EFFECTS OF ALPHA LIPOIC ACID ON BLOOD GLUCOSE, BODY WEIGHT AND HAEMATOLOGICAL PROFILE OF STREPTOZOTOCIN-INDUCED HYPERGLYCAEMIA IN WISTAR RATS

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ABSTRACT

The study investigated the effects of alpha lipoic acid (ALA) on blood glucose, body weight and haematological parameters in streptozotocin (STZ)-induced hyperglycaemia in Wistar rats. Hyperglycaemia was induced by single intraperitoneal injection of 60 mg/kg body weight dose of STZ into 18 hr fasted animals. Three days after confirmation of diabetes, the normal and diabetic rats were divided into four groups (I-IV) of five rats each. Groups I and II served as the normal and diabetic control animals that was administered with 0.5 mL of distilled water, while Group III and IVreceived 100and 2 mg/kg body weight of ALA and glibenclamiderespectively.Results obtained showed that ALA and glibenclamide produced a steady significant ($P < 0.05$) reduction in fasting blood glucose levels especially after the 6^{th} and 9^{th} day with ALA producing better effect than glibenclamide when compared with the control group. There was a significant ($P < 0.05$) improvement in body weights of diabetic animals treated with ALA with non-significant difference produced in diabetic group that were treated with glibenclamide when compared with the diabetic control group.The RBC count,Hb concentration and PCV of the diabetic control animals were not significantly ($P >0.05$) different from those of normal control group when compared. Administration with ALA and glibenclamide produced a non significant increase on the levels of above parameters when compared with the diabetic control. The total white blood cell andplatelets count obtained in diabetic control rats were significantly lower ($P < 0.05$) when compared with the normal control rats.In addition, neutrophil and monocyte countswere significantly ($P < 0.05$) reduced in the diabetic control group when compared with the normal control animals, where as the lymphocyte and eosinophil counts in the diabetic control animals were not significantly ($P > 0.05$) different when compared with those in the normal control rats. In conclusion, administration of ALA to diabetic animals controlled the increased blood glucose concentration as well as improved the body weight, but the altered haematological parameters were not significantly normalized when compared with the diabetic control group.

Keywords: Diabetes mellitus, Alpha lipoic acid, Streptozotocin, Body weight, Glibenclamide.

INTRODUCTION

Diabetes mellitus (DM) is a complex, progressive disease and metabolic disorder of the endocrine system (Li *et al*., 2004). It is defined as a sustained hyperglycaemia, a condition in which a high amount of glucose circulates in the blood (Conget, 2002). Uncontrolled chronic hyperglycaemia as a result of absolute insulin deficiency (type 1 diabetes) or insulin

resistance with or without insulin deficiency (Type 2 diabetes) is one of the primary causes of diabetic complications in a number of organs (Wang *et al.*, 2012). It is among the most common disorders in both developed and developing countries (Zhou *et al.*, 2009). It has become a global metabolic epidemic, affecting important biochemical activities in nearly every age group (Singh *et al.*, 2012). The number of people affected by diabetes was estimated to have risen by 50% by 2010, and will almost be doubled by 2025 (Bethel *et al*., 2007). It has been estimated that the number of people with diabetes will rise from the present 150 to 230 million in 2025 (Abu-Zaiton, 2010). Some of the causes of an increased risk of diabetes are due to an increase in sedentary lifestyle, consumption of an energy rich diet, obesity, higher life span (Deore*et al.*, 2012).Oxidative stress has been suggested to be a common pathway linking diverse mechanisms for the pathogenesis of complications in diabetes (Shih *et al*., 2002). It has been reported that oxidative stress participates in the progression of insulin resistance (Evans *et al*., 2002). Oxidative stress is characterized by a persistent imbalance between the production of highly reactive-oxygen species (ROS) and reactive nitrogen species (RNS) and antioxidantdefences, leads to an altered cellular redox status and subsequent tissue damage (Jakuss, 2000).

