



Antihyperglycemic and antioxidative effects of ethanolic leaf extract of *Croton zambesicus* in streptozotocin induced diabetic rats

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ABSTRACT

The aim of this study was to evaluate the antihyperglycemic and antioxidative effect of *Croton zambesicus* (*C. zambesicus*) leaf extract in experimentally induced diabetic rats. Seventy adult male wistar rats were divided into seven groups (n=10). Group A, control rats; Group B, untreated diabetic rats; Group C, diabetic rats in which *C. zambesicus* therapy started 2 weeks prior to induction of diabetes; Group D and Group E, diabetic rats administered orally with *C. zambesicus* leaf extract for 2 and 4 weeks respectively; Group F, normal rats administered orally with *C. zambesicus*; Group G, diabetic rats administered glibenclamide (2 mg/kg/day). The animals were weighed and sacrificed. Serum was obtained for catalase (CAT), glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and thiobarbituric acid reactive substances (TBARS) analyses using respective diagnostic kits. The result showed a better remediation with early commencement of the extract treatment. *C. zambesicus* exhibited significant (p<0.05) reduction in blood glucose level in the extract treated group when compared with other groups. The result also presented an improvement in the CAT, GSH, SOD, GPx and a reduction in TBARS in the extract treated groups as against a reduction in the antioxidant level and an increase in TBARS concentration in the untreated diabetic group. In conclusion, this study demonstrated that *C. zambesicus* leaf extract possesses antihyperglycemic and antioxidative activities in streptozotocin (STZ) induced diabetic rats and by extension may be useful in the management of diabetes mellitus.

Keywords: *C. zambesicus*, TBARS, antioxidant, blood glucose level, Diabetes mellitus

C. zambesicus'un etanolik yaprak özütünün streptozotocin ile indüklenmiş diyabetik sıçanlarda antihiperglisemik ve aktioksidatif etkisi

Bu çalışmanın amacı, *Croton zambesicus* (*C. zambesicus*) yaprak özütünün antihiperglisemik ve aktioksidatif etkisini deneysel olarak indüklenmiş diyabetik sıçanlarda değerlendirmektir. Yetmiş yetişkin erkek wistar sıçanlar yedi gruba ayrılmıştır (n=10). Grup A, kontrol sıçanlar; Grup B, muamele edilmiş diyabetik sıçanlar; Grup C, diyabetin indüklenmesinden önce iki hafta *C. zambesicus* terapisi başlananlar; Grup D ve Grup E, sırasıyla 2 ve 4 hafta süreyle oral olarak *C. zambesicus* yaprak özütü uygulanan diyabetik sıçanlar; Grup F, oral olarak *C. zambesicus* uygulanan normal sıçanlar; Grup G, glibenclamid (2 mg/kg/gün) uygulanan diyabetik sıçanlar. Katalaz (CAT), glutatyon (GSH), süperoksit dismutaz (SOD), glutatyon peroksidaz (GPx) ve tiyobarbiturik asit reaktif maddesi (TBARS) analizleri serumda tanı kitleri ile yapılmıştır. Sonuçlar özüt muamelesine erken başlamanın daha iyi sonuç verdiğini göstermiştir. Diğer gruplarla karşılaştırıldığında *C. zambesicus*, özüt ile muamele edilen grupta kan glukoz seviyesinde anlamlı (p<0.05) bir azalmaya yol

açmıştır. Sonuçlar aynı zamanda muamele edilmemiş diyabetik grupta TBARS konsantrasyonundaki artış ve antioksidan seviyesindeki azalmaya karşın özütü ile muamele edilmiş grupta GSH, SOD, GPx'de artış ve TBARS'ta bir azalma göstermiştir. Sonuç olarak, bu çalışma *C. zambesicus* yaprak özütünün streptozotocin (STZ) ile indüklenmiş diyabetik sıçanlarda antihiperglisemik ve antioksidatif aktiviteyi oluşturarak ve bu etkiyi uzatarak diabetes mellitus yönetiminde kullanışlı olduğunu göstermiştir.

Anahtar Kelimeler: *C. zambesicus*, TBARS, antioksidan, kan glukoz seviyesi, diabetes mellitus

Introduction

Diabetic mellitus is associated with hyperglycemia caused by defects in insulin secretion, insulin action, or both. Chronic hyperglycemia of diabetes result in long-term damage and complications of various organs (Gavin *et al.*, 1997).

