HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF *IVINGIA GABONENSIS* (IRVINGIACEAE) IN DIABETIC RATS

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Summary

The potential of dietary *Irvingia gabonensis* seed extract in ameliorating hyperglycemia and hyperlipidemia was investigated in streptozotocin (STZ)-induced diabetic rats. In a 4-week feeding trial, incorporation of *Irvingia gabonensis* seed extract (10%) in the diet of diabetic rats was found to significantly reduce the plasma glucose level of STZ diabetic rats. The total food intake was significantly reduced in the diabetic rats fed 10% *Irvingia gabonensis* seed extract compared to the control diabetic rats. However, the body weight gain over 28 days was more than 2.5 times greater in *Irvingia gabonensis* seed supplemented diabetic rats compared to the control diabetic rats. *Irvingia gabonensis* seed in the diet also decreased the elevated serum total cholesterol, triglycerides and LDL-cholesterol levels as well as atherogenic index while increasing HDL-cholesterol. The result of these studies clearly establishes the modulatory propensity of *Irvingia gabonensis* seed against diabetes induced dyslipidemia. *Irvingia gabonensis* seed could be effectively used as an ingredient in health and functional food to ameliorate certain disease states such as diabetes.

Key words: hypoglycemic activity, hyperlipidemia, *Irvingia gabonensis* seed, diabetes, streptozotocin.

Introduction

Worldwide prevalence of diabetes in adults was estimated to be 4.0% in 1995 and to rise 5.4% by the year 2025. It is higher in developed than in developing countries. The major part of this numerical increase will occur in developing countries including Cameroon. There will be a 42% increase, from 51 million to 72 million, in the developed countries and a 170% increase, from 84 million to 228 million, in the developing countries [1].

Diabetes mellitus is classified as type 1 diabetes (due to islet beta-cell destruction), type 2 diabetes (with varying degree of insulin resistance and/or insulin deficiency), gestational diabetes and other specific types of diabetes [2]. The development of a new health food for diabetic patients is important. Several dietary factors that can mitigate the symptoms of diabetes have been identified which predominantly include dietary fibbers and antioxidants [3, 4].

Irvingia gabonensis belongs to the Irvingiaceae family. The Irvingia tree, commonly known as bush mango, dikanut or African mango [5], is indigenous to West and Central Africa. Although the flesh of the fruit of *Irvingia gabonensis* is frequently consumed, its most important part is the kernel which (in its fresh or dried form) is used to add flavouring and consistency to many dishes in Cameroon and Nigeria [6].

Irvingia gabonensis extract have been shown to exert hypoglycemic effects in animal models. In rats with streptozotocin-induced diabetes, acute oral administration of *Irvingia gabonensis* methanol extract reduces blood glucose levels [7]. In the present study, we employed rats with streptozotocin-induced diabetes to examine whether, *Irvingia gabonensis* seed extracts has any attenuating effect on blood glucose level and also on lipid profile in long term treatment.

Methods

Plant material and extraction

The fruits *Irvingia gabonensis* were collected in Ebolowa, Southern province of Cameroon in April. The plant material was identified at the National Herbarium, Yaounde where a voucher specimen Nº 28054/HNC was deposited. The seeds were removed and dried at room temperature and reduced to a powder (Ig1). A part of the powder (1.9 kg) was macerated in six litters of hexane during 2 days. The solution obtained after filtration with Whatman N° 3 filter paper was concentrated under reduced pressure to obtain 1.36 kg of extract (Ig2) (yield, 71.86 %). The residue, which represents the fat-free extract (Ig3) was also used.

Animals and experimental design

Age-matched male Wistar rats weighing 210±20 g were used. Throughout the investigations the animals were fed a standardized diet (Lanavet, Garoua, Cameroon) and had free access to drinking water. Diabetes was evoked by intravenous (iv) administration of STZ, (Sigma chemical Co, St Louis, Missouri, USA) in a dose 55 mg/kg, dissolved in 0.9% saline. The control group of rats received iv saline. Seventy-two hours after STZ administration, blood was collected from the tail vein of all the animals taking part in the experiment to determine the glucose concentration in the blood. Animals which were administered STZ and in which glucose concentration was <250 mg/dl were excluded from the study. After 14 days of diabetic induction, glucose concentration was controlled with a glucometer Acutrend Glocose (Boehringer GmbH, Mannheim, Germany) [8]. The remaining rats which survived were divided into four groups of 6 rats each: Diabetic control receiving standard diet, diabetic receiving 10% *Irvingia gabonensis* seed (Ig1), diabetic rat fed with 10% *Irvingia gabonensis* seed hexane extract (Ig2) and diabetic rats fed with 10% fat free *Irvingia gabonensis* seed extract (Ig3). For comparison, one group of 6 normal rats receiving standard diet was used. At the end of the experiment, blood sample was collected. Serum was separated for the determination of serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides levels using commercial diagnostic kits (Fortress, UK). Atherogenic index was calculated following the formula used by Wakayashi and Kobaba [9].