Alpha lipoic acid (ALA) is a unique and potent antioxidant. It can deliverantioxidant activity in both fat- and water-soluble mediums, and it is capable of having an antioxidanteffect in both its oxidized and reduced forms (Goraca*et al.*, 2011). Therefore, ALA is able to exert its powerful antioxidant effectsbecause of this unique amphiphilic property (hydrophobic and hydrophilic nature).There is also evidence that ALA has the ability to directlyscavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS) (UmaandIshwarlal, 2008; Poh and Goh, 2009). This effectively allows ALA to deliver its antioxidant effect to any cell or tissue type, as well as to anysubcellular compartment, in the body (Rochette*et al.*, 2013). In addition, ALA is reported to contributeto the defense against oxidative stress by increasingthe synthesis of anti-oxidants like glutathione, one of themost abundant intra-cellular anti-oxidants in the body (Suh *et al.*, 2004). Studies have also shown that there is no significanttissue accumulation and free plasma ALA levels after anoral ingestion as there is rapid clearance rate (Pohand Goh, 2009). ALA has been documented to have positive effects on a wide variety of clinical conditions(Harding *et al.*, 2012). The anti-aging activity of ALA has been reported (Jiang *et al.*, 2013). Stoll *et al*.(1993) showed that ALA administration to animals improved memory. In addition, itsanti-inflammatory, anti-toxin, anti-proliferative effects in cancers and anti-depressant activities have been reported (Kwiecien*et al.*, 2013; Sokolowska*et al.*, 2013; Kapoor, 2013;Silva *et al.*, 2013). Furthermore, blood glucose regulating activity of ALA has been reported (Bajaj and Khan 2012; Nebbioso*et al.*, 2013), but these reports have not been extensively elucidated. This paucity of information on the anti-diabetic activity of ALA prompted the present investigation on the effects of ALA on blood glucose, body weight and haematological changes in streptozotocin-induced hyperglycaemia using Wistar rats as model.

MATERIALS AND METHODS

Materials

Animals

Adult Male Wistar rats that weighed between 150 and 250 g were procured from the AnimalHouse 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.

Chemicals and alpha lipoic acid

Streptozotocin (STZ) 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 for 18 hrs, but hadaccess to drinking water. 72 hrsafter streptozotocininjection, blood was taken from tail vein of each animal to obtain the blood glucose reading. Animals having blood glucose levels $\geq$ 200 mg/dl were considered diabetic and used in the study. Thereafter, both 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 randomized into four groups of five animals each as follows:
Group 1: Normal control animals that was administered with 0.5 mL of 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
All treatments were administered orally once daily for nine days.

Determination of fasting blood glucose level

Fasting blood glucose level was determined by collection of blood sample from the tail vein of the rats at interval of 0 day, 3rd day, 6th day and 9th day respectively by glucose-oxidase method described by Beach and Turner (1958) using digital glucometer (Accu-chekActive).

Determination of body weight of experimental animals

The body weights of control and experimental animals were determined before the commencement of treatment (initial body weight) and after the last day of administration (final body weight).

Blood sample collection

Twenty four (24) hours after the last treatmentswere given, the animals wereeuthanized by exposure to light chloroform vapour soaked in cotton wool and placed in anaesthetic box. Five(5 mL) of blood was withdrawn by cardiac puncture from the animals into heparinzedsample bottles for haematologicalprofile analysis.

Statistical analysis

All data obtained from each group were expressed as mean ± SEM. The data were statistically analyzed using ANOVA with Tukey's *post-hoc test* to compare the levels of significance between the control and treated groups. 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

Effects of Alpha Lipoic Acid on Fasting Blood Glucose Level in Streptozotocin-induced Hyperglycaemia in Wistar Rats

The results of the effect of alpha lipoic acid on fasting blood glucose level are presented in (Table 1). There was a significantly ($P < 0.05$) elevated levels of fasting blood glucose after three days of STZ injection to animals when compared with the normal control animals. Day 0 represents the day before the treatment of diabetic animals commenced. Following treatment with ALA (100 mg/kg) and glibenclamide (2mg/kg), there was a significant ($P < 0.05$) and progressive decrease on the levels of fasting blood glucose especially after the 6^{th} and 9^{th} day respectively, with ALA (100 mg/kg) producing better effect than glibenclamide when compared with the control group.

