals. Except for globulin concentration which decreased significantly ($P < 0.05$) when compared with the normal and diabetic control, the administration GLB did not significantly ($P > 0.05$) alter the levels of total bilirubin, albumin, globulin and albumin/globulin ratio in the serum of the diabetic animals. When compared with the distilled water treated diabetic control animals, administration of ALA significantly ($P < 0.05$) increased the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum of the diabetic animals. Administration of GLB significantly ($P < 0.05$) increased the activity of ALT in the serum of the diabetic animals when compared with the diabetic control. Administration of GLB significantly ($P < 0.05$) reduced the activity of AST in the serum of the diabetic animals when compared with the diabetic control. Treatment with ALA significantly ($P < 0.05$) decreased the activity of ALP in the serum of the diabetic animals when compared with the diabetic control while GLB treatment caused a significantly ($P < 0.05$) increase in ALP activity in the serum of the diabetic animalswhen compared with the diabetic control.When compared with the distilled water treated diabetic control animals, administration of 100 mg/kg body weight of ALA did not significantly ($P > 0.05$) alter the levels of urea, uric acid and creatinine in the serum of the diabetic animals. Treatment with 2 mg/kg body weight of the reference antidiabetic drug (GLB) did not significantly ($P > 0.05$) alter the concentrations of urea, uric acid and creatinine when compared with the distilled water treated normal control animals. Similarly, when compared with the distilled water treated diabetic control animals, the administration of 100 mg/kg body weight of ALA did not significantly ($P > 0.05$) affect the levels of Na^+, K^+, Ca^{2+}, Cl^-, HCO_3 and PO_4^{2-} in the serum of the diabetic animals. Treatment with 2 mg/kg body weight of GLB did not significantly ($P > 0.05$) alter the concentrations of serum Na^+, K^+, Ca^{2+}, Cl^-, HCO_3 and PO_4^{2-} when compared with the distilled water treated normal control animals. This study indicated that ALA altered the basic function of the organs and biomolecules in experimental diabetic rat model. Therefore, the100 mg/kg body weight of ALA may not be completely 'safe' as oral remedy in preventing the progression of diabetes mellitus.

Keywords: Alpha lipoic acid,Streptozotocin, Diabetes mellitus, Lipid profile, Glibenclamide

INTRODUCTION

The generation of free radicals, which results in toxicity of tissues of diabetic-induced animals cannot be over emphasized. However, Alpha lipoic acid (ALA) plays an important role in scavenging these free radicals thereby preventing oxidative stress in these animals. In this process, alterations in certain basic function of tissues may result. Diabetes mellitus is often referred to a condition in which the body either does not produce enough, or does not properly respond to insulin, a hormone produced in the pancreas [1]. It results in elevation of blood glucose level, decreased protein content, increased levels of cholesterol and triglycerides.

ALA is a cofactor of α-ketoacid dehydrogenase complexes and it plays a fundamental role in the metabolism. ALA has been found to affect cellular metabolic processes, alter redox status of cells, interacts with thiols and other antioxidants [2]. ALA is a unique antioxidant because it has beneficial effects on energy production in humans and animals, and also an essential cofactor of mitochondrial respiratory enzymes, including the pyruvate dehydrogenase (PDH) complex [3, 4]. ALA has gained considerable attention as an antioxidant for use in managing diabetic complications [5]. ALA quenches reactive oxygen species, chelates metal ions, and reduces the oxidized forms of other antioxidants such as vitamin C, vitamin E, and glutathione [6]. Alpha-lipoic acid is water and lipid soluble, a property that allows it to concentrate in cellular and extracellular environments. Exogenous ALA is rapidly absorbed from the diet, and is reduced inside the cell to dihydrolipoic acid (DHLA), the most active form of the substance [7]. It has antioxidant regenerating abilities [8]. It also protects against oxidative stress both in peripheral tissues and central nervous system [9].Previous studies by Jacob *et al* [10] reported that the antioxidant α-lipoic acid enhances insulin-stimulated glucose metabolism in insulin resistant rat skeletal muscle while Streeper*et al* [11] saw the differential effects of lipoic acid stereoisomers on glucose metabolism in insulin-resistant skeletal muscle. Sharma and Gupta [12] evaluated the effect of alpha lipoic acid on intracerebroventricular streptozotocin model of cognitive in rats while Vasdev*et al* [13] discovered that dietary alpha-lipoic acid supplementation could lower blood pressure in spontaneously hypertensive rats. Sequel to these studies, only little attention has been paid on the safety or toxic effect of the compound on tissues of diabetic animal models. Therefore, this study was designed to provide information on the toxicological effects of ALA in streptozotocin-induced diabetes in Wistar rats.

