# Hypoglycemic effect of *Irvingia gabonensis* (Aubry-Lacomate Ex. Ororke), Baill in Type 2 Diabetic Long-Evans Rats

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**ABSTRACT:** *Irvingia gabonensis* (Aubry-Lacomate Ex. Ororke), Baill (African wild mango/bush mango) seeds are widely used in cooking as a sauce in Cameroon and in most parts of tropical Africa for the treatment of a number of ailments. In this study normal rat food was incorporated with *I. gabonensis* seed powder (10%) and oil free seed powder (5%) and their chronic effects on streptozotocin induced Type 2 diabetic rats were studied. Oral consumption of food incorporated with seed powder significantly reduced serum glucose level on the 28th day ($p<0.01$) which was comparable with glibenclamide treated group. Food with oil free seed powder showed 24% fall in glucose level on the 28$^{th}$ day. Fasting serum insulin increased significantly ($p<0.001$) in glibenclamide and oil free seed powder treated ($p<0.008$) groups. No effect was observed in the seed powder treated group. Liver glycogen content increased in the glibenclamide treated group but no significant change was observed in both powder and oil free seed powder treated groups. On the 28$^{th}$ day seed powder treated group significantly lowered serum TG level ($p<0.033$) and 48% was lowered by oil free seed powder. It is concluded that seed powder as well as oil free seed powder lowered blood glucose level in Type 2 diabetic model rats. It seems to act as an insulinomimetic and/or insulin sensitizing agent.

**Key words**: Streptozotocin, serum glucose, Type 2 diabetes

## INTRODUCTION

The prevalence of diabetes is increasing globally. It has been predicted that the number of diabetics will be 300 million or more by the year 2025.[1] Furthermore, it is acknowledged that diabetes not only afflicts prosperous nations, but also often reaches its highest frequency in poor and disadvantaged communities, those which can least afford the heavy burden of its costly long-term treatment. Biguanides, derived from *Galega officinalis* is an excellent example of antidiabetic drug developed from plants[2]. Though biguanides and sulfonylureas are valuable in treating Type 2 diabetes, their use is restricted by their limited action, pharmacokinetic properties, secondary failure rates and accompanying side effects.[3] Natural products have been a source of medicinal treatment for thousands of years and plant based medicines continue to play an important role in the primary health care of 80% of worlds underdeveloped and developing countries. In continuation of our search for new leading compounding (s) to have antidiabetic property, and collaboration with the scientists of Cameroon[4] we studied the antidiabetic effect of African mango seeds.

*Irvingia gabonensis* (African mango), locally known in Nigeria as agbono[5], in Cameroon as andok, in Sierra Leone as bobo, belongs to the Irvingiaceae family. The fruits of *I. gabonensis* are rich in oil (54-64%)[6] and are commonly used in making a paste called 'dika'. The bark is used for the treatment of colic, diarrhea and dysentery.[7] In Sierra Leone, the

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Mende tribes grind the steam bark and mix it with water and apply the paste on the skin to ameliorate pain.[8] African mango seed has folkloric use to reduce body mass weight, (BMW), popular as blood thinner (blood anticoagulant effect) and are effective for diabetes and are available in the Western countries and North America over the counter. In this case, scientific studies are made to evaluate the claim. The aim of this study was to assess the chronic effect of *I. gabonensis* seed powder and oil free seed powder on body weight, serum glucose, lipid profile, serum insulin and liver glycogen content in Type 2 diabetic model rats.

## MATERIALS AND METHODS

**Collection of *I. gabonensis* (seeds).** The seeds of *I. gabonensis* were brought by one of the co-authors from Cameroon. The voucher sample (No 28054/HNC) has been kept in the National Herbarium of Cameroon. After peeling, the seeds were dried in an oven at $40^0$ C for 24 hours and powdered with motor and pestle of mesh size 100. Normal rat food was incorporated with crude powder of *I. gabonensis* (10%) and used for testing the biological activity.