Effects of Alpha Lipoic Acid on Body Weights in Streptozotocin-induced Hyperglycaemia in Wistar Rats

There was a significant ($P < 0.05$) reduction on the body weights of the STZ-treated diabetic animals when compared with those of normal control animals. Administration of ALA (100 mg/kg body) to diabetic rats recorded a significant ($P < 0.05$) increase on the body weights of the animals, with glibenclamide (2 mg/kg) treated group producing a non significant change on body weights when compared with the diabetic control animals (Table 2).

Effects of Alpha Lipoic Acid on Haematological Parameters in Streptozotocin-induced Hyperglycaemia inWistar Rats

The RBC count,Hb concentration and PCV of the untreated diabetic animals were not significantly ($P > 0.05$) different from than those of normal control group when compared. Following treatment with ALA (100 mg/kg) and glibenclamide (2 mg/kg), there was a significantincrease on the levels of above parameters, but the increase was not significant ($P > 0.05$) when compared with the diabetic control (Table 3). The total white blood cell (WBC) andplatelets count obtained in diabetic control rats were significantly lower ($P < 0.05$) than that of the normal control group when compared with diabetic control group. In addition, neutrophil and monocyte countswere significantly ($P < 0.05$) reduced in the diabetic control group when compared with the normal control animals, where as the lymphocyte and eosinophil counts in the diabetic control animals were not significantly ($P > 0.05$) different when compared with those in the normal control rats (Table 3).

Table 1: Effect of Alpha Lipoic Acid on Fasting Blood Glucose Level in Streptozotocin-induced Hyperglycaemia in Wistar Rats

	Blood Glucose Level (mg/dL)			
Treatment Groups	Day 0	Day 3	Day 6	Day 9
NC + DW	104.40 ± 3.78[a]	101.20 ± 7.14 [a]	93.75 ± 3.64[b]	92.75 ± 4.92[b]
DC + DW	371.20 ±66.64[a]	330.40 ± 54.52[b]	285.20 ± 52.74[c]	331.20 ± 61.01[b]
D + ALA	448.60 ±77.92[a]	347.80 ± 71.27[b]	293.00 ± 91.36[c]	245.60 ± 79.16[d]
D + GLB	463.20 ±83.86[a]	418.40 ± 80.17[b]	381.40 ± 71.13[c]	329.25 ± 81.09[d]

a,b,c,d = Means on the same rows 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 Body Weight in Streptozotocin-induced Hyperglycaemia in Wistar Rats

	Body Weight (g)			
	NC + DW	DC + DW	D + ALA	D + GLB
Initial Weight	211.20 ± 11.47[a]	149.00 ± 9.35[b]	160.60 ± 10.29[a]	152.00 ± 13.37 [a]
Final Weight	198.80 ± 11.79[a]	149.20 ± 6.34[b]	160.80 ± 12.42[a]	143.60 ± 12.51[b]

a,b,c,d = Means on the same rows with different superscript letters differ significantly ($P < 0.05$) compared with the control groups
NC+DW = Normal rats administered with distilled water, DC+DW = Diabetic 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)

Table3: Effects of Alpha Lipoic Acid on Haematological Parameters inStreptozotocin-induced Hyperglycaemia inWistar Rats

Parameters	NC + DW	DC + DW	D + ALA	D + GLB
RBC($\times 10^{12}$/L)	6.82±0.31[a]	6.82±0.62[a]	6.90±0.43[a]	7.86±0.39[a]
Hb (g/dL)	10.88±0.48[b]	10.72±1.04[b]	12.10±0.59[b]	12.32±0.47[b]
PCV (%)	35.40±1.47[c]	39.00±3.35[c]	38.60±2.58[c]	40.00±1.92[c]
Platelets($\times 10^9$/L)	548.00±18.60[a]	643.00±25.98[b]	152.60±16.01[a]	470.00±58.78[c]
WBC ($\times 10^9$/L)	2.50±0.44a [a]	2.92±0.19[b]	3.82±0.21[c]	4.80±1.53[c]
Neutrophil (%)	54.40±2.16[c]	41.00±5.49[d]	41.00±1.64[d]	48.80±5.97[c]
Lymphocyte (%)	31.60±3.01[d]	45.20±5.80[c]	43.00±3.02[c]	35.80±5.44[d]
Monocyte (%)	10.40±0.75[e]	9.20±0.49[d]	11.00±1.14[d]	9.60±1.70[d]
Eosinophil (%)	3.60±0.51[b]	4.20±0.20[b]	5.00±0.84[b]	6.20±0.97[c]

