

¹ Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

² Department of Biochemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

³ Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

⁴ Department of Physiological Sciences, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

EFFECT OF ETHANOLIC LEAF EXTRACT OF *CROTON ZAMBESICUS* (MÜLL. ARG.) ON LIPID PROFILE IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

David A. Ofusori¹, Omobola A. Komolafe¹, Olarinde S. Adewole¹, Efere M. Obuotor², Julius B. Fakunle³, Abiodun O. Ayoka⁴

Key words: diabetes, lipid profile, Croton zambesicus, glimepiride, streptozotocin

SUMMARY

Croton zambesicus (*C. zambesicus*) leaves have recently been identified as an antidiabetic agent in Africa. The aim of this study was to examine the effect of *C. zambesicus* leaf extract on lipid profile 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, diabetic rats administered orally with *C. zambesicus* leaf extract for 2 weeks after the initial four weeks of diabetes induction; group E, diabetic rats administered orally with *C. zambesicus* leaf extract for 4 weeks after the initial four weeks of diabetes induction; group F, normal rats administered orally with *C. zambesicus* leaf extract for four weeks;

and group G, diabetic rats administered with glimepiride (2 mg/kg/day) for four weeks after the initial four weeks of diabetes induction. At the end of the experimental period, the animals were weighed and sacrificed. Serum was obtained for lipid profile analysis using respective diagnostic kits. The results showed that the blood glucose level and body weights of extract and glimepiride treated groups were restored to the near normal level. The triglyceride, total cholesterol, low density lipoprotein and very low density lipoprotein cholesterol levels were increased significantly ($P<0.05$) in untreated diabetic group (group B) as compared with reduction in group C in which *C. zambesicus* therapy started 2 weeks prior to the induction of diabetes and group E which had *C. zambesicus* leaf extract administered for 4 weeks after the initial four weeks of diabetes induction. Also, the level of high density lipoprotein cholesterol was reduced significantly ($P<0.05$) in untreated diabetic group (group B) when compared with the group treated with the extract for four weeks (group E). In conclusion, this study showed the *C. zambesicus* leaf extract to have a lipid lowering effect in streptozotocin-induced diabetic rats and by extension may be useful in the management of diabetic hyperlipidemia.

Corresponding author: David A. Ofusori, Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria
E-mail: davidofus234@yahoo.com

INTRODUCTION

Diabetic mellitus (DM) is a chronic disease characterized by relative or absolute deficiency of insulin, resulting in glucose intolerance. It develops in 4-5 million persons in the United States (approximately 2% of the population) (1,2). The classic symptoms of DM result from abnormal glucose metabolism (1). The lack of insulin activity results in failure of glucose transfer from the plasma into the cells. This situation is also called “starvation in the midst of plenty”. The body responds as if it were in the fasting state, with stimulation of glucogenolysis, gluconeogenesis and lipolysis producing ketone bodies (3)

The glucose absorbed during a meal is not metabolized at the normal rate and therefore accumulates in the blood (hyperglycemia) to be excreted in the urine (glycosuria) (2). Glucose in the urine causes osmotic diuresis, leading to an increase in urine production (polyuria). Stimulation of protein breakdown to provide amino acids for gluconeogenesis results in muscle wasting and weight loss (2,4). These classic symptoms occur only in patients with severe insulin deficiency, most commonly in type 1 diabetes. Many patients with type 2 diabetes do not have these symptoms and present with one of the complications of diabetes (5).

Clinically, diabetes is characterized by hyperlipidemia, which may eventually result in severe cardiovascular complications (4). This necessitates the use of safe medication for diabetic management to replace current drugs, which often bring about side effects.

Herbal therapy has become a potent alternative means of managing hyperlipidemia in diabetes with lesser side effects. Several herbal medicines have been reported to exhibit hypoglycemic or hypolipidemic effect (3-5).