**Extraction.** The seed powder (1.5 kg) of *I. gabonensis* was extracted with hexane at room temperature for 24 hours and solvent was removed. The residue was extracted again with hexane two times more. All extracts were mixed and dried completely by rotavapor and seed powder was collected. Normal rat food was incorporated with oil free seed powder of *I. gabonensis* (5%) and used for biological testing.

**Animals.** Male Long Evans rats, bred at BIRDEM animal house and weighing between 180-200 g were used in the study. Type 2 diabetic model of rats were produced with intraperitoneal injection of streptozotocin using conventional methods and following the procedure standardized in BIRDEM.[9] To carry out the experiment, Type 2 diabetic rats were divided into four groups; (i) Control group receiving normal food. (ii) Treated with standard drug glibenclamide (5 mg/kg body wt, once daily for 28 consecutive days). (iii) Treated with food containing seed powder (crude powder) of *I. gabonensis*, 10%). (iv) Treated with food containing oil free seed powder of *I. gabonensis*, 5%, for 28 consecutive days). Number of rats in each group was 6.

**Blood collection and analytical procedure.** After an overnight fast, blood was collected by cutting the tail tip at the beginning and middle ($14^{th}$ day) of the study period under mild ether anesthesia and by decapitation after 28 days. The serum was separated by centrifugation at 3000 rpm for 15 min. The clear non-hemolyzed supernatant fresh serum was then carefully taken into a set of clean, dried and sterilized glass vials. Serum glucose was estimated by glucose-oxidase (GOD-PAP) method.[10] Serum insulin was measured by a rat insulin ELISA kit.[11] Total serum cholesterol and triglyceride (TG) were determined by enzymetic-colorimetric (Cholesterol CHOD-PAP) method.[12,13] On the $28^{th}$ day after decapitation, abdomen was opened by laporotomy and liver was taken out, washed in ice-cold saline, patted dry and processed for glycogen estimation. Liver glycogen was measured by anthrone-reagent method.[14]

**Statistical analysis.** Statistical analysis was performed using Statistical Package for Social Sciences (SPSS, version 12, Chicago, IL, USA). Results are expressed as mean ± SD for a given number of observations (n). Statistical evaluation of data was performed by using one-way analysis of variance (ANOVA) followed by Duncan's test.[15] The level of significance was set at 0.05.

## RESULTS AND DISCUSSION

Effects of *I. gabonensis* seed powder and oil free seed powder on body weight (B.W.) of Type 2 diabetic model rats is presented in Table 1. The body weight of rat did not change significantly in any of the groups during the 28 days of study period. The standard drug glibenclamide significantly reduced serum glucose level of Type 2 diabetic model rats on the $14^{th}$ day ($p<0.001$) as well as on the $28^{th}$ day ($p<0.001$). It was evident from Figure 1 that oral

consumption of food incorporated with *I. gabonensis* crude powder (10%) resulted in a significant reduction in serum glucose level (serum glucose, M ± SD; 7.52 ± 1.23 mmol/l on the 0 day vs. 5.10±0.58 on the 28$^{th}$ day, $p$<0.01). The oil free seed powder (5%) showed 24% fall in serum glucose level on the 28$^{th}$ day, 7.63±1.50 mmol/l vs. 5.86±1.21 mmol/l although the reduction was not significant ($p$=NS).

It is evident from Table 2 that fasting serum insulin significantly increased in glibenclamide treated group (serum insulin M ± SD in ng/ml; 0.37 ± 0.16 on the 0 day vs. 0.62±0.14 on the 28$^{th}$ day, $p$<0.001). *I. gabonensis* defatted seed powder (5%) treated group showed the same effect on fasting serum insulin level, as did glibenclamide, (0.42 ± 0.12 on the 0 day vs. 0.69 ± 0.59 ng/ml on the 28$^{th}$ day, $p$<0.008). No effect was observed in *I. gabonensis* powder treated group. Liver glycogen content increased in the glibenclamide treated group in comparison with the control group. No change was observed in both *I. gabonensis* powder and defatted seed powder treated groups (Table 2).