a,b,c,d = Means on the same rows with different superscript letters differ significantly ($P < 0.05$) when 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

STZ-induced diabetes in rats represents well-established animal models of type 1 insulin dependent, diabetes mellitus (Maritim*et al.*, 2003). STZ selectively destroys the insulin producing β-cells by inducing necrosis, and this is accompanied by characteristic alterations in blood insulin and glucose concentrations (Szkudelski, 2001; Sharma, 2010). In the present study, there was a significant ($P < 0.05$) increase on the levels of fasting blood glucose after three days of STZ injection to animals when compared with the normal control animals. This observation may be due to destruction to insulin producing β-cells of the islet of langerhans resulting to sustained elevated blood glucose levels. Following oral treatment of diabetic animals with ALA and glibenclamide, there was a steady reduction of the fasting blood glucose levels in diabetic rats when compared with the control group. Our present finding corroborates the report of Samar *et al.* (2010), who showed that supplementation of ALA significantly reduced STZ-induced hyperglycaemia in animals. The anti-diabetic activity of ALA has been reported to be due its ability to regulate glucose metabolism (Singh and Jialal, 2008). In addition, Khamaisi*et al.*(1997) showed that ALA administration to diabetic animals reduced blood glucose concentration by enhancing muscle GLUT4 protein content and thus increased muscle glucose utilization. Bodyweight is normally investigated as a sensitive indicator of chemically induced changes (Peters and Boyd, 1996). STZ-induced diabetes is usually characterized by a severe loss of body weight (Andrade Cetto and Wiedenfeld, 2001). A significant decrease in the body weights of diabetic animals were observed after induction of diabetes with streptozotocin. This finding agrees with the report of Oyedemi*et al*. (2011), who observed similar effect on diabetic animals treated with streptozotocin. Therefore, the reduction in body weights may be linked to degradation of structural proteins and increased muscle wasting due to loss of tissue proteins (Swanston-Flatt*et al.*, 1990; Daye *et al.*, 2013). Administration of ALA to diabetic animals produced a significant improvement on the body weights of diabetic animals when compared with the control group, which indicated that ALA prevented muscle tissue damage due to diabetic condition. However, the body weights of glibenclamide treated diabetic animals did not differ significantly when compared with the diabetic control group. The haematological parameters assessment have been reported to be a useful tool employed to study the deleterious effect of foreign compounds including chemical compounds on the blood constituents of animals (Oyedemi*et al.*, 2011; Okoro*et al.*, 2014). In the present investigation, STZ-induced diabetes did not produce a significant change in the RBC count, Hb concentration and PCV when compared with normal control animals. However, treatment of diabetic animals with ALA (100 mg/kg body) resulted to a significant elevation on the levels of above mentioned parameters, but the increase was not significant, when compared with the diabetic control. The total white blood cell (WBC) and platelets count obtained in diabetic control rats were significantly lower ($P < 0.05$) than that of the normal control group when compared. In addition, neutrophil and monocyte counts were significantly ($P < 0.05$) reduced in the diabetic control group when compared with the normal control animals, where as the lymphocyte and eosinophil counts in the diabetic control animals were not significantly ($P > 0.05$) different when compared with those in the normal control rats.

CONCLUSION

Following available findings from the present study, it can be concluded that the administration of ALA and glibenclamide lowered blood glucose level as well as restored the body weights of diabetic animals; however the altered haematological parameters were not significantly normalized when compared with the diabetic control group.

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