Destruction of the β -cells is implicated in the development of diabetes. These eventually lead to insulin deficiency. Impairment of insulin secretion and defects in insulin action occurs simultaneously in diabetic patient (Cantor *et al.*, 1995). It should be noted as well that abnormalities in carbohydrate, fat, and protein metabolism in diabetes is due to deficient action of insulin on target tissues (Cantor *et al.*, 1995; Gavin *et al.*, 1997). Diabetes is associated with significant oxidative stress (Kakkar *et al.*, 1995). Reactive oxygen species are an important part of the defense mechanisms against infection or injury (Manonmani *et al.*, 2005). Oberley (1988) and Garg *et al.* (1996) have shown that diabetic patients have an increased incidence of vascular disease and it has been shown that free radical activity is elevated during diabetes.

Experimentally induced diabetes in animals has provided considerable insight into the physiologic and biochemical derangements of the diabetic state (Ananthan *et al.*, 2003). Significant changes in lipid metabolism and structure is also evident in diabetes (Ananthan *et al.*, 2003). In diabetic rats, increased lipid peroxidation is associated with hyperlipidemia (Morel and Chisolm, 1989).

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or nonketotic hyperosmolar syndrome (Malone *et al.*, 1995).

The genus *Croton* is well known for its diterpenoid content and a lot of different types of diterpenes (phorbol esters, clerodane, labdane, kaurane, trachylobane, pimarane, etc.) have been

isolated from this genus (Block *et al.*, 2004). Ngadjui *et al.* (2002) revealed that compounds isolated from *C. zambesicus*, which are used in traditional medicine for the treatment of a number of diseases including malaria, were characterized and screened for anti-plasmodial activity using inhibition of growth of *Plasmodium berghei* in mice (Ngadjui *et al.*, 2002; Okokon and Nwafor, 2009). The compounds include abiatane diterpenoids, quinines, triterpenoids and flavonoid (Ngadjui *et al.*, 2002). The analgesic, anti-inflammatory, antipyretic (Okokon *et al.*, 2005) and antidiabetic (Okokon *et al.*, 2006) properties of *C. zambesicus* has been reported. Block *et al.* (2002) identified a new cytotoxic trachylobane diterpene from the leaves of *C. zambesicus*. In order to continue their investigations on the composition of the cytotoxic dichloromethane extract of the leaves, Block *et al.* (2004) have isolated and characterised two new trachylobane and one isopimarane diterpenes together with transphytol, α -amyrin and sterols. This study therefore examined the antioxidative effect of ethanolic leaf extract of *C. zambesicus* in streptozotocin-induced diabetic rats.

Materials and Methods

Materials

Streptozotocin was purchased from Tocris Bioscience, UK. Glimperide was purchased from Obafemi Awolowo University pharmacy shop, Nigeria. 10% Tween 80 was purchased from Sigma chemical company, St Louis, Missouri, USA. Glutathione, glutathione peroxidase, catalase, thiobaburic acid reactive substance (TBARS) were purchased from BioAssay Systems, USA. Superoxide dismutase (SOD) was purchased from Abor assays, USA.

Animal care

Seventy adult male albino rats of the Wistar strain were procured and acclimatized for two weeks at the Animal Holdings of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria before the commencement of the research

work. Animals were fed with standard rat feed (Capfeeds, Ibadan) and given water liberally. All the animal experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health, USA (NIH, 1985).

Preparation of plant extract

The leaves of *C. zambesicus* Müll. Arg. (Euphorbiaceae) were collected from Dramatic art garden of the Obafemi Awolowo University, Nigeria, and taken to the herbarium of Botany Department, Obafemi Awolowo University for authentication and identification. Herbarium specimen number of the plant (UHI 16511) was obtained. The fresh leaves of the plant were air dried on a laboratory table for 30 days and reduced to powder using squeezing and crushing machine (Daiki Rika Kogyo Co-ltd, Japan). The powder (400 g) was extracted with absolute ethanol (2.8L) for 72 hours. The extract was filtered using a filter paper. The filtrate obtained was concentrated *in vacuo* at 20°C using a vacuum rotary evaporator (BÜchi Rotavapor R110, Schweiz). The extract obtained was partitioned between dichloromethane and water. The dichloromethane fraction was oven dried at 37°C. The fraction obtained (13.8 g, 3.5%) was dissolved in 10% Tween 80 and administered orally at a dose of 200 mg/kg as the plant extract.