Croton zambesicus (*C. zambesicus*), a component of tiger bush, is a medicinal plant grown in villages and towns in Nigeria (6). This is a shrub, often branching low down with a spreading crown and characteristic hanging leaves and silvery beneath and with whitish to

pale grayish bark. Compounds such as abiatane diterpenoids, quinines, triterpenoids and flavonoids labdane, clerodane and trachylobane diterpenes have been identified in the stem bark of *C. zambesicus* (7). Recent reports confirm that *C. zambesicus* leaf extract displays antidiabetic and gastroprotective activity as demonstrated by significant inhibition of the formation of ulcers induced through the three different ulcer models studied (8).

Information available within a limited search did not show any study on the effects of *C. zambesicus* on lipid profile in diabetic rats. This study was thus initiated to examine the effect of *C. zambesicus* leaf extract on lipid profile in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

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 commencement of the research work. Animals were fed with standard rat feed (Capfeeds, Ibadan) and given water *ad libitum*.

All 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) (9).

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. 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

(2.8 L) 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, Switzerland). 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

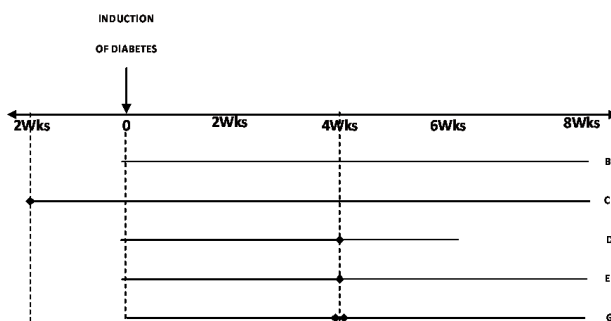
The animals were divided into seven groups as follows, ten animals *per* group (Fig. 1): group A: control rats intraperitoneally administered 0.1 M sodium citrate buffer (pH 4.5); group B: diabetic rats orally administered 10% Tween 80 for 4 weeks after the initial four weeks of diabetic induction; group C: diabetes rats in which *C. zambesicus* leaf extract (200 mg/kg body weight/day/rat) in 10% Tween 80 therapy started 2 weeks prior to diabetes induction and continued throughout the experimental period (8 weeks); group D: diabetic rats orally administered *C. zambesicus* leaf extract (200 mg/kg body weight/day/rat) in 10% Tween 80 for 2 weeks after the initial four weeks of diabetes induction (withdrawal group); group E: diabetic rats orally administered *C. zambesicus* leaf extract (200 mg/kg body weight/day/rat) in 10% Tween 80 for 4 weeks after the initial four weeks of diabetes induction; group F: normal rats orally administered *C. zambesicus* leaf extract (200 mg/kg body weight/day/rat) in 10% Tween 80 for four weeks; and group G: diabetic rats administered glimepiride (2 mg/kg body weight/day/rat) in 10% Tween 80 solution orally for four weeks (10) after the initial four weeks of diabetes induction.

Induction of experimental diabetes

The animals in groups B, C, D, E and G were injected intraperitoneally with streptozotocin (Tocris Bioscience, UK, 65 mg/kg body weight) dissolved in 0.1M sodium citrate buffer (pH 4.5). All animals were kept and maintained under laboratory conditions of light, humidity and temperature. Before induction of

diabetes, all animals were fasted for 16 h, but still allowed free access to water throughout the experiment. 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 (Fig. 1). Meanwhile, group C animals were pretreated with *C. zambesicus* extract therapy 2 weeks prior to induction of diabetes.

Figure 1. Diabetes induced experimental group



- ◆◆ Point of glimepiride administration
- ◆ Point of *Croton zambesicus* extract administration

Blood glucose determination

Blood glucose level was determined in all animal groups using a digital glucometer (Accu-chek® Advantage, Roche Diagnostic, Germany) and a compatible test strip.

Body weight monitoring

Body weights of all animals in each group were monitored using a top loader weighing balance throughout the experimental period.

Sacrifice of animals

At the end of the experimental period, all animals were sacrificed by chloroform inhalation. A blood sample was obtained through cardiac puncture following incision made in the thoracic cage.

Serum collection and preparation

Serum was obtained after centrifugation of blood samples for 5 min at 5000 rpm in a Benchtop refrigerated centrifuge (Centurion Scientific centrifuge, R8000 series, UK). The serum obtained was used for determination of triglycerides, total cholesterol and high-density lipoprotein cholesterol using a commercially available kit (Randox, Northern Ireland).

