Vol. 26 (1) 1–8 31 March 2014

Biokemistri
An International Journal of the Nigerian Society for Experimental Biology

Original Article

Long-term anti-diabetic and anti-hyperlipidaemic effects of aqueous stem bark extract of *Irvingia gabonensis* in streptozotocin-induced diabetic rats

Akhere A. Omonkhua [1*], Iyere O. Onoagbe [2], Israel A. Fajimeye [3], Mukaila B. Adekola [3], Zainab A. Imoru [3]

[1] Department of Medical Biochemistry, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, PMB 1154, Benin City, Nigeria.

[2] Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

[3] Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

*Corresponding author: Akhere Omonkhua. E-mail: aaomonkhua@yahoo.com; Tel.: +2348053447581

Received: 08 February 2014: Revised 25 February 2014; Accepted: 25 February 2014

ABSTRACT: *Irvingia gabonensis* is used traditionally to treat diabetes. The antidiabetic effect of the seed extract has been demonstrated in human and animal models. This study was designed to evaluate the long-term anti-diabetic and anti-hyperlipidaemic effects of aqueous stem bark extract of *I. gabonensis* in streptozotocin-induced diabetic rats. Twenty four Wistar rats in three groups, normal control, diabetic control and *I. gabonensis* treated diabetic rats (TDR) were used for this study. Diabetes was induced in 16 rats by intraperitoneal injection of streptozotocin (STZ) at 65mg/kg body weight. Upon confirmation of diabetes, the treated diabetic rats were orally (by gavage) given aqueous extract of *I. gabonensis* bark at 200 mg/kg body weight daily for 24 weeks. Body weight was monitored weekly, while fasting blood sugar (FBS) and serum lipid profile (triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol) were assessed at specific intervals for 24 weeks. *I. gabonensis* significantly (P<0.05) reduced the FBS of the treated diabetic rats to normal control levels 2 weeks after the commencement of treatment. The reduction of FBS was sustained till the end of the study (24 weeks). Furthermore, at various stages of monitoring, the extract reduced the STZ-induced elevation of serum triglycerides, total cholesterol and LDL-cholesterol, and significantly (p<0.05) increased the STZ-induced decrease in HDL-cholesterol. Our study concludes that aqueous stem bark extract of *I. gabonensis* possess significant long-term anti-diabetic and hypolipidaemic effects. These anti-hyperlipidaemic effects as well as the presence of phytochemicals with recognizable anti-oxidant effects will be useful in the treatment of diabetic complications.

KEYWORDS: *Irvingia gabonensis*, Diabetes mellitus, Anti-diabetic, Anti-hyperlipidaemic, Medicinal plants.

INTRODUCTION

Diabetes mellitus is recognized as a major healthcare problem worldwide. The growing incidence of diabetes in sub-Sahara Africa is a major concern as there is no concurrent increase in healthcare provision, since the disease is still largely considered a Western problem (Motala and Ramaiya, 2010). Data gathered from epidemiological studies have clearly shown increased incidence, particularly in type 2 diabetes, which could be attributed to rising rates of obesity, physical inactivity, urbanization and ageing (Levitt, 2008; Mbanya *et al.,* 2010). The rate of increase in diabetes in rural African societies is far less than that seen in urban areas (Assah *et al.*, 2011), supporting diet and other lifestyle choices as causative factors. Many people in traditional African societies treat diabetes with medicinal plants that are thought to have fewer side effects (Akah *et al*., 2011). Hundreds of medicinal plants are available in literature with recognizable anti-diabetic effects (Bnouham *et al*., 2006; Neelesh *et al*., 2010). It is therefore imperative to study these plants for efficacy and safety, as well as providing a background for developing drugs that have superior therapeutic or even curative effects on diabetes.

Irvingia gabonensis is used widely in Nigeria and other African countries as food and medicinal plant (Ekpe *et al*., 2007; Awono *et al*., 2009), it is also used traditionally to treat diabetes (Ogunwande *et al*., 2007). The seed extract has been reported to have anti-diabetic effects on human type 2 diabetes (Adamson *et al*., 1990) and streptozotocin-induced diabetic rats (Ngondi *et al*., 2006). The aqueous stem bark extract has also been demonstrated to have sustained anti-obesity and hypoglycaemic effects in normal rabbits (Omonkhua and Onoagbe, 2012). Since diabetes is a chronic disease and most diabetics who use medicinal plant extracts to manage diabetes consume these extracts for a long period of time, this study was designed to evaluate the long-term (24 weeks) anti-diabetic and anti-hyperlipidaemic effects of aqueous stem bark extract of *I. gabonensis* in streptozotocin diabetic rats.

MATERIALS AND METHODS

Reagents

Streptozotocin (Sigma, London), Randox kits for glucose, total cholesterol, total triglycerides and HDL-cholesterol (product of Randox Laboratory Ltd, Ardmore, Diamond Road, Crumlin, Co. Anrtim, United Kingdom) and other analytical grade chemicals were used for this study.