Experimental design

The animals were divided into seven groups as follows, with ten animals in each group. Group A were control rats administered 10% tween 80 for 4 weeks after the initial four weeks of intraperitoneal administration of 0.1 M sodium citrate buffer (pH 4.5), Group B were diabetic rats administered orally with 10% Tween 80 for 4 weeks after the initial four weeks of diabetic induction, Group C were diabetic rats, for which *C. zambesicus* leaf extract (200 mg/kg body weight/day/rat) in 10% Tween 80 therapy started 2 weeks prior to induction and continued throughout the period the experiment lasted (8 weeks), Group D were diabetic rats administered orally with *C. zambesicus* leaf extract (200 mg/kg body weight/day/rat) in 10% tween 80 for 2 weeks after the initial four weeks of diabetic induction (Withdrawal group), Group E were diabetic rats administered orally with *C. zambesicus* leaf extract (200 mg/kg body weight/day/rat) in 10% tween 80 for 4 weeks after the initial four weeks of diabetic

induction, Group F were normal rats administered orally with *C. zambesicus* leaf extract (200 mg/kg body weight/day/rat) in 10% tween 80 for four weeks, Group G were diabetic rats administered glimepiride (2 mg/kg body weight/day/rat) in 10% Tween 80 solution orally for four weeks (Mir *et al.*, 2008) after the initial four weeks of diabetic induction.

Induction of experimental diabetes

The animals in groups B, C, D, E and G were injected intraperitoneally with streptozotocin (Tocris Bioscience, UK, 65mg/kg body weight) dissolved in 0.1M sodium citrate buffer (pH 4.5). All the animals were kept and maintained under laboratory conditions of light, humidity and temperature. Before induction of diabetes, all the animals were fasted for 16 h, but still allowed free access to water throughout. These animals were stabilized for four weeks after which the leaf extract in 10% tween 80 was administered orally through gavages at a concentration of 200 mg/kg body weight/rat/day to groups D and E for another 2 and 4 weeks respectively. Meanwhile animals in group C were pretreated with *C. zambesicus* extract therapy 2 weeks prior to induction of diabetes.

Determination of blood glucose level

The animals were fasted for a period of 16 hours and their fasting blood glucose level was determined using a digital glucometer (Accu-chek® Advantage, Roche Diagnostic, Germany) consisting of a digital meter and the test strips. Blood sample was obtained from the tail vein.

Biochemical assays in the serum

The serum levels of GSH, GPx, SOD, CAT and TBARS were estimated using respective diagnostic kits.

Statistical analysis

Data were expressed as Mean ± Standard Error of Mean (S.E.M). The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 17.0 (SPSS, Cary, NC, USA) with Duncan's Multiple Range Test (DMRT) option. A value of $p < 0.05$ was considered to indicate a significant difference between groups.

Results

Blood Glucose Level (Bgl)

Forty-eight hours (day 2) after the injection with STZ in groups B, D, E and G, BGLs were far beyond hyperglycemic state of 250 mg/dl. The BGLs reached significantly high level in groups B, D, E and G only- 510.00 ± 33.70 mg/dl, 370.38 ± 28.18 mg/dl, 586.88 ± 6.77 mg/dl, 531.25 ± 55.33 mg/dl respectively vis-à-vis control group (104.25 ± 2.59 mg/dl). It was however interesting to note that of all the animal groups induced with STZ, only the group which received *C. Zambesicus leaf* extract (200 mg/kg) two weeks prior to STZ induction did not attain hyperglycemic status. At the end of the four weeks after a single dose of diabetic induction, the BGL in this group reached 118.86 ± 2.24 mg/dl which is not significantly different when compared with control 77.63 ± 2.11 mg/dl (Fig. 1).

Four weeks after diabetic induction (28 days), the animals in groups B, D, E and G still maintained their hyperglycemic state respectively (403.83 ± 47.20 mg/dl, 480.38 ± 35.78 mg/dl, 404.14 ± 34.31 mg/dl, 419.25 ± 45.58 mg/dl). Animals in group C at this stage still maintained their normoglycemic state (118.86 ± 2.24 mg/dl).

At the end of the experimental period (56 day), animals in group B (Diabetic rats administered orally with 10% tween 80 for 4 weeks after the initial four weeks of diabetic induction) maintained hyperglycemic state of 469.33 ± 30.63 mg/dl, which is significantly different ($p < 0.05$) from the control group (109.75 ± 2.14 mg/dl). Group C animals which received *C. Zambesicus leaf* extract (200 mg/kg) two weeks prior to STZ induction, maintain their normoglycemic state of 113.71 ± 3.29 mg/dl, which is not significantly different ($p > 0.05$) when compared with the control group (109.75 ± 2.14). However, animals in group D (Diabetic rats administered orally with *C. Zambesicus leaf* extract (200 mg/kg body weight/day/rat) in 10% tween 80 for 2 weeks after the initial four weeks of diabetic induction (Withdrawal group), had a 86.72% increment in BGLs as soon as the extract administration was withdrawn, which is significantly different ($p < 0.05$) from the control group. The BGL of animals in group E was observed to have gone down by 44.77% during the four weeks of extract administration. The final BGL of animals in group E (223.20 ± 15.42 mg/dl) was not only reduced to normoglycemic state but also compares favourably with group G (198.33 ± 52.41 mg/dl), which received 2 mg of glimepiride (antidiabetic drug) in a non-significant manner (Fig. 1).

