

# Hypoglycemic and Lipidemic Effects of *Irvingia gabonensis* Seeds in Long-Evans Rats

Md. Shakhawoat Hossain<sup>1</sup>, Md. Kamrul Hasan<sup>2</sup>, Nilufar Nahar<sup>2</sup>  
and Begum Rokeya<sup>3</sup>

<sup>1</sup>Department of Arts and Sciences, Ahsanullah University of Science and Technology, 141-142, Love Road, Tejgaon Industrial Area, Dhaka-1208, Bangladesh

<sup>2</sup>Department of Chemistry, University of Dhaka, Dhaka-1000, Bangladesh

<sup>3</sup>Department of Pharmacology and Research, Bangladesh University of Health Sciences, 125/1, Darus Salam, Mirpur, Dhaka-1216, Bangladesh

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**ABSTRACT:** The n-hexane extract and 80% ethanol extract of defatted residue (dfr) of *Irvingia gabonensis* (seeds) were evaluated for their hypoglycemic and hypolipidemic effects on the rats with type 2 diabetes. In addition, GC analysis of n-hexane extract was also investigated. A rat insulin ELISA kit was used to assess the serum insulin level. The measurements of total serum cholesterol and triglycerides (TG) were made using the enzymatic-colorimetric (cholesterol CHOD-PAP) method. The anthrone-reagent was used to measure liver glycogen level. The obtained results indicated that oral treatment of both extracts, 80% ethanol extract of dfr and n-hexane extract, significantly reduced serum glucose levels on the 14th day ( $p=0.000$ ;  $p=0.001$ ) and 21st day ( $p=0.001$ ;  $p=0.000$ ), respectively. The glucose level reduction was similar to that of the positive standard glibenclamide-treated group. The n-hexane extract significantly raised fasting serum insulin ( $p=0.02$ ) but 80% ethanol extract of dfr showed no significant effect. 80% ethanol extract of dfr considerably raised serum HDL levels ( $p=0.004$ ) and decreased serum TG levels ( $p=0.03$ ). There were essentially the same effects were observed with n-hexane extract. Neither LDL and serum cholesterol levels nor body weight showed any change over the course of the study. The glycemic and lipidemic state of type 2 diabetes model rats are found to be improved by n-hexane extract and 80% ethanol extract of dfr.

**Key words:** *Irvingia gabonensis* seed, streptozotocin, insulin, type 2 diabetes mellitus.

## INTRODUCTION

An important worldwide health issue is diabetes mellitus. Globally, there are expected to be 366 million diabetics by 2030, compared to 171 million in 2000.<sup>1</sup> It is a metabolic disease characterized by hyperglycemia. It arises from either inadequate insulin production from the pancreas or from concomitant impairment of insulin action.<sup>2</sup> Insulin resistance and the eventual malfunction of pancreatic beta cells are the causes of type 2 diabetes.<sup>3</sup> Over the past few decades, there has been a significant increase in the prevalence of type 2 diabetes.<sup>4</sup>

Plants and their extracts have been utilized as a diabetic treatment since ancient times. In fact, for

diabetic patients living in underdeveloped nations, this may be their only option for treatment. According to a recent study, supplementary and alternative medicine is used by up to 30% of diabetes patients.<sup>5</sup> An excellent example of a plant-based antidiabetic medication is the biguanides derived from *Galega officinalis*.<sup>6</sup> Although insulin and oral hypoglycemic medications such as sulfonylureas and biguanides remain the mainstays of type 2 diabetes treatment, their low effectiveness limits their use.<sup>7</sup> Therefore, scientists are in search of more effective anti-diabetic agents for diabetes management. In continuation of our research for new anti-diabetic compounds and collaboration with scientists from home and abroad<sup>8</sup> we studied the hypoglycemic effect of two different extracts of *I. gabonensis* seeds.