Biochemical assays in serum

Serum levels of triglycerides (TG) (11), total cholesterol (TC) (12) and high-density lipoprotein cholesterol (HDL-C) (12) were determined by the respective diagnostic commercial kits from Randox, Northern Ireland. Very low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) were calculated according to Friedewald's equation (13).

Statistical analysis

Data were expressed as mean \pm standard error of mean (SEM). 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 significant difference between groups.

RESULTS

Blood glucose level

The blood glucose level of animals pretreated (group C) and treated (group E) with *C. zambesicus* extract was significantly ($P < 0.05$) lowered as compared with the untreated diabetic group at the end of the experimental period (day 56). The results as presented in Table 1 also showed a significant ($P < 0.05$) elevation of blood glucose level in group D animals following withdrawal of the extract administration as compared with group E.

Body weight

At the expiration of the experimental period, the body weight of group A animals (control rats intraperitoneally administered 0.1M sodium citrate buffer (pH 4.5) presented 48.35% increment when compared with 16% decrement obtained in group B animals (diabetic rats orally administered 10% Tween 80 for 4 weeks after the initial four weeks of diabetes induction). Group C animals (diabetic rats in which *C. zambesicus* therapy started 2 weeks prior to diabetes induction and continued throughout the experimental period (8 weeks)) showed 54% increment in body weight during the experimental period (Table 2). Meanwhile, diabetic rats orally administered *C. zambesicus* leaf extract (200 mg/kg body weight/day/rat) in 10% Tween 80 for 2 weeks after the initial four weeks of diabetes induction (withdrawal group D) lost 9% of body weight within the 8 weeks of experimental period. Diabetic rats orally administered *C. zambesicus* leaf extract (200 mg/kg body weight/day/rat) in 10% Tween 80 for 4 weeks after the initial four weeks of diabetes induction (group E) and normal rats orally administered *C. zambesicus* leaf extract (200 mg/kg body weight/day/rat) in 10% Tween 80 for four weeks gained 47% and 45% of body weight over the experimental period, respectively. Diabetic rats administered antidiabetic drug glibenclamide (2 mg/kg body weight/day/rat) in 10% Tween 80 solution orally for four weeks after the initial four weeks of diabetes induction (group G) also presented a body weight gain of 38.8% (Table 2).

Comparison of body weights at the end of the 8-week experimental period (day 56) showed a significant difference ($P < 0.05$) between the control group (group A, 168.75 \pm 7.49 g) and untreated diabetic group (group B, 128.33 \pm 8.43 g). Also, the body weights did not differ significantly ($P < 0.05$) when groups C, E, F and G were compared with control animals, with the exception of group D (withdrawal group) which differed significantly ($P < 0.05$, 115.00 \pm 9.35 g) (Table 2).

Table 1. Effects of *C. zambesicus* on blood glucose level (BGL) in STZ induced diabetic rats

Day	-14	2	14	28	42	56
Group A	100.00±3.91 ^a	104.25±2.59 ^a	121.00±5.60 ^a	77.63± 2.11 ^a	133.13±7.54 ^a	109.75±2.14 ^a
Group B	100.75±2.24 ^a	510.00±33.70 ^c	554.14±29.89 ^c	403.83±47.20 ^b	353.67±26.69 ^c	469.33±30.63 ^c
Group C	98.00±2.10 ^a	104.50±4.55 ^a	67.13±1.74 ^a	118.86±2.24 ^a	120.43±6.25 ^a	113.71±3.29 ^a
Group D	90.75±3.19 ^a	370.38±28.18 ^b	515.75±14.22 ^{bc}	480.38±35.78 ^b	267.25±43.79 ^b	499.00±65.67 ^c
Group E	96.63±3.93 ^a	586.88±6.77 ^c	450.25±39.68 ^b	404.14±34.31 ^b	183.86±32.79 ^a	223.20±15.42 ^b
Group F	91.25±3.27 ^a	99.25±6.66 ^a	114.50±2.62 ^a	69.38±3.44 ^a	118.20±4.50 ^a	108.00±1.87 ^a
Group G	91.75±3.67 ^a	531.25±55.33 ^c	499.20±63.21 ^{bc}	419.25±45.58 ^b	255.67±22.59 ^b	198.33±52.41 ^b