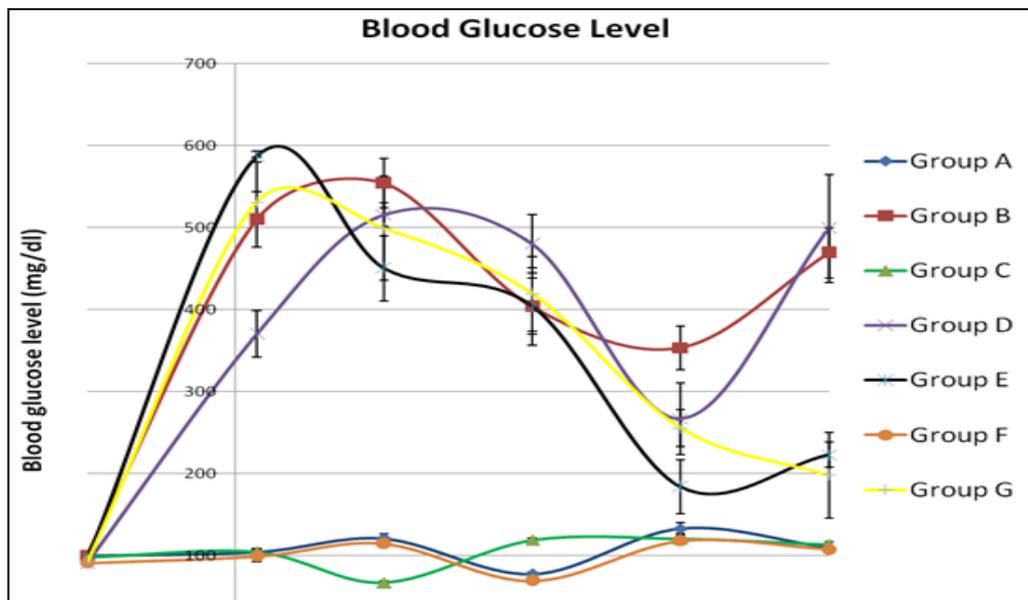


Figure 1. Effect of *C. zambesicus* on blood glucose level of animals (mg/dl) in STZ induced diabetic rats. Values are given as Mean ± SEM and are considered significant at $p < 0.05$ (using one way ANOVA with Duncan multiple range test).

Lipid peroxidation

TBARS: The activities of TBARS concentrations in the serum of all animal groups were measured. Untreated diabetic group ($18.80 \pm 6.46 \mu\text{M}$) showed a non significant ($p > 0.05$) increase in the concentration of TBARS when compared with the control animals ($13.60 \pm 5.67 \mu\text{M}$). With early commencement of extract administration two weeks prior to STZ induction, the concentration of TBARS was reduced to $12.26 \pm 5.19 \mu\text{M}$ (34.79%). Administration of the extract for four weeks after four weeks of diabetic stabilization (group E) also reduced the concentration of TBARS to $15.68 \pm 4.77 \mu\text{M}$ (16.60%). Withdrawal of the extract administration for two weeks significantly raised the concentration of TBARS by 24.65% when compared with group E (group which was administered extract for four weeks after four weeks of diabetic stabilization). Group administered glimepiride (group G) showed a reduction in TBARS concentration when compared with the group which was administered extract for four weeks after four weeks of diabetic stabilization (group E). The group administered extract alone also showed a non significant ($p > 0.05$) decrease in the concentration of TBARS vis-à-vis untreated diabetic group (group B). (Table 1).