MATERIALS AND METHODS

MATERIALS

Experimental Animals:

Male Wistar rats that weighed between 150 and 250 g were procured from the Animal House of Federal College of Animal Husbandry, Kuru, Jos, Plateau State, Nigeria. The animals were kept and maintained under laboratory condition and were allowed to acclimatize for eight weeks prior to the commencement of the study. They were fed on standard commercial rat pellets (Vital Feeds) with free access to water.

2.2.1 Chemicals and Reagents:

Streptozotocin was purchased from Sigma Chemicals Company Ltd (St Louis, U.S.A.), while alpha lipoic acid was purchased from General Nutrition Corporation, Pittsburgh, U.S.A.300 mg of alpha lipoic acid was diluted in appropriate volumes of distilled water to obtained working dose concentration used in the study. All other chemicals andsolvents used were of analytical grade.

METHODS

Induction of Experimental Diabetes Mellitus:

Experimental diabetes was induced by giving single intraperitoneal injection of 60 mg/kg body weight dose of streptozotocin (STZ) that was dissolved in freshly prepared 0.1M cold citrate buffer of pH 4.5 to animals fasted 18 hrs, but had free access to drinking water. 72 hrs after streptozotocin injection, blood was taken from tail vein of each animal to obtain the blood glucose reading. Animals having blood glucose levels ≥ 200mg/dL were considered diabetic and used in the study. Thereafter, normal and diabetic animals were randomly assigned into different groups.

Animal Grouping and Treatment:

A total of twenty (20) Wistar rats which included both normal and diabetic animals were randomly divided into four groups of five animals each as follows:

Group 1: Normal control animals that was administered with distilled water

Groups 2: Diabetic control group that received distilled water

Group 3: Diabetic animals that were treated with 100 mg/kg body weight of ALA

Group 4: Diabetic rats that were administered with 2 mg/kg body weight of glibenclamide

The treatments were given orally once daily for nine days.

Collection of Blood Sample:

Twenty four (24) hours after the last treatment was given to animals, the animals wereplaced inanaesthetic box and euthanized by exposure to light chloroform vapour. Five (5) mL of blood was withdrawn by cardiac puncture from the animals into EDTA sample bottles for the analysis of biochemical parameters.

Determination of Serum Total Protein, Albumin, Globulin and Albumin-Globulin Ratio:

The serum total protein was determined by the Biuret method of Reinhold [14] using a commercial kit (Randox Laboratories Ltd., U.K.). The serum albumin which quantitatively binds bromocresol green (BCG) to form an albumin-BCG complex was measured as an endpoint reaction at 596 nm was determined according to the method of Doumaset al. [15]. Globulin was obtained by subtracting the albumin from the total protein, while albumin-globulin ratio was obtained according to the method of Coles [16]. Urea was determined according to the method of Kingsley et al. [17]. The method was based on the pH increase resulting from the ammonia released by urease hydrolysis of urea. *O*-Cresolphthalein complexone was used to monitor the pH change colorimetrically.

Evaluation of Renal Function:

The level of serum urea was determined using the method of Tietz*et al* [18]. Serum sodium and potassium ions were measured by the flame photometry method of Vogel [19], and bicarbonate ion was determined using the titration method of Segal [20]. Chloride ion was analyzed using the method of Schales and Schales [21].Serum uric acid and calcium ion were determined by adopting the procedure described by Tietz [22].Serum phosphate ion and creatinine were determined by using the method described by Owen *et al* [23] and Fiske and Subbarow [24]respectively.

Determination of Serum Liver Enzymes Activities:

Activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated by the method adopted by Tietz [22] and Henderson and Moss [25] respectively. Alkaline phosphatase was determined according to the method of Scherwin [26]. Serum total bilirubin was determined by the method of Malloy and Evelyn [27].