Statistical analysis

Results are expressed as the mean ± SD. The difference between the groups was compared using one-way analysis of variance (ANOVA) followed by the Dunnett's post hoc test. A value of P < 0.05 was considered statistically significant.

Results

Effects of *Irvingia gabonensis* seed on blood glucose

The levels of change in blood glucose in experimental rats are given in table 1. Blood glucose levels were elevated in untreated diabetic rats as compared to normal rats. The results obtained showed an improvement of the blood glucose levels in rats with 7, 14, 21 and 28 days feeding of *Irvingia gabonensis* seed. Ig1 showed 46.04%, 67.18% and 73.89% antidiabetic activity for 7, 14 and 21st days, respectively. For the same period of time, Ig2 exhibited 51.90%, 60.66% and 60.45% reduction in blood glucose as compared to initial value. With 10% incorporation of Ig3 in diet, the reduction was 47.01%, 54.76% and 48.70% respectively, at days 7, 14 and 21. In experimental animals of group Ig1 and Ig2, the 28th day blood glucose levels tended to increase as compared to 21st day values. In group Ig3 in contrast, we obtained the maximal blood glucose decreased (79.80%).

Table 1: Effects of *Irvingia gabonensis* seed on blood glucose levels

Groups	Blood glucose levels (mg/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal	84.66±2.92	73.00±2.40	81.83±5.70	84.66±4.09	82±1.84
Diabetic	265.16±15.41***	312.33±18.17***	317.33±14.03**	352.60±17.73***	353.60±16.85***
Diabetic+Ig1	270.60±19.96***	146.20±16.62**	88.80±4.99^{aaa}	70.40±4.96^{aaa}	112.80±17.67^{aaa}
Diabetic+Ig2	283.20±14.07***	136.20±17.67**^{aaa}	111.40±12.32^{aaa}	112.00±18.42^{aa}	71.40±1.96^{aaa}
Diabetic+Ig3	271.00±12.02***	143.60±11.52**^{aaa}	122.60±13.74^{aaa}	139.00±14.84**^{aaa}	180.00±13.81**^{aaa}

Values represent means ± SD, n=6. **p<0.01; ***p<0.001 significantly different as compared to normal control rats; ^{aa}p<0.01, ^{aaa}p<0.001, significantly different as compared to diabetic control rats. Day 0 represent the first day of experiment, 14 days after diabetes induction.

Effect of *Irvingia gabonensis* seed on body weight, food and water intake

The body weight gain, total food and water intake of rats of various groups at the end of 4 weeks are presented in Table 2. Body weight gain in STZ diabetic rats was significantly lower than the body weight gain in nondiabetic control and *Irvingia gabonensis* seed fed rats. Diabetic rat fed with Ig1, Ig2 and Ig3 sowed a weight gain as compared to diabetic rat receiving standard diet. Food intake was increased significantly (87.42%) in diabetic rats compared to that of non-diabetic control rats, while incorporation of 10% Ig1, Ig2 and Ig3 in the diet of diabetic rats significantly reduced the food intake, respectively by 13.42%, 17.44% and 9.25% when compared to that of the diabetic rats. In diabetic control rat, the increased in water intake was 53.67% higher when compared to normal control rats. As compared to diabetic control rats, 10% *Irvingia gabonensis* in food exhibited a significant (almost 18% in all groups) reduction in water intake as compared to diabetic control rats.

Table 2: Effect of *Irvingia gabonensis* seed on body weight, food and water intake of experimental rats

Groups	Body weight gain (%)	Total food intake (g/rat/28 days)	Total water intake (ml/rat/28 days)
Normal	+23.88	556.50±5.19	619.50±3.13
Diabetic	-18.65	1043.00±55.43***	952.00±40.03***
Diabetic+Ig1	-7.23	903.00±28.55**[aa]	777.00±22.90*[aa]
Diabetic+Ig2	-6.11	861.00±33.42**[aa]	780.56±60.22*[aa]
Diabetic+Ig3	+4.52	946.50±58.38**[a]	776.12±80.06*[aa]

Values of food and water intakes represent means ± SD, n=6. *** p<0.001 significantly different as compared to normal control rats; [a]p<0.05, [aa]p<0.01, significantly different as compared to diabetic control rats.