Values are given as mean ± SEM for reference days coded as days -14, 2, 14, 28, 42 and 56 in each group; a, b, c, bc within column signify that means with different letters differ significantly at $P<0.05$, while means with the same letters do not differ significantly at $P<0.05$ (using one-way ANOVA with Duncan multiple range test)

Table 2. Effects of *C. zambesicus* on body weight (g) in STZ induced diabetic rats

Day	-14	2	14	28	42	56
Group A	113.75±3.24 ^a	104.75±0.726 ^a	127.50±5.26 ^{ab}	151.88±6.26 ^c	165.00±8.02 ^b	168.75±7.49 ^b
Group B	153.13±16.15 ^b	157.50±17.074 ^c	141.43±12.38 ^b	141.67±7.49 ^{bc}	125.83±6.76 ^a	128.33±8.43 ^a
Group C	120.00±4.23 ^a	138.13±5.17 ^{bc}	142.50±3.41 ^b	145.00±1.89 ^{bc}	163.13±5.25 ^b	185.71±3.85 ^b
Group D	127.50±8.61 ^a	137.50±7.79 ^{bc}	124.38±4.671 ^{ab}	112.50±5.59 ^a	125.00±10.61 ^a	115.00±9.35 ^a
Group E	111.88±4.43 ^a	121.25±3.87 ^{ab}	108.75±7.55 ^a	125.71±10.88 ^{ab}	150.00±7.85 ^b	165.17±9.28 ^b
Group F	123.75±8.44 ^a	145.63±9.09 ^{bc}	133.13±7.61 ^b	145.00±6.27 ^{bc}	168.33±6.54 ^b	180.20±12.71 ^b
Group G	128.75±3.63 ^a	138.13±3.40 ^{bc}	136.00±6.00 ^b	154.67±7.31 ^c	166.67±10.93 ^b	178.67±11.33 ^b

Values are given as mean ± SEM for reference days coded as -14, 0, 14, 28, 42 and 56 in each group; a, b, c, ab, bc within column signify that means with different letters differ significantly at $P<0.05$, while means with the same letters do not differ significantly at $P<0.05$ (using one-way ANOVA with Duncan multiple range test)

Values are given as mean ± SEM for reference days coded as days -14, 2, 14, 28, 42 and 56 in each group; a, b, c, bc, within column signify that means with different letters differ significantly at $P<0.05$, while means with the same letters do not differ significantly at $P<0.05$ (using one-way ANOVA with Duncan multiple range test)

Values are given as mean ± SEM for reference days coded as -14, 0, 14, 28, 42 and 56 in each group; a, b, c, ab, bc within column signify that means with different letters differ significantly at $P<0.05$, while means with the same letters do not differ significantly at $P<0.05$ (using one-way ANOVA with Duncan multiple range test)

Lipid profile

The results showed an increment in serum levels of TG, TC, LDL-C and VLDL-C in group B, diabetic control (108.60±28.36 mg/dL, 115.83±8.09 mg/dL, 65.32±15.27 mg/dL and 21.72±5.67 mg/dL,

respectively), which was lowered when treated with *C. zambesicus* (200 mg/kg) for 4 weeks (75.51±17.49 mg/dL, 72.12±26.71 mg/dL, 30.65±21.41 mg/dL and 15.10±3.49 mg/dL). The levels of TG and VLDL-C were observed to be further reduced to 68.72±10.69 mg/dL and 13.74±2.13 mg/dL, respectively, with early commencement of *C. zambesicus* two weeks prior to STZ induction (group C). Following the withdrawal of the extract for two weeks after the initial two weeks of administration, the TG and VLDL-C levels were elevated (91.61±33.22 mg/dL and 18.32±6.64 mg/dL, respectively) when compared with group E animals (75.51±17.49 mg/dL and 15.10±3.49 mg/dL, respectively), which received the extract for 4 weeks. However, there was a significant fall in the level of high-density lipoprotein-cholesterol (HDL-C) in the diabetic group (28.79±4.46 mg/dL) where, when treated with ethanolic extract of *C. zambesicus* (200 mg/kg) for 4 weeks, serum levels of HDL-C significantly increased (43.32±7.02 mg/dL, $P<0.05$). Following withdrawal of the extract in group D