Determination of Serum Total Cholesterol:

This was determined spectrophotometrically, using enzymatic colometric assay kits (Randox Laboratories Limited kits, Unite Kingdom) as follows: The serum level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by the method of Stein [28]. 1000 μL of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 ^{0}C after mixing and the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was measured against the reagent blank within 30 minutes at 546 nm. The value was expressed in the unit of (mg/dL).

Determination of Serum Triglyceride:

The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by the method of Tietz [29]. 1000 μL of the reagent was added to each of the sample and standard. This was then incubated for 10 minutes at 20-25 ^{0}C after mixing and the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was measured against the reagent blank within 30 minutes at 546 nm. The value was expressed in the unit of (mg/dL).

Determination of Serum High-density Lipoprotein Cholesterol:

The serum level of HDL-C was measured by the method of Wacnic and Albers [30]. Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample was precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes at 2000 g. The supernatant represented the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined. The value was expressed in the unit of (mg/dL).

Determination of Serum Low-density Lipoprotein cholesterol (LDL-C):

The serum level of (LDL-C) was measured according to the protocol of Friedewald *et al* [31].

Statistical Analysis:

All data obtained from each group were expressed as mean ± SEM of five replicates. The data were statistically analyzed using (ANOVA) with Tukey's*post-hoc test* to compare the levels of significant between the control and treated animals. All statistical analysis was evaluated using SPSS version 17.0 software and Microsoft Excel (2007). The values of $P \leq 0.05$ were considered as significant.

RESULTS

Compared with the diabetic control, while treatment with 100 mg/kg body weight of alpha lipoic acid (ALA) did not significantly (P > 0.05) affect the concentrations of high density lipoprotein cholesterol (HDLC) and low density lipoprotein cholesterol (LDLC), the administration of100 mg/kg body weight of ALA significantly (P < 0.05) decreased the concentrations of total cholesterol and triglyceride in the serum of the diabetic animals (Table 1). The administration of 2 mg/kg body weight of glibenclamide (GLB) did not significantly (P > 0.05) alter the concentrations of total cholesterol and HDLC when compared with the normal control. However, when compared with the normal control, the administration of 2 mg/kg body weight of GLB significantly (P < 0.05) reduced the concentration of triglyceride and significantly (P < 0.05) increased the concentration of LDLC in the serum of the diabetic animals (Table 1).When compared with the normal and diabetic controls, administration of ALA did not significantly (P > 0.05) affect the levels of total bilirubin, albumin, globulin and albumin/globulin ratio in the serum of the diabetic animals. Except for globulin concentration which decreased significantly (P < 0.05) when compared with the normal and diabetic control, the administration GLB did not significantly (P > 0.05) alter the levels of total bilirubin, albumin, globulin and albumin/globulin ratio in the serum of the diabetic animals (Table 2).

When compared with the distilled water treated diabetic control animals, administration of ALA significantly (P < 0.05) increased the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum of the diabetic animals. Administration of GLB significantly (P < 0.05) increased the activity of ALT in the serum of the diabetic animals when compared with the diabetic control. Administration of GLB significantly (P < 0.05) reduced the activity of AST in the serum of the diabetic animals when compared with the diabetic control (Table 2). Treatment with ALA significantly (P < 0.05) decreased the activity of ALP in the serum of the diabetic animals when compared with the diabetic control while GLB treatment caused a significantly (P < 0.05) increase in ALP activity in the serum of the diabetic animalswhen compared with the diabetic control (Table 2).

When compared with the distilled water treated diabetic control animals, administration of 100 mg/kg body weight of ALA did not significantly (P > 0.05) alter the levels of urea, uric acid and creatinine in the serum of the diabetic animals. Similarly, treatment with 2 mg/kg body weight of the reference antidiabetic drug (GLB) did not significantly (P > 0.05) alter the concentrations of urea, uric acid and creatinine when compared with the distilled water treated normal control animals (Table 3).

When compared with the distilled water treated diabetic control animals, the administration of 100 mg/kg body weight of ALA did not significantly (P > 0.05) affect the levels of Na^+, K^+, Ca^{2+}, Cl^-, HCO_3 and $PO4^{2-}$ in the serum of the diabetic animals. Treatment with 2 mg/kg body weight of GLB did not significantly (P > 0.05) alter the concentrations of serum Na^+, K^+, Ca^{2+}, Cl^-, HCO_3 and $PO4^{2-}$ when compared with the distilled water treated normal control animals (Table 3).