Antioxidants

CAT: The activities of catalase concentration in the serum of all animal groups were measured. Untreated diabetic group ($9.13 \pm 6.06 \text{ U/L}$) showed a significant ($p < 0.05$) decrease in the activity of catalase when compared with the control animals ($47.79 \pm 10.37 \text{ U/L}$). With early commencement of extract administration two weeks prior to STZ induction, the activity of catalase was increased to $24.66 \pm 4.05 \text{ U/L}$. Administration of the extract for four weeks after four weeks of diabetic stabilization (group E) also raised the activities of catalase to $22.81 \pm 6.38 \text{ U/L}$. Withdrawal of the extract administration for two weeks significantly reduced the activities of catalase by 63.09% and 22.67% when compared with the control and the group E (group which was administered extract for four weeks after four weeks of diabetic stabilization) respectively. Group administered glimepiride showed a significant ($p < 0.05$) reduction in the activities of catalase concentration when compared with the group which was administered extract for four weeks after four weeks of diabetic stabilization (group E) and a significant increase ($p < 0.05$) when

compared with the untreated diabetic group. The group administered extract alone also showed a significant ($p < 0.05$) increment in the activities of catalase vis-à-vis untreated diabetic group (group B) (Table 1).

GSH: The activities of glutathione concentration in the serum of all animal groups were measured. Untreated diabetic group ($7.98 \pm 5.23 \mu\text{M}$) showed a significant ($p < 0.05$) decrease in the concentration of glutathione when compared with the control animals ($15.50 \pm 7.60 \mu\text{M}$). With early commencement of extract administration two weeks prior to STZ induction, the concentration of glutathione was increased to $17.12 \pm 8.57 \mu\text{M}$ (114.54%). Administration of the extract for four weeks after four weeks of diabetic stabilization (group E) also raised the concentration of glutathione to 19.96 ± 10.17 . Withdrawal of the extract administration for two weeks significantly reduced the concentration of glutathione by 13.42% and 32.77% when compared with the control and group E. Group administered glimepiride showed a non significant ($p > 0.05$) reduction in the activities of glutathione concentration when compared with the group which was administered extract for four weeks after four weeks of diabetic stabilization (group E). The group administered extract alone also showed a significant ($p < 0.05$) increment in the activities of glutathione vis-à-vis untreated diabetic group (group B) (Table 1).

SOD: The activity of SOD in the serum of all animal groups was measured. Untreated diabetic group ($0.21 \pm 0.15 \text{ U/mL}$) showed a non significant ($p > 0.05$) decrease in the activity of SOD when compared with the control animals ($0.31 \pm 6.79 \text{ U/mL}$). With early commencement of extract administration two weeks prior to STZ induction, the activity of SOD was increased to $0.50 \pm 6.79 \text{ U/mL}$. Administration of the extract for four weeks after four weeks of diabetic stabilization (group E) also increased the activities of SOD to $0.46 \pm 6.79 \text{ U/mL}$ when compared with the untreated diabetic group. Withdrawal of the extract administration for two weeks significantly reduced the activities of SOD by 32.26% and 54.35% when compared with the control and the group E (group which was administered extract for four weeks after four weeks of diabetic stabilization) respectively. Group administered glimepiride showed a non-significant ($p > 0.05$) increment in the activities of SOD concentration when compared with the group which

was administered extract for four weeks after four weeks of diabetic stabilization (group E). The group administered extract showed a significant ($p < 0.05$) increment in the activities of SOD vis-à-vis untreated diabetic group (group B) and control group (Table 1).

GPX: The activity of GPX in the serum of all animal groups was measured. Untreated diabetic group (0.69 ± 0.29 U/L) showed a non significant ($p > 0.05$) decrease in the activity of GPX when compared with the control animals (0.78 ± 0.34 U/L). With early commencement of extract administration two weeks prior to STZ induction, the activity of GPX was increased to 0.81 ± 0.33 U/L. Administration of the extract for four weeks after four weeks of diabetic stabilization (group E)

also raised the activities of GPX to 1.45 ± 0.30 U/L. Withdrawal of the extract administration for two weeks significantly reduced the activities of GPX by 26.92% and 60.69% when compared with the control and the group E (group which was administered extract for four weeks after four weeks of diabetic stabilization) respectively. Group administered glimepiride showed a reduction in the activities of GPX concentration when compared with the group which was administered extract for four weeks after four weeks of diabetic stabilization (group E) and the diabetic group. The group administered extract alone also showed a significant increase ($p < 0.05$) in the activity of GPX vis-à-vis untreated diabetic group (group B) (Table 1).

Table 1. Effects of *C. Zambesicus* on lipid peroxidation and antioxidants in STZ induced diabetic rats.