Table 3. Effects of *C. zambesicus* on serum lipid profile in STZ induced diabetic rats

Day	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Group A	68.26±19.46 ^a	77.47±2.21 ^{ab}	53.86±8.33 ^c	7.01±2.87 ^a	13.65±3.89 ^a
Group B	108.60±28.36 ^a	115.83±8.09 ^b	28.79±4.46 ^{ab}	65.32±15.27 ^b	21.72±5.67 ^a
Group C	68.72±10.69 ^a	76.20±10.38 ^{ab}	26.22±2.15 ^{ab}	39.14±11.02 ^{ab}	13.74±2.13 ^a
Group D	91.61±33.22 ^a	42.61±15.61 ^a	15.85±9.15 ^a	42.14±1.14 ^{ab}	18.32±6.64 ^a
Group E	75.51±17.49 ^a	72.12±26.71 ^{ab}	43.32±7.02 ^b	30.65±21.41 ^{ab}	15.10±3.49 ^a
Group F	55.74±10.89 ^a	44.71±10.11 ^a	34.19±2.64 ^{ab}	7.80±2.67 ^a	11.14±2.17 ^a
Group G	98.00±27.45 ^a	48.69±9.01 ^a	37.73±5.39 ^b	3.09±3.05 ^a	19.60±5.48 ^a

Values are given as mean ± SEM for 5 biochemical parameters (TG, TC, HDL-C, LDL-C and VLDL-C) in each group; a, b, c, ab within column signify that means with different letters differ significantly at $P<0.05$, while means with the same letters do not differ significantly at $P<0.05$ (using one-way ANOVA with Duncan multiple range test)

animals after 2 weeks of administration, HDL-C levels decreased significantly ($P<0.05$) to 15.85±9.15 mg/dL when compared with group E (43.32±7.02 mg/dL), which had extract administration continued for 4 weeks after diabetes induction. The antidiabetic group had their TG reduced from 108.60 ± 28.36 mg/dL to 98.00 ± 27.45 mg/dL, TC from 115.83 ± 8.09 mg/dL to 48.69 ± 9.01 mg/dL, LDL-C from 65.32 ± 15.27 mg/dL to 3.09 ± 3.05 mg/dL and VLDL-C from 21.72 ± 5.67 mg/dL to 19.60 ± 5.48 mg/dL. However, HDL-C increased from 28.79 ± 4.46 mg/dL to 37.73 ± 5.39 mg/dL. The group administered the extract only presented a similar but different pattern of alteration in the lipid profile when compared with controls, as shown in Table 3.

Values are given as mean ± SEM for 5 biochemical parameters (TG, TC, HDL-C, LDL-C and VLDL-C) in each group; a, b, c, ab, within column signify that means with different letters differ significantly at $P<0.05$, while means with the same letters do not differ significantly at $P<0.05$ (using one-way ANOVA with Duncan multiple range test)

DISCUSSION

Plant extract has been used in recent times for the management of diabetes. The hypoglycemic and antihyperglycemic effect of the *C. zambesicus* leaf extract further corroborates previous study (14). This result may be due to the presence of hypoglycemic agent and/or insulin-like substances such as flavonoid previously identified in *C. zambesicus* (7). Studies have shown that the aromatic hydroxyl groups present

in flavonoids have strong antioxidant properties and consequently protect against lipid peroxidation and chelating metal ions, an effect which is capable of lowering blood glucose (15).

Diabetic condition has been known to be associated with weight loss (16). The weight loss recorded in untreated diabetic animals could be a symptom of ill health, which must have been caused by the release of free radicals. This is related to a previous investigation (16). Halliwell and Gutteridge (17) also related systemic toxicity to the release of free radicals and hence the loss in weight, which is an index of ill health.