Effects of *Irvingia gabonensis* seed on protein level and lipid profile

As shown in table 3, the administration of streptozotocin to rats has significantly increased the total cholesterol by 29.74%, triglycerides by 99.52%, LDL-cholesterol by 1.66% and artherogenic index by 29.89% as well as a decreased in HDL-cholesterol by 22.64%, as compared to normal rats. These rises in total cholesterol, LDL-cholesterol, triglycerides and artherogenic index were prevented by the incorporation of *Irvingia gabonensis* seed in diet. The most potent effect on lipid profile was obtained with Ig3 where we obtained 13.20%, 23.28%, 29.90% and 13.17% reduction respectively for total cholesterol, triglycerides, LDL-cholesterol and artherogenic index and an increased of 26.31% for HDL-cholesterol as compared to standard diet fed diabetic rats. Serum total protein was decreased in all experimental rats as compared to normal control rats.

Table 3: Effects of *Irvingia gabonensis* seed on protein level and lipid profile

Groups	Protein	Total cholesterol	HDl-cholesterol	Triglycerides	LDL-cholesterol	Atherogenic index
Normal	110.00±8.02	97.90±6.33	56.30±6.45	50.03±3.61	41.51±4.44	97.32±5.31
Diabetic	97.12±4.06	127.02±9.32**	43.55±3.96**	98.32±6.30***	65.20±8.79	126.41±8.87**
Diabetic+Ig1	80.15±4.99*	120.36±8.44	72.01±5.50** [aaa]	65.92±8.20*[aa]	41.91±6.25	119.76±7.79**
Diabetic+Ig2	91.25±6.88	113.66±6.01* [a]	71.56±6.32** [aaa]	74.85±4.12**[aa]	51.34±3.22[a]	113.03±4.96[a]
Diabetic+Ig3	80.92±5.41*	110.25±8.75[a]	53.69±5.24[aa]	72.45±8.06** [aa]	45.70±12.50	109.76±8.75[aa]

Values represent means ± SD, n=3. *p<0.05, **p<0.01, ***p<0.001 significantly different as compared to normal control rats; [a]p<0.05, [aa]p<0.01, significantly different as compared to diabetic control rats.

Discussion

Medicinal plants are widely used by the populations of underdeveloped countries as alternative therapy. In Africa, hundreds of plants are used traditionally for the management and/or control of diabetes mellitus. Unfortunately only a few of such African medicinal plants have received scientific scrutiny.

Streptozotocin is widely used for the induction of experimental diabetes, involving weight lost and lipid disturbance [8, 10]. Therefore, we employed a streptozotocin-induced diabetic model system to examine whether *Irvingia gabonensis* seed has hypoglycemic and hypolipidemic effects against experimental diabetes. Increase in blood glucose is a typical feature in diabetes and the extent of diabetic complication correlates with elevated blood glucose concentrations. Therefore, *Irvingia gabonensis* seed would be expected to prevent diabetic complications by controlling blood glucose level. In the present study, the blood glucose level of the diabetic rats fed dietary *Irvingia gabonensis* seed was significantly lower than that of the diabetic control group, clearly suggesting the efficacy of *Irvingia gabonensis* seed and extracts to suppress elevation of blood glucose concentrations induced by streptozotocin on long-term (28-day) treatment in rats. In fact, *Irvingia gabonensis* seeds delay stomach emptying, leading to a more gradual absorption of dietary sugar. This effect can reduce the elevation of blood sugar levels that is typical after a meal [11].

Loss of body weight and increase in blood glucose level are typical features found in diabetes mellitus and, as anticipated, rats administered streptozotocin showed about 42.53% less body weight gain by the end of the experiment. However, the weight loss of the diabetic rats groups receiving 10% *Irvingia gabonensis* seed and extracts was less compared to the diabetic control group and consequently, the decrease of the body weight gain in the diabetic group was significantly suppressed by dietary *Irvingia gabonensis*.

In our study, the body weight gain of diabetic treated rats was not positively correlated to food intake. The total food intake of normal group rats were found to be 556.50±5.19 g/rat/28day, whereas the food intakes were significantly (1043.00±55.43) increased in the diabetic control group of rats (compared with normal rats). Although there was a significant increase in food intake of diabetic treated rats as compared to normal rats, we also observed a significant decrease of this parameter as compared to diabetic standard diet rats. *Irvingia gabonensis* seed diet (Ig1) was more consumed than *Irvingia gabonensis* seed hexane extract (Ig2), but lead to less weight gain. The most consumed diet was 10% Ig3, which produced a positive effect on the body weight of diabetic rat. This could be probably due to its fat free composition. It is well known that fatty diets are less consumed due to their high energy contained. Water intake was also increased in diabetic rats as compared to normal rats. However, 10% supplementation of *Irvingia gabonensis* seed and extracts significantly ($p<0.01$) rise the water intake, confirming its positive effect on diabetic statue.