	TBARS (μM)	CAT (U/L)	GSH (μM)	SOD (U/mL)	GPX (U/L)
Group A	13.60 ± 5.67^a	47.79 ± 10.37^b	15.50 ± 7.60^{ab}	0.31 ± 0.19^{ab}	0.78 ± 0.34^a
Group B	18.80 ± 6.46^a	9.13 ± 6.06^a	7.98 ± 5.23^{ab}	0.21 ± 0.15^{ab}	0.69 ± 0.29^a
Group C	12.26 ± 5.19^a	24.66 ± 4.05^{ab}	17.12 ± 8.57^{ab}	0.50 ± 0.12^c	0.81 ± 0.33^a
Group D	20.81 ± 10.44^a	17.64 ± 8.82^a	13.42 ± 6.42^{ab}	0.22 ± 0.12^{ab}	0.57 ± 0.21^a
Group E	15.68 ± 4.77^a	22.81 ± 6.38^{ab}	19.96 ± 10.17^{ab}	0.46 ± 0.17^c	1.45 ± 0.30^{ab}
Group F	16.72 ± 5.45^a	20.40 ± 7.08^{ab}	33.65 ± 12.90^b	0.75 ± 0.23^b	1.40 ± 0.80^{ab}
Group G	14.98 ± 9.45^a	14.30 ± 10.30^a	11.14 ± 5.14^{ab}	0.43 ± 0.28^{ab}	0.60 ± 0.16^a

Values are given as Mean \pm SEM for 5 biological parameters called anti-oxidants in each group. a, b, c, ab, within column signifies that means with different letters differs significantly at $p < 0.05$, while means with the same letters does not differ significantly at $p < 0.05$ (using One way ANOVA with Duncan multiple range test).

Discussion

Medicinal herbs have been known to have hypoglycemic properties (Kakkar *et al.*, 1995; El-Missiry and El-Gindy, 2000; Pari and Saravanan, 2002; Vessal *et al.* 2003; Okokon *et al.* 2006; Un *et al.* 2006). Induction of STZ in groups B, D, E and G, led to an increase in BGL. STZ are known to be widely used to induce experimental diabetes in animals (Szkudelski, 2001). STZ enters the β cells through glucose transported and cause alkylation of DNA. The DNA damage induces activation of poly ADP-ribosylation which leads to the depletion of cellular NAD⁺ and ATP (Manonmani *et al.*, 2005). ATP dephosphorylation leads to the generation of hydrogen peroxide and hydroxyl radicals, thus, making the β cells undergo destruction by necrosis. Animals in group C that was pretreated with *C. zambesicus* leaf extract two weeks before STZ induction were not hyperglycemic. This observation was unique, but not surprising as some other medicinal herbs have also been reported to present the same effect (Hii and Howell 1984; Abdelmoaty *et al.*, 2010). The mechanism by which *C. zambesicus* leaf extract stalled the development of hyperglycemia in the STZ-induced animals is yet to be understood, but information has it that cytotoxic action of diabetogenic agents are mediated by generation of reactive oxygen species, which in turn leads to hyperglycemic state (Szkudelski, 2001). As in the case of STZ, the process leading to the generation of reactive oxygen species include the STZ entering the β cell via a glucose transporter, causing alkylation of DNA, activation of poly ADP-ribosylation, depletion of cellular NAD⁺ and ATP, generation of superoxide radicals, hydrogen peroxide and hydroxyl radicals, thus resulting in the destruction of β cells by necrosis. Any of the above processes may have been targeted and halted by the systemic presence of *C. zambesicus*, thus preventing the development of hyperglycemia in the experimental animals pretreated with *C. zambesicus* (group C). Ngadjui *et al.* (2002) identified compounds such as abiatane, diterpenoids, quinines, triterpenoids and flavonoid, labdane, clerodane, trachylobane and diterpenes in *C. zambesicus*. The flavonoid contained in *C. zambesicus* with or without some other compounds, whose pharmacological actions are yet to be ascertained may also have a role to play in the inhibition of hyperglycemia in group C. Hii and Howell (1984) and Abdelmoaty *et al.* (2010) reported that exposure of isolated rat islets to certain flavonoids such as epicatechin or

quercetin, enhances insulin release by 44–70%. They further emphasized that such flavonoids may act on islet function, at least in part, via alteration in Ca²⁺ fluxes and cyclic nucleotide metabolism. Due to the presence of aromatic hydroxyl groups, flavonoids have strong antioxidant properties and consequently protect against lipid peroxidation and chelating metal ions (Laughton *et al.*, 1991; du Thie and Crozier, 2000; Abdelmoaty *et al.*, 2010). They scavenge reactive oxygen and nitrogen species thus inhibiting peroxidation reactions. Reports have also shown that flavonoids protect macrophages from oxidative stress by maintaining glutathione in its reduced form (Fuhrman and Aviram, 2001; Abdelmoaty *et al.*, 2010). Flavonoids shield normal rat islets from STZ, normalize blood glucose levels and promote β -cell regeneration in islets of STZ-treated rats (Un *et al.*, 2006; Abdelmoaty *et al.*, 2010).