Large edible tree *I. gabonensis* (*Irvingiaceae* family) produces delicious, edible fruit pulp and edible seeds. It is extensively distributed throughout tropical West Africa and North America and also in

**Correspondence to:** Md. Kamrul Hasan  
E-mail address: kamrul\_du79@du.ac.bd  
Mobile: +880 1610001662

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the rainforests. The fruit is natively known as bobo in Sierra Leone, andok in Cameroon, and agbono in Nigeria. It is generally known as African mango, Dikanut, or Bush mango.<sup>9-11</sup> *I. gabonensis* seeds extracted with solvents produced 68–75% fat.<sup>12</sup> In a recent study, it was found that at 7.5 and 10 % w/v, IGS-gum proved to be a better binder than acacia in metformin tablets formulation.<sup>13</sup> The seeds are added to soups and stews to thicken them or to add flavor.<sup>14</sup> The seed also reduce plasma glucose levels of diabetic subjects and improves dyslipidemia.<sup>15</sup> In one of our studies, we have reported that, seed powder and oil-free seed extract of *I. gabonensis* reduced blood glucose levels in type 2 diabetic rats.<sup>16</sup> In this study, type 2 rats were used to assess the chronic effects of n-Hexane extract (fat) and an 80% ethanol extract of the defatted residue (dfr) of *I. gabonensis* seeds on body weight, serum insulin, serum glucose, lipid profile and liver glycogen content. Additionally, n-hexane extract from *I. gabonensis* seeds was subjected to GC analysis.

## MATERIALS AND METHODS

**Collection of *I. gabonensis* seeds.** *I. gabonensis* seeds were collected from Cameroon. The voucher No. 28054/HNC as a sample is stored in the Cameroonian National Herbarium. The seeds were peeled first, oven dried for 24 hours at 40 °C and then powdered using a motor and a pestle with a mesh size of 100.

**Extraction.** *I. gabonensis* seed powder (1.5 kg) was extracted for 24 hours at room temperature using n-hexane, and the solvent was then removed. Two more times, the same residue was extracted using hexane. All the extracts were combined and thoroughly dried using rotavapor and solid fat was gathered in order to assess the biological activity. Oil free seed powder, also known as defatted residue, was extracted using 80% ethanol and left overnight before the solvent was collected. Two further extractions of the same residue were conducted using 80% EtOH. All the extracts were mixed, thoroughly dried using a freeze-drier and rotavapor, and then used for biological activity.

**Animals.** In the study we used male Long-Evans rats (150–220 g) that were bred at the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) animal house. The Ethical Review Committee of the Bangladesh University of Health Sciences (BUHS) has given the ethical clearance (No: BUHS/ERC/EA/14/01) for the conduction of the study. Using the Bonner-Weir method, 48 hours old pups were given a single intraperitoneal injection of streptozotocin (90 mg/kg body weight) to induce type 2 diabetes.<sup>17</sup> The rats were caged in groups with 12-hour light-dark cycles, and were given unlimited rat food and water. Three months later, the rats used in the experiment were checked with an oral glucose load of 2.5 g/kg body weight. Four groups were formed with 6 type 2 rats in each group; (1) Control rats receiving water (WC group). (2) Treated with standard drug glibenclamide (5 mg/kg body wt). (3) Treated with 80% ethanol extract of defatted residue of *I. gabonensis* seeds (625 mg/kg body wt). (4) Treated with hexane extract of *I. gabonensis* seeds (625 mg/kg body wt).

## Blood collection and analytical Procedure.

There was just one dose oral feeding of the extracts for 21 days in every rat. After an overnight fast, in order to collect the blood, the tail tip was cut on days 0, 7 and 14 under a mild ether anesthesia and on 21<sup>st</sup> day by decapitation. Body weights of the rats were checked every week. Centrifugation was used for 15 minutes at 3000 rpm in order to separate the serum. The glucose-oxidase (GOD-PAP) technique was employed to assess serum glucose level.<sup>18</sup> An insulin ELISA kit for rats was used to assess serum insulin.<sup>19</sup> Triglyceride (TG) and total serum cholesterol were assessed using the enzymatic-colorimetric (cholesterol CHOD-PAP) method.<sup>20-21</sup> The abdomen was opened by laparotomy on the 21<sup>st</sup> day after decapitation, and the liver was removed. Subsequently, it was washed in ice-cold saline, dried by patting, weighed, and processed to estimate the amount of glycogen. The anthrone-reagent technique was used to quantify liver glycogen.<sup>22</sup>

**Statistical analysis.** The statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS, version 12, Chicago, IL, USA). For a specific number of observations (n), the results are presented as mean  $\pm$  SD. The statistical analysis of the data was carried out using one-way analysis of variance (ANOVA) and Duncan's test.<sup>23</sup>  $p < 0.05$  was used as the significance threshold.

