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

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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-Saharan 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 (Brouham 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 (Omonkhu 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. Antrim, 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 °C) 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.](image)

Data are means of 4-8 determinations ±SEM. Values carrying different letters are statistically different at p<0.05.

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.](image)

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.

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.](image)

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.

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 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.](image)

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.

![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.](image)

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
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.

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**Conclusion**

The availability of orthodox anti-diabetic drugs in sub-Saharan Africa remains a major health care challenge. The development of drugs from local sources to combat the 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 antihyperlipidaemic 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.


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