The hypoglycemic effect of *C. zambesicus* observed in this investigation (for animals in group E which was treated with *C. zambesicus* leaf extract for four weeks after four weeks of diabetic stabilization) corroborates earlier investigation (Okokon *et al.*, 2006). STZ-induction in animals selectively destroys the pancreatic insulin secreting β cells thus leaving less active pancreatic cells resulting in a type 2 DM model (Ivorra *et al.*, 1988; Sharma *et al.*, 1997; Amrani *et al.*, 2009).

The hypoglycemic effects of *C. zambesicus* leaf extract might be by regeneration and/or stimulation of the β cells which are not totally destroyed to produce insulin. Insulin is a hormone that is produced by specialized cells (beta cells) of the pancreas (Maritim *et al.*, 2003). In addition to helping glucose to enter the cells, insulin is also important in tightly regulating the level of glucose in the blood. After a meal, the blood glucose level rises. In response to the increased glucose level, the pancreas normally releases more insulin into the bloodstream to help glucose enter the cells and lower blood glucose levels after a meal. When the blood glucose levels are lowered, the insulin release from the pancreas is reduced. Vessal *et al.* (2003) and Abdelmoaty *et al.* (2010) in their respective study proposed that flavonoid is advantageous in lowering blood glucose level, promoting regeneration of the pancreatic islets and increasing insulin release in STZ-induced diabetic rats; thus exerting its beneficial antidiabetic effects. Animals in group D (withdrawal group) however, could not achieve the hypoglycemic status in the present investigation. 86.72% increment in BGLs was

noticed as soon as the extract administration was withdrawn two weeks to the expiration of this study which is significantly different ($p < 0.05$) from control group. The reason for this may be that there was a decrease in the systemic presence of *C. zambesicus* thus, reducing the regenerative potential and stimulating influence of the extract on the few β cells left to produce insulin which consequently resulted in hyperglycemic state. The fact that the hypoglycemic activities of the extract in group E is not significant vis-à-vis group treated with glimepiride, corroborate the efficacy of *C. zambesicus* in the management of DM.

Hypoinsulinemia in diabetes increases the activity of the enzyme fatty acyl coenzyme A oxidase which initiates beta oxidation of fatty acids, resulting in lipid peroxidation (Horie *et al.*, 1981; Kumar *et al.*, 2008). This present study presented an increase in serum concentration of TBARS in diabetic rats. Increased TBARS concentration has been reported to be associated with diabetes (Bagnyukova *et al.*, 2005; Manonmani *et al.*, 2005; Jin *et al.*, 2008; Kumar *et al.*, 2008; Matsunami *et al.*, 2010). This increment in TBARS is indicative of peroxidative injury. Increased lipid peroxidation impairs membrane function by decreasing membrane fluidity and changing the activity of membrane bound enzymes and receptors (Acworth *et al.*, 1997; Manonmani *et al.*, 2004). Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of both types of DM (Maritim *et al.*, 2003). Maritim *et al.* (2003) reported that free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance (Maritim *et al.*, 2003).

Pretreatment of animals with *C. zambesicus* two weeks prior to STZ induction and treatment of diabetic animals with *C. zambesicus* for four weeks reduced the TBARS concentration in the animal groups vis-à-vis the untreated diabetic group. Following the withdrawal of the extract (group D), the TBARS concentration was increased by 32.72%. This shows that *C. zambesicus* is a potent inhibitor of oxidative damage in diabetic state. Treatment of diabetic animals with glimepiride also lowered the TBARS concentration in a non

significant manner when compared with the group pretreated with extract two weeks prior to STZ induction and the group treated with extract for four weeks after four weeks of STZ induction.

Antioxidant plays a vital role in preventing diabetic complications by fighting or destroying free radicals or oxidants. They also enhance insulin secretion and insulin sensitization. Antioxidants like catalase, glutathione peroxide, superoxidase dismutase, albumin, bilirubin, uric acid, selenium and vitamins are very necessary to avoid long-term diabetic complications.

Reduced activities of GSH, CAT, GPx and SOD in the untreated diabetic group were observed in this investigation. Reduction in antioxidants results in lethal effects due to the accumulation of superoxide radicals and hydrogen peroxide (Halliwell and Gutteridge, 1989; Harris, 1992; Lushchak *et al.*, 2005a; Lushchak *et al.*, 2005b).