Plant materials

The bark of *I. gabonensis* was obtained from the local forest at Akungba-Akoko, Ondo State, Nigeria. The identity of the plant was authenticated by Dr A. E. Ayodele of the Department of Microbiology and Botany, University of Ibadan, Ibadan, Nigeria. Herbarium specimen, with voucher number UIH 22286 was deposited at the Herbarium of the University of Ibadan, Nigeria.

The plant material was prepared by a modification of the method described previously (Onoagbe *et al*., 1999). Briefly, pulverized dry plant material was soaked in distilled water for 72 hours in a plastic container and covered with cheesecloth. The content was stirred several times a day and at the end of the third day the content was filtered through two layers of cheesecloth. To ascertain the yield of the extract, 1 ml of the homogeneous filtrate was dried by controlled heating (below 40 ^oC) in a pre-weighed watch glass to constant weight; this was done in triplicates and the average determined. The average yield of extract obtained was 32 mg/ml. The extract was kept frozen until use, when it was allowed to thaw at room temperature.

Animals

Twenty-four (24) adult rats of the Wistar strain, with average weight of 215.7g obtained from the Animal Unit of the University of Ibadan Teaching Hospital (UCH), Ibadan, Nigeria, were used for this study. They were kept in a well aerated room, with 12h light and 12h dark cycles. They were allowed food (standard pelleted feed) and water *ad libitum* and allowed to acclimatize for three weeks before the commencement of the study. Treatment of the animals conformed to the guidelines in the Principles of Laboratory Animal Care (NIH Publication 85-23, revised 1985). The study was reviewed and approved by the Local Institutional Review Board.

Induction of Diabetes

Rats were injected (i.p.) with streptozotocin dissolved in acidified (pH 4.5) normal saline at a dose of 65 mg/kg body weight after a 12-hour fast. Seven (7) days later, diabetes was confirmed by measuring fasting blood sugar. Rats with FBS higher than 8.2 mmol/l and glucosuria were randomly distributed into groups 2 and 3.

Experimental Design

Three groups of eight rats each were used for this study, namely: Group 1: normal control rats given water for 24 weeks, Group 2: diabetic control rats given water for 24 weeks and Group 3: *I. gabonensis* treated diabetic rats (TDR), orally given 200 mg/kg body weight of *I. gabonensis* aqueous bark extract daily for 24 weeks. The rats were weighed weekly.

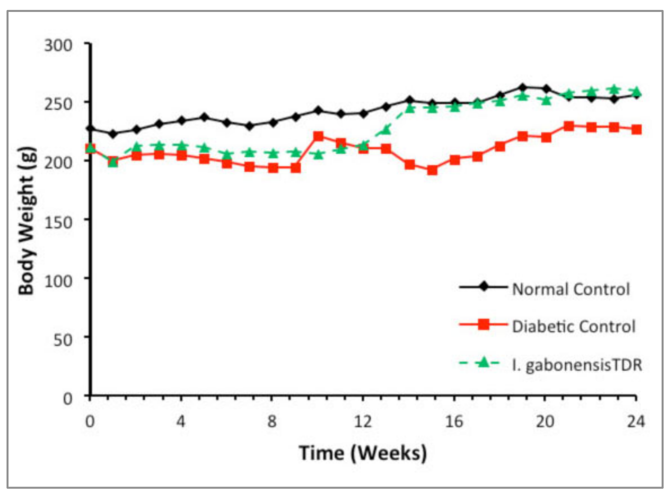

Figure 1: Effect of *I. gabonensis* on body weight of STZ-induced diabetic rats. Data are means of 4-8 determinations ± SEM. Error bars were less than 15% of mean values and are omitted for lucidity.

Before the administration of STZ, blood was collected from the tail vein of each rat to obtain the basal levels of all parameters. After the confirmation of diabetes (FBS ≥ 8.2 mmol/l) and the commencement of treatment with *I. gabonensis* bark extract; FBS was assessed at week 2 and then week 4, thereafter, once every four (4) weeks. Other parameters were assessed once every 4 weeks. During the period of monitoring, blood was collected from the tail vein of each rat. At the end of the monitoring phase, the rats were sacrificed and blood was obtained through heart puncture. Blood for glucose assays was collected in fluoride bottles while that for serum lipid profile was collected in plain bottles. Blood samples for glucose and biochemical assays were allowed to clot on ice and centrifuged at 1,000 X g for 5 minutes; the serum was then separated for analysis.

Biochemical Analyses

Fasting blood glucose was measured by the glucose oxidase method of Barham and Trinder (1972), while serum total triglyceride concentration was measured by the Tietz (1990) method. Serum total cholesterol level and serum HDL-cholesterol concentration were analyzed by the Richmond (1973) and Lopes-Virella *et al.* (1977) methods respectively. Serum LDL-cholesterol level was calculated by the Friedewald *et al.* (1972) method, as described in the manual of the Randox HDL-cholesterol kit.