In streptozotocin-induced diabetic rats, the rise in blood glucose is accompanied by an increase in the serum cholesterol, triglyceride, LDL-cholesterol, and the decrease in HDL, whereas the treatment with seeds and extracts of *Irvingia gabonensis* reduced cholesterol, triglycerides, LDL-cholesterol, atherogenic index and improved HDL-cholesterol in diabetic rats. The low serum cholesterol concentrations observed in rats treated with *Irvingia gabonensis* diet as compared to standard diet might be due to the reduction of lipogenesis. Thus, the reduced biosynthesis of fatty acids in turn will reduce the production of VLDL particles, thus limiting the formation of LDL particles and resulting in low serum triglycerides and cholesterol concentrations [12]. Our results are consistent with those of Ngondi et al. [13] who reported an increased in HDL-cholesterol and a decreased in LDL-cholesterol in normal rats treated with *Irvingia gabonensis* Kermel oil.

The effects of *Irvingia gabonensis* seed fractions varied on evaluated parameters. *Irvingia gabonensis* seed (Ig1) had the best effect on reducing LDL-cholesterol and increasing HDL-cholesterol while free-fat extract (Ig3) was more potent on reducing total cholesterol, atherogenic index and improving body weight of diabetic rats. Hexane extract (Ig2) exhibited the best hypoglycaemic activity. This effect could not be attributed to the amount of food intake (consequently *Irvingia gabonensis*) but to the extract composition since food consumption in this group is lower as compared to Ig3.

On the basis of the current investigation it was noted that the hexane extract of *Irvingia gabonensis* posses a blood glucose lowering activity while whole *Irvingia gabonensis* seed and free-fat extract exhibit hypolipidemic effect and it can be suggested that these results provide pharmacological evidence for its folklore claim as an anti-diabetic agent.

References

1.King H, Aubert RE, Herman WH. Global burden of diabetes. 1995–2025: Prevalence. numerical estimates and projections. Diab Care 1998; 219: 1414–1431.
2.American Diabetes Association. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diab. Care 1997; 20:1183-1197.
3.Vedavanam K, Srijayanta S, O'Reilly J, Raman A, Wiseman H. Antioxidant action and potential antidiabetic properties of an isoflavonoids-containing soybean phytochemical extract SPE. Phyt Res 1999; 13: 601–608.
4. Ou S, Kwok K, Li Y, Fu L. In vitro study of possible role of dietary fibres in lowering post prandial serum glucose. J Agric Food Chem 2001; 49: 1026–1029.
5.White L, Albernethy K: Guide de la végétation de la réserve de la Lopé. Gabon. ECOFAC Gabon 1996.
6.Okafor J. Okolo HC: Potentials of some indigenous fruit trees of Nigeria. Paper presented at the 5th Annual Conference of the Forestry Association of Nigeria Jos 1974. 60-71.
7.Ngondi JL, Djiotsa EJ, Fossouo Z, Oben J. Hypoglycaemic effect of the methanol extract of *irvingia gabonensis* seeds on streptozotocin diabetic rats. AJTCam 2006 ; 3:74-77.
8.Dzeufiet DPD, Tedong L, Dimo T, Assongalem EA, Sokeng DS; Kamtchouing P. Hypoglycaemic effect of themetylene chloride/methanol root extracts of *Ceiba pentandra* in normal and diabetic rats. Indian J Pharmacol 2006; 38(3) : 194-197.
9.Wakayashi I, Kobaba WR. Effet de l'âge sur le rapport entre le boire et les rapports artherosclerotiques. Girontology 2002; 48. 151-156.
10. Kamtchouing P, Kahpui M, Dzeufiet DPD, Tedong L, Assongalem E,A, DimoT. Antidiabetic activity of the methanol/metylene chloride stem bark extracts of Terminalia superba and Canarium schweinfurthii on streptozotocin-induced diabetic rats. J Ethnopharmacol 2006; 104(3):306-309.
11. Ngondi JL, Fossouo Z, Djiotsa EJ, Oben J. Glycaemic variations after administration of irvingia gabonensis seeds fractions in normoglycemic rats. AJTCam 2006 ; 3 4: 94 – 101.
12. Ali AA, Velasquez MT, Hansen CT, Mohamed AI, Bhathena SJ. Effects of soybean isoflavones, probiotics, and their interactions on lipid metabolism and endocrine system in an animal model of obesity and diabetes. J Nutr Biochem 2004, 15:583-590.
13. Ngondi JL, Mbouobda HD, Etame S, Oben J. Effect of *irvingia gabonensis* Kernel Oil on blood and liver lipids on leam and overweight rats. Journal of food technology 2005; 34: 592-594.