**Table 1. Chronic effect of *I. gabonensis* on body weight of Type 2 diabetic Long Evans male rats.**

| Group | B.W. 0 day (g) | B.W. 14 days (g) | B.W. 28 days (g) |
|---|---|---|---|
| Normal Food (n=6) | 148 ± 4 (100) | 154 ± 5 (104) | 158 ± 4 (107) |
| Food + Glibenclamide (n=6) | 212 ± 19 (100) | 197 ± 20 (93) | 179 ± 66 (84) |
| Food + *I gabo* powder (10%) (n=6) | 163 ± 8 (100) | 165 ± 5 (101) | 167 ± 9 (102) |
| Food + Oil free seed powder ( 5%) (n=6) | 154 ± 15 (100) | 161 ± 18 (104) | 164 ± 23 (106) |

Data are presented as Mean ± SD and ANOVA was done followed by Duncan test with significance level at 0.05.

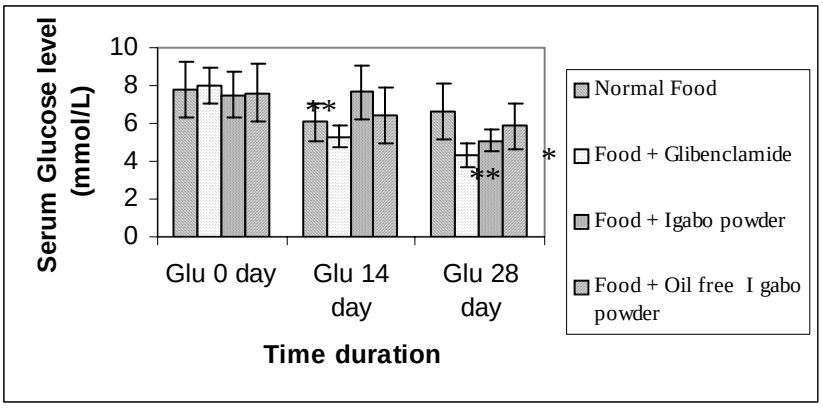


**Figure 1.** Chronic effect of *Irvingia gabonensis* seed powder and defatted seed powder on glycemic status of Type 2 diabetic model rats. *p<0.05; **p<0.01

**Table 2 Chronic effect of *I. gabonensis* on insulinemic status and hepatic glycogen content of Type 2 diabetic model rats.**

| Group | Insulin 0 day (ng/ml) | Insulin 28 day (ng/ml) | Hepatic glycogen content (g/100 g tissue) |
|---|---|---|---|
| Normal Food (n=6) | 0.56 ± 0.36 (100) | 0.39 ± 0.28 (69) | 2.77 ± 1.52 |
| Food + Glibenclamide (n=6) | 0.37 ± 0.16 (100) | 0.62 ± 0.14** (167) | 3.41 ± 2.17 |
| Food + *I gabo* powder (10%) (n=6) | 0.70 ± 0.27 (100) | 0.59 ± 0.26 (84) | 1.25 ± 0.68 |
| Food + Oil free seed powder (5%) (n=6) | 0.42 ± 0.12 (100) | 0.69 ± 0.59** (164) | 2.03 ± 0.41 |

Data are presented as Mean ± SD and ANOVA was done followed by Duncan test with significance level 0.05.

The changes in the level of serum lipids in control and experimental rats are illustrated in Table 3. Glibenclamide significantly lowered serum cholesterol level ($p<0.02$) and serum TG level ($p<0.002$). *I. gabonensis* powder (10%) significantly lowered serum TG level on the 28$^{th}$ day ($p<0.033$). There was a 48% decrease in the serum TG level after 28 days feeding of *I. gabonensis* oil free seed (5%) although the fall was not significant. On the contrary, serum cholesterol level was increased by both the treated groups with *I. gabonensis* after 28 days of feeding although glibenclamide lowered the cholesterol level on 28 days.