**Sample preparation and compound identification using GC analysis:**

**Saponification.** 2.0 ml of 0.5 M methanolic NaOH were poured to a pear-shaped flask containing 100 mg of dry n-hexane extract (fat). It was then well shaken and heated for 30 minutes in a boiling water bath at 95°C. The mixture was dried by rotavapor and 5.0 ml distilled water was added to it and hexane was used to extract the mixture. The hexane part was taken in a conical flask and anhydrous Na<sub>2</sub>SO<sub>4</sub> was added to it to make it completely free from water. Then the solution was filtered and the hexane part was dried.

**Esterification.** 2.0 ml BF<sub>3</sub>-MeOH complex was added to the dried hexane part in a pear-shaped flask and heated for 10 minutes in a boiling water bath to convert fatty acids to fatty acid methyl esters (FAMEs). Then hexane was added to it and hexane part containing FAMEs was taken in a small vial for GC analysis.

**GC analysis.** A small volume (1  $\mu$ l) of the prepared FAMEs sample is injected into the GC system (GC-FID, Shimadzu, Japan), using a split injector. The injector is heated to 250°C to vaporize the sample. The vaporized sample is carried through the column (Length = 30 m, i.d. = 0.32 mm) by nitrogen (N<sub>2</sub>) gas at a flow rate of 1 ml/min. The column is located within a temperature-controlled oven of initial temperature 100°C. The ramp rate was 7°C/min and the final temperature of the oven was set to 280 °C. As the FAMEs travel through the column, they interact with the stationary phase based on their volatility and chain length and eluted at different times. FAMEs were detected by the Flame Ionization Detector (FID) of temperature 295°C for its high sensitivity to organic compounds.

**Compound confirmation.** A standard mixture containing known concentrations of various fatty acid methyl esters (including capric, lauric, myristic, palmitic, oleic, stearic, and linolenic FAMEs) was run under the exact same GC conditions as the experimental samples. The retention time for each peak in the experimental chromatogram is then compared to the retention time of the corresponding peak in the standard chromatogram. The retention times matched closely provides strong evidence for the identity of the compound. Once identified, the relative percentage of each fatty acid is calculated based on the area of its peak in the chromatogram. Results are given in the Table 3.

## RESULTS AND DISCUSSION

The effects of *I. gabonensis* seed extract on the body weight (BW) of type 2 diabetes rats over the course of the 21 days investigation are depicted in figure 1. As it is seen from the figure, WC group of rats gained body weight by 2%. Rats from other groups were found decreased body weight but the reduction was not statistically significant (P = NS).

The effect of 80% ethanol extract of dfr and n-hexane extract of *I. gabonensis* on fasting blood glucose levels of type 2 diabetic rats are given in Table 1. As the Table shows, administering both extracts orally led to a statistically considerable drop in blood glucose levels on day 14 ( $p = 0.000$ ;  $p = 0.001$  for the ethanol extract of dfr and the n-hexane extract of *I. gabonensis*, respectively). The group treated with glibenclamide experienced a similar reduction in glucose levels. While comparing between groups hexane extract of *I. gabonensis* on 21 day showed the best result although decrease in glucose level was not significant ( $p = 0.097$ ).

Chronic effect of *I. gabonensis* extracts on serum insulin level and liver glycogen content are presented in Table 2. It is evident that baseline serum insulin level was similar between groups 1, 2, and 3 but group 4 had comparatively lower serum insulin level. After 21 days of chronic administration of the extracts, serum insulin level decreased in group 1, 2 and 3 but there was a notable rise in serum insulin in

group 4 i.e. n-hexane extract treated group. The amount of liver glycogen in the rats of the various

experimental groups did not significantly alter.