GSH is the most abundant intracellular antioxidant, dysregulation of which is widely implicated in disease states (Davis, 2007). A significant decrease in GSH was observed in untreated diabetic rats in this investigation. Evidence from *in vitro* and clinical perspectives have shown that abnormal glutathione status is involved in β -cell destruction and pathogenesis of complications associated with diabetes (Burk, 1983; Kaplowitz *et al.*, 1985; Davis, 2007). Reduction of glutathione has also been reported in diabetic rats (Manonmani, 2004; Ani *et al.*, 2011). Pretreatment of animals with *C. zambesicus* two weeks prior to STZ induction and treatment of diabetic animals with *C. zambesicus* for four weeks presented an improvement in GSH concentration. This may be due to the presence of GSH antioxidant compounds in *C. zambesicus* as evident in the significant increase in GSH status in the group administered extract alone. Over the years, interest has shifted to the invention of therapeutic modification of GSH status in the treatment of diabetes (Davis, 2007). Following the withdrawal of extract treatment two weeks to the expiration of the research (group D), the concentration of GSH was reduced by 32.77% as compared with the group treated with extract for four weeks (group E). This justifies earlier findings of Davis (2007) who supports the use of glutathione pro-drugs, lipoic acid and vitamin supplementation in the management of diabetes. The group treated with glimepiride also increased the glutathione concentration but not as much as the extract treated groups.

CAT and GPx are the two scavenging enzymes that remove free radicals generated during oxidative stress (Maritim *et al.*, 2003). While CAT is located in peroxisomes, GPx is found in the cytoplasm, mitochondria, and nucleus. These enzyme activities were inactivated by ROS during diabetes (Ahmed *et al.*, 2000). CAT and GPx are responsible for reducing hydrogen peroxide to water. GPx exhibit its function by reducing hydrogen peroxide to water via oxidation of glutathione. Reduction of the oxidized form of glutathione is then catalysed by glutathione reductase. In this study, the activities of CAT and GPx were significantly reduced in the untreated diabetic rats. Pretreatment of animals with *C. zambesicus* two weeks prior to STZ induction (group C) and treatment of diabetic animals with *C. zambesicus* for four weeks (group E), presented a significant ($p < 0.05$) increase in CAT and a non-significant increase in GPx activities. Following the withdrawal of extract treatment (group D), the activities of CAT and GPx was significantly reduced by 22.67% and 60.69% respectively. This suggests that antidiabetic efficacy of the *C. zambesicus* apart from other mechanism yet to be known, is by increase in CAT and GPx activities thus reducing oxidative stress in diabetes as seen in this study. This was also justified in view of the significant increase in CAT and GPx activities in the extract treated groups vis-à-vis group treated with glimepiride.

SOD converts superoxide anion radicals produced in the body to hydrogen peroxide, thereby reducing the likelihood of superoxide anion interacting with nitric oxide to form reactive peroxynitrite (Maritim *et al.*, 2003). Isoforms of SOD are variously located within the cell. SOD activity is undoubtedly important to the regulation of oxidative status in diabetes. However, there is variation as to the status of this enzyme in the diabetic state. Some studies have reported decreased SOD activity (Kedziora-Kornatowska *et al.*, 2003; Maritim *et al.*, 2003), others have shown increases (Rauscher *et al.*, 2001) or no change in the enzyme (Mekinova *et al.*, 1995; Maritim *et al.*, 2003). In this investigation, a significant reduction was observed in SOD activity when compared with the control group. Treatment of animals with *C. zambesicus* two weeks prior to STZ induction (group C) and treatment of diabetic animals with *C. zambesicus* for four weeks (group E), presented a significant ($p < 0.05$) increase in SOD activity. Following the withdrawal of extract treatment (group D), the activities of SOD was significantly

reduced by 57.17%. This shows that *C. zambesicus* may contain SOD-like antioxidant compounds as evident in the significant improvement in SOD status in the group administered extract alone. This further affirms that, the antidiabetic efficacy of the *C. zambesicus* could be by increase in the antioxidant status as evident in this study.

In conclusion, ethanolic leaf extract of *C. zambesicus* possesses antihyperglycemic and antioxidative activities in streptozotocin-induced diabetic rats in the extract treated animals and this effect may be due to some important compounds such as abiatane, diterpenoids, quinines, triterpenoids, flavonoid, labdane, clerodane, trachylobane and diterpenes identified in *C. zambesicus*.

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