Table 1: Effect of Alpha Lipoic Acid on Serum Lipids in Streptozotocin-induced Diabetes inWistar Rats

Treatment Groups	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	High-density lipoprotein (mg/dL)	Low-density lipoprotein (mg/dL)
NC + DW	51.64 ± 6.09^a	76.30 ± 4.31^a	11.66 ± 0.84^a	24.70 ± 5.23^a
DC + DW	52.94 ± 3.07^a	65.20 ± 3.70^b	12.10 ± 0.31^a	27.84 ± 2.11^a
D + ALA	45.06 ± 1.63^b	52.33 ± 0.89^c	11.60 ± 0.44^a	25.00 ± 1.60^a
D + GLB	59.34 ± 3.47^a	66.96 ± 5.24^b	13.16 ± 0.35^a	32.76 ± 2.78^b

a,b,c,d = Means on the same column with different superscript letters differ significantly (P < 0.05) compared with the control groups

NC+DW = Normal control rats administered with distilled water, DC+DW = Diabetic control rats administered with distilled water, D+ALA = Diabetic rats treated with Alpha Lipoic acid (100 mg/kg b w), D + GLB = Diabetic rats treated with glibenclamide (2 mg/kg b w)

Table 2: Effects of Alpha Lipoic Acid on Liver Function Parameters and Enzyme Activities in Streptozotocin-induced Diabetic Wistar Rats

Treatment Groups	Total Bilirubin (µmol/L)	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Protein (g/L)	Albumin (g/L)	Globulin (g/L)	Albumin/ Globulin Ratio (g/L)
NC+DW	3.10±0.07[a]	99.08±7.26[a]	14.42±82[a]	329.74±35.63[a]	64.22±3.59[a]	32.22±2.08[a]	32.00±3.77[a]	1.07±0.17[a]
DC+DW	3.77±0.05[a]	112.68±13.81[a]	257.17±5[b]	442.22±53.22[b]	68.70±3.27[a]	36.68±1.56[a]	32.02±1.76[a]	1.15±0.02[a]
D+ALA	3.70±0.17[a]	156.28±0.12[b]	273.86±0.57[c]	427.86±68.80[b]	58.56±0.70[b]	31.16±1.13[a]	27.40±1.81[b]	1.17±0.14[a]
D+GLB	3.50±0.09[a]	165.50±42.20[b]	120.68±4[b]	545.30±62.90[c]	60.98±3.47[a]	33.10±1.60[a]	27.82±2.04[b]	1.20±0.05[a]

a,b,c,d = Means on the same column with different superscript letters differ significantly (P < 0.05) compared with the control groups

NC+DW = Normal control rats administered with distilled water, DC+DW = Diabetic control rats administered with distilled water, D+ALA = Diabetic rats treated with Alpha Lipoic acid (100 mg/kg b w), D + GLB = Diabetic rats treated with glibenclamide (2 mg/kg b w)

Table 3: Effects of Alpha Lipoic Acid on Kidney Function Parameters in Streptozotocin-induced Diabetic Wistar Rats

Treatment Groups	Na+ (mmol/L)	K+ (mmol/L)	Cl- (mmol/L)	HCO3- (mmol/L)	Ca2+ (mmol/L)	PO4 2- (mg/dL)	Uric Acid (mg/dL)	Urea (mmol/L)	Creatinine (µmol/L)
NC+DW	199.46±1.31[a]	7.94±0.42[a]	101.72±1.07[a]	29.76±0.15[a]	2.22±0.09[a]	7.06±0.87[a]	4.46±0.60[a]	6.62±0.40[a]	113.44±3.09[a]
DC+DW	192.12±6.48[a]	9.40±0.46[a]	103.60±2.01[a]	26.77±1.32[a]	2.10±0.06[a]	7.02±0.04[a]	4.00±0.08[a]	7.12±0.35[a]	100.18±5.40[a]
D+ALA	188.26±0.41[a]	5.96±0.35[a]	107.62±0.91[a]	23.61±0.13[a]	2.26±0.25[a]	7.02±0.04[a]	4.22±0.49[a]	7.76±0.64[a]	97.34±1.91[a]
D+GLB	183.30±4.50[a]	6.30±0.62[a]	96.92±2.74[a]	30.26±0.19[a]	2.36±0.81[a]	7.04±0.03[a]	2.96±0.42[a]	8.24±0.71[a]	103.78±3.48[a]

a,b,c,d = Means on the same column with different superscript letters differ significantly (P < 0.05) compared with the control groups