Statistical analysis

Data are means of 4-8 determinations ± standard error of mean (SEM). The differences among groups were analyzed by the one-way analysis of variance (ANOVA). Inter-group comparisons were done using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals. The SPSS 11.0, SPSS Inc., Chicago, Illinois, USA, was used for this analysis.

RESULTS

The results obtained from this study are displayed in Figures 1 to 7. Figure 1 shows the effect of streptozotocin (STZ) diabetes and its treatment with *I. gabonensis* on body weight of rats. For the duration of this study, comparison between groups showed that the body weight gain of the untreated STZ-diabetic rats insignificantly reduced compared to normal control. The body weight reduction of the diabetic control group was more than that of the treated diabetic group for most parts of the study. The body weight increase of the treated diabetic rats improved from week 13 (Normal control - 246.8 ± 19, Diabetic control - 210.8 ± 14.6 and *I. gabonensis* TDR - 227.6 ± 22), a point from which the body weight of the *I. gabonensis* treated diabetic rats (TDR) were similar to that of normal control.

There were no statistically significant differences observed in the liver-body weight ratio of all groups of rats (Figure 2). However, the relative liver weight of the diabetic control group was insignificantly higher than normal control and *I. gabonensis* TDR. The relative kidney weight of the diabetic control rats was significantly ($p<0.05$) higher than normal control, while the kidney-body weight of the *I. gabonensis* TDR was significantly lower than the diabetic control group but similar to normal control. No statistical difference was observed between normal and diabetic control groups in the heart-body weight ratio. The relative heart weight of the *I. gabonensis* TDR was significantly ($p<0.05$) lower than normal and diabetic controls. The relative pancreas weight of the untreated diabetic rats was significantly higher than normal control, the medicinal plant treated rats had values that were similar to normal control, and significantly ($p<0.05$) lower than diabetic control.

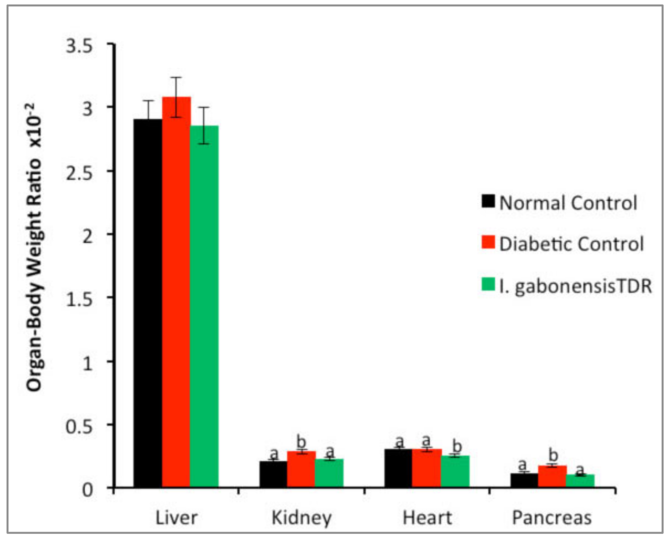

Figure 2: Effect of *I. gabonensis* on organ-body weight ratio of STZ-induced diabetic rats. Data are means of 4-8 determinations ±SEM. Values carrying different letters are statistically different at $p<0.05$.

Figure 3 shows the effects of *I. gabonensis* on fasting blood glucose levels of STZ-induced diabetic rats. After the administration of streptozotocin (Week 0), the FBS of test rats increased significantly ($p<0.05$) compared to normal control. Two (2) weeks after medicinal plant treatment, the FBS levels of the diabetic control remained high, while that of the *I. gabonensis* TDR reduced to the levels of normal control. This reduction was sustained till the end of the study (week 24).

As presented in Figure 4, significantly ($p<0.05$) higher serum total triglyceride levels in the untreated diabetic groups were recorded in weeks 8 and 12, with insignificantly higher values in week 24 compared to normal control. At week 8, a significantly higher serum triglyceride level was recorded for the *I. gabonensis* TDR compared to normal and diabetic controls but the value was significantly lower than that of diabetic control at week 12. At weeks 20 and 24, the serum triglyceride levels of this group were insignificantly lower than the diabetic control group.

The serum total cholesterol levels of the untreated diabetic rats were significantly ($p<0.05$) higher than normal control at weeks 4, 12 and 20 (Figure 5). The *I. gabonensis* TDR had serum cholesterol concentrations that were slightly higher (week 20) and lower (weeks 12 and 16) than diabetic control.