Alterations in serum lipid profile increase the risk of coronary heart disease (2,4,18). In this investigation, marked hyperlipidemia characterized animals in the untreated diabetic group. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats (18-23). This marked hyperlipidemia noticed in the untreated diabetic state may be due to unrestrained actions of lipolytic hormones on the fat depot (18,23).

Increase in serum levels of triglycerides has been traced to insulin deficiency, thus resulting in hyperglycemia and mobilization of fatty acids from adipose tissue (24). The fatty acids from adipose tissue are mobilized for energy purpose and excess fatty acids are accumulated in the liver and converted to triglycerides (25). Suryawanshi *et al.* (26) report an increase in the number of LDL receptor in diabetic patients. It is therefore envisaged that insulin deficiency might be associated with a diminished level

of LDL receptor, thus leading to an increase in LDL-C concentration in diabetic state, as corroborated in this investigation.

Ethanol extract of *C. zambesicus* reduced serum total cholesterol and triglycerides and increased HDL-C levels as compared with untreated diabetic rats (group B). The antihyperlipidemic activity of *C. zambesicus* was comparable with the group treated with glimepiride, a standard antidiabetic drug, but glimepiride is a stronger LDL-C reducer than *C. zambesicus*. One of the most important metabolic actions of insulin or insulin-like agent such as *C. zambesicus* is the inhibition of lipolytic activity in fat cells. Activation of the adipocyte cGMP-inhibited cAMP phosphodiesterase by insulin and insulin-like agent is believed to be the major mechanism whereby cellular cAMP is reduced, which then leads to inactivation of the cAMP-dependent protein kinase, net dephosphorylation of hormone-sensitive lipase and antilipolysis (27). The antihyperlipidemic effect of *C. zambesicus* may be due to the presence of flavonoids as previously investigated (7). It has been reported that

quaternary alkaloids, flavonoids and glycoside components reduce lipid levels in animals (28). Another antihyperlipidemic process of *C. zambesicus* may be down-regulation of NADPH and NADH, cofactors in the fat metabolism as reported in a similar investigation by Ananthan *et al.* (20). These activities of *C. zambesicus* are beneficial in preventing diabetic complications in addition to improving lipid metabolism in diabetes.

Thus it can be concluded that oral administration of the ethanolic leaf extract of *C. zambesicus* has a significant antihyperlipidemic effect in streptozotocin-induced diabetes in adult Wistar rats and by extension can be used in the management of diabetic hyperlipidemia.

Acknowledgment

We sincerely express our profound gratitude to Mr. Idowu Olawuni of the Biochemistry Department, Obafemi Awolowo University, Ile-Ife, Nigeria, for his biochemical input.

REFERENCES

1. Bolzan AD, Bianchi MS. Genotoxicity of streptozotocin. *Mutat Res* 2002;512:121-134.
2. Laakso M. Lipids and lipoproteins as risk factors for coronary heart disease in non-insulin dependent diabetes. *Ann Med* 1996;28:341-345.
3. Freener EP, King GL. Vascular dysfunction in diabetes mellitus. *Lancet* 1997;350 Suppl 1:S19-S113.
4. Steiner G. Risk factors for macrovascular disease in type 2 diabetes: classic lipid abnormalities. *Diabetes Care* 1999;22(3):c6-c9.
5. Nukatsuka M, Yoshimura Y, Nishida M, Kawada J. Allopurinol protects pancreatic beta cells from the cytotoxic effect of streptozotocin: *in vitro* study. *J Pharmacobiodyn* 1990;13:259-262.
6. Okokon JE, Ofodum KC, Ajibesin KK, Danladi B, Gamaniel KS. Pharmacological screening and evaluation of antiplasmodial activity of *Croton zambesicus* against *Plasmodium berghei berghei* infection in mice. *Ind J Pharmacol* 2005;37:243-246.
7. Ngadjui BT, Abegaz BM, Keumedjio F, Folefoc GN, Kapche GWF. Diterpenoids from the stem bark of *Croton zambesicus*. *Phytochemistry* 2002;60:345-349.
8. Okokon JE, Umoh UF, Udobang JA, Etim EI. Antiulcerogenic activity of ethanolic leaf extract of *Croton zambesicus* Muell. Arg. *Afr J Biomed Res* 2011;14:43-47.
9. National Institute of Health Guide for the Care and Use of Laboratory Animals. DHEW Publication (NIH), revised. Bethesda, MD: Office of Science and Health Reports, DRR/NIH, 1985.