**Table 3. Chronic effect of *I. gabonensis* on lipidemic status of Type 2 diabetic model rats.**

| Group | Chol 0 day (mg/dl) | Chol 28 day (mg/dl) | TG 0 day (mg/dl) | TG 28 day (mg/dl) |
|---|---|---|---|---|
| Normal Food | 64 ± 6 (100) | 77 ± 14 (120) | 106 ± 21 (100) | 163 ± 14* (153) |
| Glibenclamide | 76 ± 6 (100) | 57 ± 22* (75) | 80 ± 25 (100) | 54 ± 15* (67) |
| I gabo powder (10%) | 67 ± 12 (100) | 89 ± 17 (132) | 119 ± 55 (100) | 65 ± 21* (54) |
| Oil free seed powder (5%) | 80 ± 16 (100) | 99 ± 12 (123) | 93 ± 26 (100) | 49 ± 9 (52) |

Data are presented as Mean ± SD. ANOVA was done followed by Duncan test with significance level 0.05.

The crude powder (10%) and oil free extract (5%) of *I. gabonensis* seed was incorporated in the normal food of rats for 28 days. The findings on the body wt. of Type 2 diabetic model rats showed no significant effects (an increase of 2-4% was noticed). On the contrary, it was claimed that *I. gabonensis* seeds significantly reduces body wt. in obese subjects.[16] Our Type 2 diabetic rats were not obese, probably that is the reason why no change was observed in body weight during the treatment period. *I. gabonensis* was evaluated for biological activities including diabetes and significant blood glucose lowering effect was found in Type I diabetic model rats.[16-17] *I. gabonensis* seed crude powder as well as extract lowered blood glucose level in Type 2 diabetic model rats. Administration of *I. gabonensis* seed crude powder significantly decreased the blood glucose level in these rats suggesting that it has hypoglycemic properties. Our results are in accordance with the findings of other investigators where it has been claimed that three weeks consumption of a viscous preparation of *I. gabonensis* resulted in a decreased blood glucose ($P<0.05$), pyruvate kinase and lactate dehydrogenase activities ($P<0.05$); and also increased glucose-6-phosphatase activity ($P<0.05$) in dikanut-fed diabetic rats compared with others not fed dikanuts.[18]

It is well established that dyslipidemia, especially hypertriglyceridemia is a common finding in patients with diabetes mellitus and is responsible for vascular complications.[19] It has also been claimed that deficiency of lipoprotein lipase activity may contribute significantly to the elevation of triglyceroides in diabetes.[20] The effects of the seeds of *I. gabonensis* on serum lipids was studied and it was found that obese subjects under *I. gabonensis* treatment for 4 weeks had a significant decrease in total cholesterol, triglycerides, LDL-cholesterol and an increase in HDL-cholesterol.[17] Significant ($P<0.05$) decrease in serum Tg level by *I. gabonensis* powder and 48% decrease by oil free seed extract were also observed in our experiment. This decrease in triglyceroides level by *I. gabonensis* seeds may be due to control of hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues. However, serum total cholesterol increased by both of them which is not beneficial. This increase in total cholesterol yet remains to be explained. It is known that *I. gabonensis* seeds may contain soluble dietary fibers that are bulk-forming

laxatives. It is well established that dietary fibers delay stomach emptying; leading to a more gradual absorption of dietary[18] sugar which is probably responsible for glucose lowering effect seen in Type 2 diabetic model rats with the treatment of *I. gabonensis* seeds (both powder and extract).

Moreover, it has got insulinomimetic effect since *I. gabonensis* powder significantly increased serum insulin level. Hence, in addition to dietary fiber effect, it has insulinomimetic or insulin sensitizing effect. Since the seeds did not change liver glycogen contents significantly, therefore it seems that lowering of blood glucose by *I. gabonensis* seeds is not accomplished through glycogenesis.

## CONCLUSIONS

The obtained results suggest that *I. gabonensis* seed powder as well as extract lowered blood glucose level in Type 2 diabetic model rats. In addition to dietary fiber like action it seems to act as an insulinomimetic and/or insulin sensitizing agent. The plant has triglyceride lowering effect. The plant merits further exploration both chemically and biologically to identify the active principle(s) and mechanism of action.

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