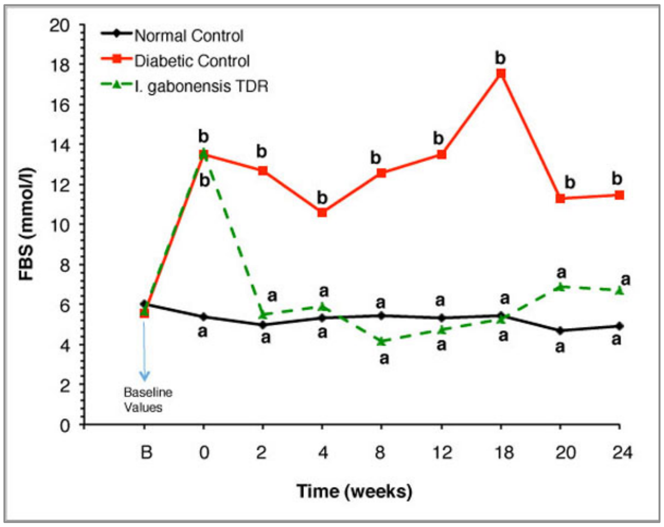

Figure 3: Effect of *I. gabonensis* on fasting blood sugar (FBS) of STZ-induced diabetic rats. Data are means of 4-8 determinations ± SEM. Error bars were less than 15% of mean values and are omitted for lucidity. Values carrying different letters are statistically different at $p<0.05$.

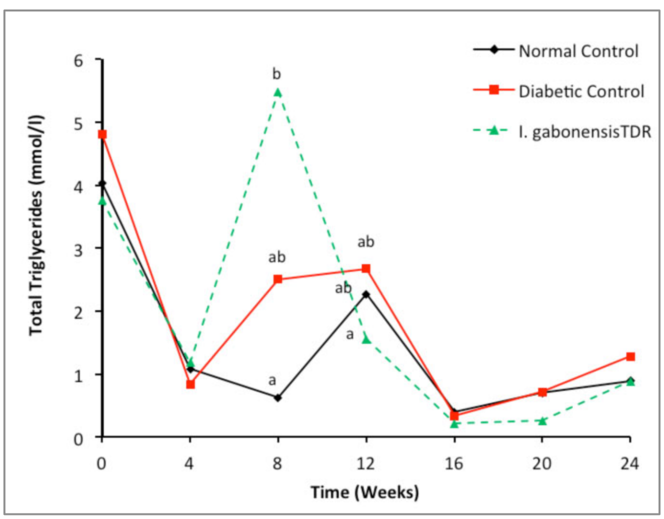

Figure 4: Effect of *I. gabonensis* on serum total triglycerides (mmol/l) of STZ-induced diabetic rats. Data were obtained from serum at pre-determined intervals and are means of 4-8 determinations ± SEM. Error bars were less than 15% of mean values and are omitted for lucidity. Values carrying different letters are statistically different at $p<0.05$

Serum LDL-cholesterol levels of the untreated diabetic rats were significantly ($p<0.05$) higher than normal control in weeks 12, 16 and 20 and insignificantly higher for other weeks except week 24 (Figure 6). For the *I. gabonensis* TDR, significantly higher values were seen only in week 20 compared to diabetic control; weeks 4 and 8 (insignificantly), and weeks 12 and 16 (significantly) recorded reduced values.

The serum HDL-cholesterol levels of the diabetic control rats were consistently lower than normal control with significant values seen in weeks 4 and 16. At week 4, the serum HDL-cholesterol levels of the treated diabetic rats were significantly ($p<0.05$) higher than normal and diabetic controls (Figure 7). At week 16, the HDL-cholesterol levels of the *I. gabonensis* TDR was similar to normal control but significantly higher than diabetic control.

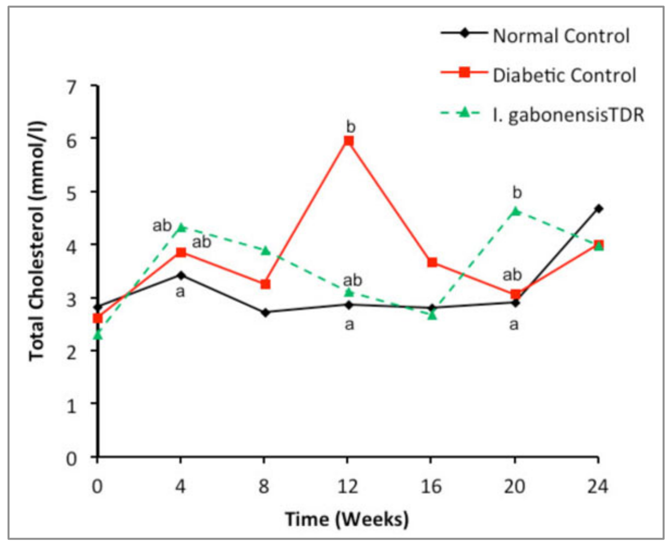

Figure 5: Effect of *I. gabonensis* on serum total cholesterol (mmol/l) of STZ-induced diabetic rats. Data were obtained from serum at pre-determined intervals and are means of 4-8 determinations ± SEM. Error bars were less than 15% of mean values and are omitted for lucidity. Values carrying different letters are statistically different at $p<0.05$.