10. Mir SH, Darzi MM, Ahmad F, Chishti MZ, Mir MS. The influence of glimepiride on the biochemical and histomorphological features of streptozotocin-induced diabetic rabbits. *Pakistan J Nutr* 2008;7(3):404-407.
11. Footsati S, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem* 1982;28:2077-2080.
12. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974;20:470-475.
13. Friedewald VT, Levy RI, Fredrickson DS. Estimation of low-density lipoprotein cholesterol in plasma, without use of the preparative centrifuge. *Clin Chem* 1972;18:499.
14. Okokon JE, Basse AL, Obot J, *et al.* Antidiabetic activity of ethanolic leaf extract of *Croton zambesicus* Muell. (thunder plant) in alloxan diabetic rats. *Afr J Trad Comp Alt Med* 2006;3(2):21-26.
15. Laughton M, Evans P, Moroney M, *et al.* Inhibition of mammalian 5-lipoxygenase and cyclo-oxygenase by flavonoids and phenolic dietary additives. Relationship to antioxidant activity and to iron ion-reducing ability. *Biochem Pharmacol* 1991;42:1673-1681.
16. Abdelmoaty MA, Ibrahim MA, Ahmed NS, Abdelaziz MA. Confirmatory studies on the antioxidant and antidiabetic effect of quercetin in rats. *Ind J Clin Biochem* 2010;25(2):188-192.
17. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine, 3rd ed. Oxford: Oxford University Press, 1999.
18. Massing MW, Sueta CA, Chowdhury M, Biggs DP, Simpson RJ. Lipid management among coronary artery disease patients with diabetes mellitus or advanced age. *Am J Cardiol* 2001;87:646-664.
19. Trivedi NA, Mazumdar B, Bhatt JD, Hemavathi KG. Effect of shilajit on blood glucose and lipid profile in alloxan-induced diabetic rats. *Ind J Pharmacol* 2004;36:373-376.
20. Ananthan R, Latha M, Ramkumar KM, Pari L, Baskar C, Narmatha BV. Effect of *Gymnema montanum* leaves on serum and tissue lipids in alloxan diabetic rats. *Exp Diab Res* 2003;4:183-189.
21. Pushparaj P, Tan CH, Tan BKH. Effects of *Averrhoa bilimbi* leaf extract on blood glucose and lipids in streptozotocin diabetic rats. *J Ethnopharmacol* 2000;72:69-76.
22. Austin MA, Hokanson JE. Epidemiology of triglyceride, small dense low density lipoprotein and lipoprotein (a) as risk factors for coronary heart disease. *Med Clin North Am* 1994;78:99-115.
23. Kraus-Friedmann N. Hormonal regulation of hepatic gluconeogenesis. *Physiol Rev* 1984;64:170-176.
24. Brown MS, Goldstein JL. Lipoprotein receptor in the liver. Control signals for plasma cholesterol traffic. *J Clin Invest* 1983;72:743-747.
25. Goodman LS, Gilman A. The pharmacological basis of therapeutics, 7th ed. New York: MacMillan, 1985;1490-1510.
26. Suryawanshi NP, Bhutey AK, Nagdeote AN, Jadhav AA, Manoorkar GS. Study of lipid peroxide and lipid profile in diabetes mellitus. *Ind J Clin Biochem* 2006;21(1):126-130.
27. Morimoto C, Tsujita T, Okuda H. Antilipolytic actions of insulin on basal and hormone-induced lipolysis in rat adipocytes. *J Lipid Res* 1998;39:957-962.
28. Abdel-Barry JA, Abdel-Hassan IA, Jawad AM, al-Hakim MH. Hypoglycaemic effect of aqueous extract of the leaves of *Trigonella foenum-graecum* in healthy volunteers. *East Mediterr Health J* 2000;6:83-88.