NC+DW = Normal control rats administered with distilled water, DC+DW = Diabetic control rats administered with distilled water, D+ALA = Diabetic rats treated with Alpha Lipoic acid (100 mg/kg b w), D + GLB = Diabetic rats treated with glibenclamide (2 mg/kg b w)

DISCUSSION

The changes in serum lipids can provide useful information about functioning of the heart and coronary artery as it relates with susceptibility to atherosclerosis [32]. In comparison with the distilled water control, the non-significant effect of 100 mg/kg body weight of ALA on the concentrations of serum LDLC and HDLC may be adduced to lack of effect of the dose. The decrease in serum levels of total cholesterol and triglyceride by 100 mg/kg body weight of ALA may be an indication that membrane of the myocytes are intact and will not expose the animals to cardiovascular disease. The non-effect of GLB on serum total cholesterol concentration is an indication that GLB may not affect the β-oxidation of fatty acids, as fatty acid is the precursor for cholesterol biosynthesis. The alteration in serum triglyceride and LDLC concentrations by GLB may not be beneficial to the animals as it may not enhance obesity, atherosclerosis and hypertension [33, 34].

Liver function tests are often done to ascertain the effectiveness of the hepatocytes. The non-significant effect of 100 mg/kg body weight of ALA onserum levels of total bilirubin, albumin, globulin and albumin/globulin ratio may be adduced to normal functioning effect of the liver cell [35]. Similarly, the non-significant effect of 2 mg/kg body weight of GLB on serum levels of total bilirubin, albumin, and albumin/globulin ratio may be attributed to maintenance of the integrity of the hepatocytes. The decreased serum globulin concentration by GLB in the present study may be an indication of diminished synthetic and secretory function of the liver. These alterations in biomolecules by ALA may adversely affect liver function.

Alkaline phosphatase (ALP) is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum [36]. The decrease in serum ALP activity by ALA in the diabetic animals when compared with the diabetic control may be attributed to either inhibition of the enzyme activity at the cellular/molecular level. It may also affect other metabolic processes where the enzyme is involved. The increase in serum ALP activity by GLB in the diabetic animals when compared with the diabetic control may be adduced to an increase in the functional activity of the organs leading to *De novo* synthesis of the enzyme molecule [37].The increase in the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum of the diabetic animals by ALA when compared with the distilled water treated diabetic control animals may be adduced to damage in liver cell leading to compromise in membrane integrity which causes leakage of the enzyme from the cell into the blood [38]. The increase in serum ALT activity and decrease in serum AST activity by GLB when compared with the diabetic control group may account for the selective effect of the drug (GLB) which would result in alteration in enzyme activity. The increase and decrease in enzyme activity by ALA are alterations, and would have consequential effect on integrity of the membrane.

The functional capacity of the kidney can be measured by determining the concentrations of excretory products and electrolyte constituents [39]. The non-significant effect of 100 mg/kg body weight ALA and 2 mg/kg body weight GLB on serum levels of urea, uric acid and creatinine when compared with the distilled water treated normal and diabetic control animals suggest no impairment in renal function, glomerular dysfunction of the nephron and creatinine clearance functioning of the kidney [40]. The non-significant effect of 100 mg/kg body weight ALA and 2 mg/kg body weight GLB on serum levels of Na^+, K^+, Ca^{2+}, Cl^-, HCO_3 and PO_4^{2-} when compared with the distilled water treated normal as well as diabetic control animals suggest no dysfunction at the renal, tubular or glomerular level of the kidney [41].The absence of an effect by ALA and GLB on biomolecules could be beneficial as it will maintain the functioning capacity of the kidney.

CONCLUSION

Available evidence from the present study indicated that the non-significant effect of ALA on biomolecules could maintain the functioning capacity of the kidney. Also, ALA altered functional indices of the liver and serum. These alterations might be responsible for some toxic and deleterious effects on basic function of the organs and biomolecules in diabetic animals. Therefore, the 100 mg/kg body weight of ALA may not be completely 'safe' as oral remedy in preventing the progression of diabetes mellitus.

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