DISCUSSION

Treatment of adult rats with streptozotocin (STZ) produces a diabetic state that is characterized by loss of weight, polydipsia, polyuria, glucosuria, polyphagia, hypoinsulinaemia and hyperglycaemia (Hakim *et al.,* 1997). The hyperglycaemia produced by STZ can be sustained for a long period of time. Previous reports show that STZ induced hyperglycaemia can persist for twenty-four (24) weeks (Howarth *et al*., 2005). This agrees completely with this study where the hyperglycaemic state of the untreated STZ-diabetic rats was sustained for 24 weeks. This study also revealed that aqueous bark extract of *I. gabonensis* possess potent anti-diabetic effects, since the FBS levels of the treated STZ diabetic rats returned to normal control levels two weeks after the commencement of the treatment. This anti-diabetic effect was also sustained for the duration of the study (24 weeks). This implies that *I. gabonensis* possess substances that have anti-diabetic effect. Indeed proximate and phytochemical analyses have shown that *I. gabonensis* bark contains nutrients (fibre and carbohydrates) and phytochemicals (tannins, saponins and anthraquinones) with recognizable anti-diabetic effects (Omonkhua and Onoagbe, 2010). Fibre (Ashutosh and Jha, 2011) and plant polysaccharides (Morada *et al.*, 2011) retard the rate of absorption of carbohydrates; also polyphenolics, such as tannins, saponins and anthraquinone, have been demonstrated to have inhibitory effects on carbohydrate digestion and glucose absorption in the intestine (Hanhineva *et al.,* 2010). These effects can collectively suppress post-prandial hyperglycaemia, thus ameliorating the effects of dietary carbohydrates on glycaemic control and modulating the existing hyperglycaemia. Such fibres have also been reported for *I. gabonensis* seed extract (Ngondi *et al*., 2005).

Some medicinal plants have been demonstrated to restore β-cell function in experimental diabetes (Ahmed *et al*., 2010; Kumari *et al*., 2012). Indeed, studies have shown that plant derived polysaccharides and polyphenolics stimulate insulin secretion from pancreatic β-cell (Mao *et al*., 2009; Hanhineva *et al*., 2010). It is therefore possible that the nutrients and phytochemicals present in *I. gabonensis* could ameliorate pancreatic cell destruction and/or stimulate insulin secretion from the pancreas. These suggested mechanisms of *I. gabonensis* anti-diabetic action i.e. glycaemic control and restoration/stimulation of pancreatic cell function, are not necessarily mutually exclusive, but may act together to establish and sustain its anti-diabetic effect.

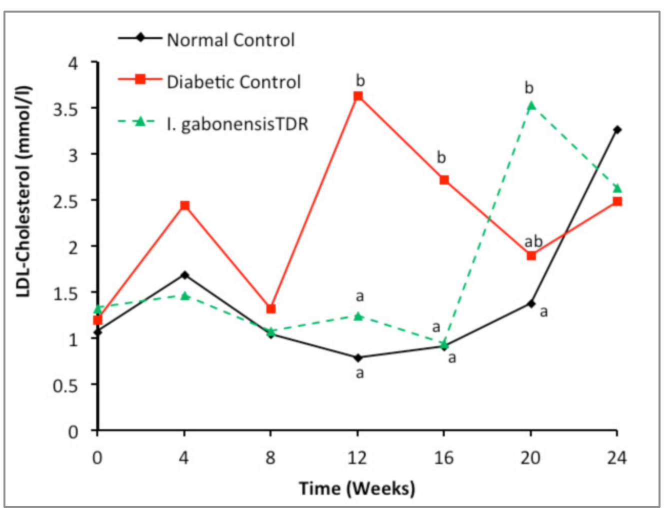

Figure 6: Effect of *I. gabonensis* on serum LDL-cholesterol (mmol/l) of STZ-induced diabetic rats. Data were obtained from serum at pre-determined intervals and are means of 4-8 determinations ± SEM. Error bars were less than 15% of mean values and are omitted for lucidity. Values carrying different letters are statistically different at p<0.05.

Weight reduction in STZ diabetes is related to weight reduction in type 1 diabetes which STZ mimics and is a result of the negative caloric effect of diabetes. Treatment of diabetic rats with *I. gabonensis* countered the weight loss caused by STZ diabetes. STZ-diabetes has been shown to cause weight reduction in rats (Zafar *et al*., 2012) and several medicinal plants have been shown to improve STZ-diabetes weight reducing effect (Singh *et al*., 2011; Haidari *et al*., 2012). It had previously been demonstrated that aqueous stem bark extract of *I. gabonensis* possess anti-obesity effect in normal rabbits (Omonkhua and Onoagbe, 2012). This may be relevant in the treatment of type 2 diabetes which is more prevalent in Africa. Furthermore, several mechanisms have been proposed for the anti-diabetic effect of *I. gabonensis* which may play important roles in the management of both type 1 and type 2 diabetes.

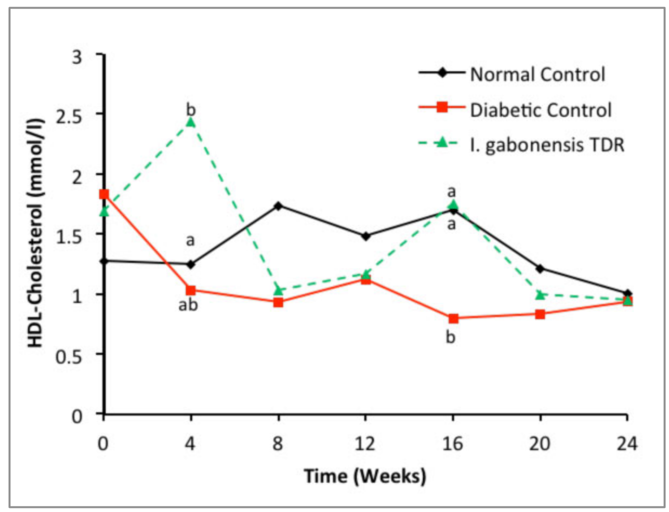

Figure 7: Effect of *I. gabonensis* on serum HDL-cholesterol (mmol/l) of STZ-induced diabetic rats. Data were obtained from serum at pre-determined intervals and are means of 4-8 determinations ± SEM. Error bars were less than 15% of mean values and are omitted for lucidity. Values carrying different letters are statistically different at p<0.05.

The relative liver, kidney and pancreas weights of untreated STZ diabetic rats were brought to normal control levels by treatment with *I. gabonensis.* It can thus be inferred that treatment of diabetic rats with *I. gabonensis* was able to alleviate the negative effect of STZ diabetes on the relative weights of these tissues. This may be related to the presence of several antioxidant phytochemicals in *I. gabonensis* stem bark which may protect the tissues from STZ-induced oxidative damage.

STZ diabetes results in dyslipidaemia i.e. increase in serum triglycerides, total cholesterol and LDL-cholesterol as well as decrease in HDL-cholesterol. The consistently higher triglyceride levels of the diabetic control group compared to normal control clearly indicate that STZ diabetes resulted in hyper-triglyceridaemia. At week 8, the serum triglyceride concentration of the *I. gabonensis* TDR was unexpectedly significantly (P<0.05) higher than diabetic control. This was however the only point where such an increase occurred; other values were either significantly (week 12) or insignificantly (weeks 16, 20 and 24) lower. This shows that *I. gabonensis* had a lowering effect on STZ-induced hyper-triglyceridaemia. Our results also show that STZ diabetes caused an almost consistent increase in serum total cholesterol and LDL-cholesterol compared to normal control. Again treatment with *I. gabonensis* caused reductions in these parameters for most part of the period of monitoring. Perhaps the most obvious anti-hyperlipidaemic effects of *I. gabonensis* treatment in this study, was the consistently higher serum HDL-cholesterol concentration of the *I. gabonensis* TDR compared to diabetic control. The anti-hyperlipidaemic effects of many anti-diabetic plants are well documented (Singh *et al*., 2011**;** Maruthupandian and Mohan, 2011; Ramachandran *et al*., 2012). Indeed, *I. gabonensis* seed extract has been shown to have significant anti-hyperlipidaemic effects in STZ diabetic rats (Dzeufiet *et al*., 2009). Adamson *et al*. (1990) reported that type 2 diabetics given *I. gabonensis* seed extract, had significantly lower LDL and VLDL-cholesterol and triglycerides levels, while HDL-cholesterol concentration was increased. A similar trend was observed in this study. Soluble fibres from plants have been shown to reduce serum total cholesterol, LDL-cholesterol and triglycerides (Saeed *et al*., 2011). In fact it has been reported in a study in the US that increasing intakes of refined carbohydrates and decreasing intakes of fibre paralleled the increasing prevalence of type 2 diabetes mellitus (DM) which had risen to near epidemic proportions in that country (Gross *et al*., 2004). Phytochemicals such as saponins (Francis *et al*., 2002; Gupta *et al*., 2009) have been reported to have hypolipidaemic effects. Plant derived saponins interact with bile acids to form large mixed micelles which have a higher excretion rate, with the consequent enhancement of the conversion of cholesterol to bile acids in the liver, thus reducing its serum concentration (Francis *et al*., 2002). The presence of these phytochemicals in *I. gabonensis* stem bark, may contribute greatly to the anti-hyperlipidaemic effects observed in this study. The chronic complications of diabetes mellitus, especially cardiovascular diseases, are responsible for most of the morbidity and mortality of the disease. The long term antidiabetic and antihyperlipidaemic effects observed in this study, as well as the presence of several antioxidant phytochemicals in *I. gabonensis* bark, could provide a sustainable means of treating diabetes and its complications particularly in Africa where the availability of drugs is a limiting factor.

Conclusion

The availability of orthodox anti-diabetic drugs in sub-Sahara Africa remains a major health care challenge. The development of drugs from local sources to combat the impending diabetes pandemic is a step in the right direction. This necessitates the building of a body of knowledge to investigate, validate and assess the safety of folkloric anti-diabetic remedies. The significant long-term anti-diabetic and anti-hyperlipidaemic effects of *I. gabonensis* aqueous stem bark extract observed in this study, presents an opportunity for further studies to understand and utilize these effects.

ACKNOWLEDGEMENT

The authors acknowledge the technical support of the laboratory staff of the Central Research Laboratory, Faculty of Science, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

REFERENCES

Adamson I, Okafor C, Abu-Bakare A (1990) A supplement of Dikanut (*Irvingia gabonensis*) improves treatment of type II diabetics. *West African Journal of Medicine 9: 108–115.*

Ahmed BA, Rao AS, Rao MV (2010) *In vitro* callus and *in vivo* leaf extract of *Gymnema sylvestre* stimulate β-cells regeneration and anti-diabetic activity in Wistar rats. *Phytomedicine 17: 1033–1039.*

Akah PA, Uzodinma SU, Okolo CE (2011) Antidiabetic activity of aqueous and methanol extract and fractions of *Gongronema latifolium* (Asclepidaceae) leaves in alloxan diabetic rats. *Journal of Applied Pharmaceutical Sciences 1: 99–102.*

Ashutosh M, Jha S (2011) *In vitro* postprandial glucose lowering effects of dietary fibers isolated from *Tamarindus indica* and *Cassia fistula* seeds. *American Journal of Food Technology 6: 435–440.*

Assah FK, Ekelund U, Brage S, Mbanya JC, Wareham NJ (2011) Urbanization, physical activity and metabolic health in sub-Saharan Africa. *Diabetes Care 34: 491–496.*

Awono A, Djouguep A, Zapfack L, Ndoye O (2009) The potential of *Irvingia gabonensis*: can it contribute to the improvement of the livelihoods of producers in southern Cameroon? *International Journal of Social Forestry 2: 67–85.*

Barham D, Trinder P (1972) An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst 97: 142–145.*

Bnouham M, Ziyyat A, Mekhfi H, Tahri A, Legssyer A (2006) Medicinal plants with potential antidiabetic activity–A review of ten years of herbal medicine research (1990-2000). *International Journal of Diabetes and Metabolism 14: 1–25.*

Dzeufiet DPD, Ngeutse DF, Dimo T, Tédong L, Ngueguim TF, Tchamadeu M, Nkouambou NC, Sokeng DS, Kamtchouing P (2009) Hypoglycemic and hypolipidemic effects of *Irvingia gabonensis* (Irvingiaceae) in diabetic rats. *Pharmacologyonline 2: 957–962.*

Ekpe OO, Umoh IB, Eka OU (2007) Effect of a typical rural processing method on the proximate composition and amino acid profile of bush mango seeds *(Irvingia gabonensis). African Journal of Food Agriculture and Nutrition 7(1).*

Francis G, Kerem Z, Makkar HPS, Beckerm K (2002) The Biological action of saponins in animal systems: A Review. *British Journal of Nutrition 88: 587–605.*

Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge**.** *Clinical Chemistry 18**:** 499–505.*

Gross LS, Li L, Ford ES, Liu S (2004) Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: an ecologic assessment. *American Journal of Clinical Nutrition* 79: *774–779.*

Gupta AK, Ganguly P, Majumder UK, Ghosal S (2009) Improvement of lipid and antioxidant status in hyperlipidaemic rats treated with steroidal saponins of *Solanum nigrum* and *Solanum xanthocarpum. Pharmacologyonline 1: 1–14.*

Haidari F, Shahi MM, Zarei M, Rafiei H, Omidian K (2012) Effect of green tea extract on body weight, serum glucose and lipid profile in streptozotocin-induced diabetic rats. A dose response study. *Saudi Medical Journal 33: 128–133.*

Hakim ZS, Patel BK, Goyal RK (1997) Effects of chronic ramipril treatment in streptozotocin-induced diabetic rats. *Indian J Physiol Pharmacol 41: 353–360.*

Hanhineva K, Torronen K, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkanen H, Poutamen K (2010) Impact of dietary polyphenols on carbohydrate metabolism. *International Journal Molecular Science 11: 1365–1402.*

Howarth FC, Jacobson M, Shafiullah M, Adeghate E (2005) Long-term effects of streptozotocin-induced diabetes on the electrocardiogram, physical activity and body temperature in rat*s. Experimental Physiology 90: 827-835.*

Kumari S, Wanjari M, Kumar P, Palani S (2012) Antidiabetic activity of *Pandanus fascicularis* Lamk–aerial roots in alloxan-induced hyperglycemic rats. *International Journal Nutrition Pharmacology and Neurological Diseases 2: 105–110.*

Levitt NS (2008) Diabetes in Africa: epidemiology, management and healthcare challenges. *Heart* 94: *1376–1382.*

Lopes-Virella MF, Stone P, Ellis S, Colwell JA (1977) Cholesterol determination in high-density lipoproteins separated by three different methods. *Clinical Chemistry 23: 822–824*.

Mao XQ, Yu F, Wang N, Wu Y, Zou F, Wu K, Liu M, Ouyang JP (2009) Hypoglycemic effect of polysaccharide enriched extract of *Astragalus membranaceus* in diet induced insulin resistant C57BL/6J mice and its potential mechanism. *Phytomedicine 16: 416–425.*

Maruthupandian A, Mohan VR (2011) Antidiabetic, antihyperlipidaemic and antioxidant activity of *Pterocarpus marsupium* Roxb. in alloxan induced diabetic rats. *International Journal Pharmaceutical Technical Research 3: 1681–1687.*

Mbanya JC, Motala AA, Sobngwi E, Assah FK, Enoru ST (2010) Diabetes in sub-Saharan Africa. *Lancet 375: 2254–2266.*

Morada NJ, Metillo EB, Uy MM, Oclarit JM (2011) Anti-diabetic polysaccharide from mangrove plant, *Sonneratia alba* Sm. International conference on Asia Agriculture and Animal. *International Proceedings of Chemical Biology Environmental Engineering (IPCBEE) 13: 197–200.*

Motala A and Ramaiya K (eds) (2010) Diabetes: the hidden pandemic and its impact on sub-Saharan Africa. Diabetes Leadership Forum, Africa 2010.

Neelesh N, Jain S, Malviya S (2010) Antidiabetic potential of medicinal plants. *Acta Poloniae Pharmaceutica–Drug Research 67: 113–118.*

Ngondi JL, Djiotsa EJ, Fossouo Z, Oben J (2006) Hypoglycaemic effect of the methanol extract of *Irvingia gabonensis* seeds on streptozotocin diabetic rats. *African Journal Traditional CAM 3: 74–77.*

Ngondi JL, Oben JE, Minka SR (2005) The effect of *Irvingia gabonensis* seeds on body weight and blood lipids of obese subjects in Cameroon. *Lipids in Health Disease* 4: 12.

Ogunwande IA, Matsui T, Fujise T, Matsumoto K (2007) Alpha-glucosidase inhibitory profile of Nigerian medicinal plants in immobilized assay system. *Food Science and Technology Research* 13: 169–172.

Omonkhua AA, Onoagbe IO (2010) Preliminary proximate and phytochemical analyses of some medicinal plants used to treat diabetes mellitus in Nigeria. *Inventi Impact: Ethnopharmacology 1: 68–70.*

Omonkhua AA, Onoagbe IO (2012) Effects of long-term oral administration of aqueous extracts of *Irvingia gabonensis* bark on blood glucose and liver profile of normal rabbits. *Journal of Medicinal Plant Research 6: 2581–2589.*

Onoagbe IO, Ebhota AO, Udegbe HC, Omondia M, Edeni D, Ebengho SO (1999) Assessment of some medicinal plants for hypoglycemic activities in rats and rabbits. *Bioscience Research Communications 11: 159–163*

Ramachandran S, Rajasekaran A, Manisenthilkumar KT (2012) Investigation of hypoglycemic, hypolipidemic and antioxidant activities of aqueous extract of *Terminalia paniculata* bark in diabetic rats. *Asian Pacific Journal of Tropical Biomedicine 2: 904–909.*

Richmond V (1973) Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. *Clinical Chemistry 19: t350–t356*

Saeed S, Mosa-Al-Reza H, Atiyeh SD, Marziyeh A (2011) Reduction of serum cholesterol in hypercholesterolemic rats by Guar gum. *Avicenna Journal of Phytomedicine 1: 36–42.*

Singh V, Singh M, Shukla S, Singh S, Mansoori MH, Kori ML (2011) Antidiabetic effect of *Flacouria indica* Merr in streptozotocin induced diabetic rats. *Global Journal of Pharmacology 5: 147–152.*

Tietze NW (1990) Clinical guide to laboratory tests, 2nd Edition. Philadelphia, USA: WB Saunders Company.

Zafar M, Naeem-Ul-Hassan NS (2010) Effects of streptozotocin-induced diabetes on the relative weights of kidney, liver and pancreas in albino rats: a comparative study. *International Journal of Morphology 28: 135–142.*