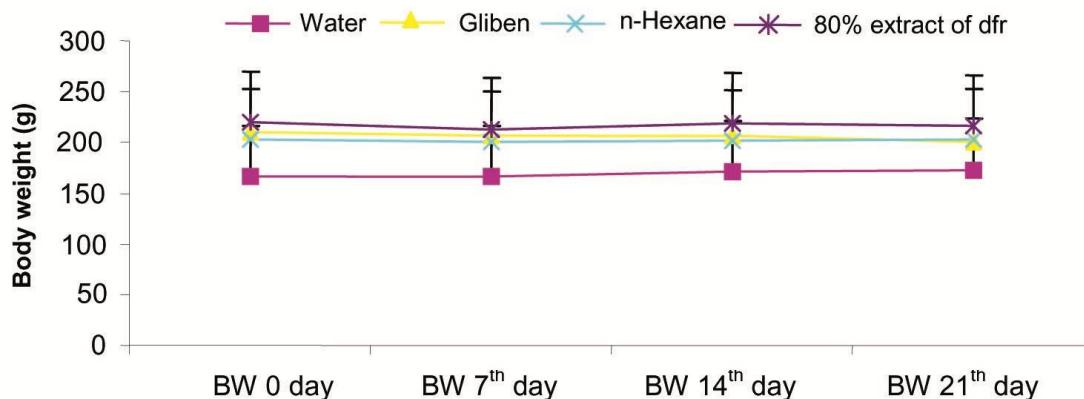


Figure 1. Chronic effect of *I. gabonensis* seed extracts on body weight of type 2 diabetic rats.

**Table 1.** Chronic effects of *I. gabonensis* seed extracts on the glycemic state of type 2 diabetic model rats.

Treatment	Experimental period (days)			t/p value	
	Gl 0 (mmol/l)	Gl 14 (mmol/l)	Gl 21 (mmol/l)	0 day vs 14 day	0 day vs 21 day
Group					
Water	7.63 ± 1.26	6.61 ± 1.05	7.62 ± 1.74	4.33/0.008	0.005/0.996
Glibenclamide	7.97 ± 1.58	6.14 ± 1.69	6.43 ± 1.19	3.14/0.026	3.24/0.023
80% ethanol extract of dfr	9.89 ± 0.48	5.33 ± 0.38***	5.70 ± 0.64	11.88/0.00	8.88/0.001
n-Hexane extract	9.02 ± 1.24	4.78 ± 1.24***	5.73 ± 1.05	6.34/0.001	10.57/0.000

#### Bonferroni p value

Group	0 day	14 day	21 day
WC vs Gliben	1.000	1.000	0.675
WC vs 80% ethanol extract	0.041	0.584	0.117
WC vs n-Hexane extract	0.394	0.104	0.097***
Gli vs 80% ethanol extract	0.112	1.000	1.000
Gli vs n-Hexane extract	0.940	0.407	1.000
80% ethanol extract vs n-Hexane extract	1.000	1.000	1.000

Results are shown as Mean ± SD, and an ANOVA was performed before the Duncan test at p<0.05.

**Table 2.** Chronic effects of *I. gabonensis* seed extracts on the amount of hepatic glycogen and insulinemic state in type 2 diabetic rats.

Group	Insulin 0 day (ng/ml)	Insulin 21 day (ng/ml)	Glycogen (mg/g tissue)
Water	0.64 ± 0.32	0.53 ± 0.25	1.07 ± 0.36
Glibenclamide	0.66 ± 0.25	0.61 ± 0.18	1.65 ± 1.17
80% ethanol extract of dfr	0.78 ± 0.37	0.39 ± 0.21	1.28 ± 0.66
n-Hexane extract	0.24 ± 0.39*	0.56 ± 0.25*	1.07 ± 0.21

Results are shown as Mean ± SD, and an ANOVA was performed before the Duncan test at p<0.05-0.02.

Figure 2 shows the effects of *I. gabonensis* seed extracts on lipidemic status of type 2 rats. It is seen from the figure that at the end of the study period in

control group serum cholesterol level increased and serum TG level decreased. Glibenclamide decreased both serum cholesterol and TG level on 21<sup>st</sup> day.

Ethanol extract lowered serum cholesterol level but the reduction was not significant. On the other hand, serum TG level was reduced significantly by ethanol

extract of *I. gabonensis*. n-hexane extract raised serum cholesterol levels but decreased serum TG levels at the end of the trial.

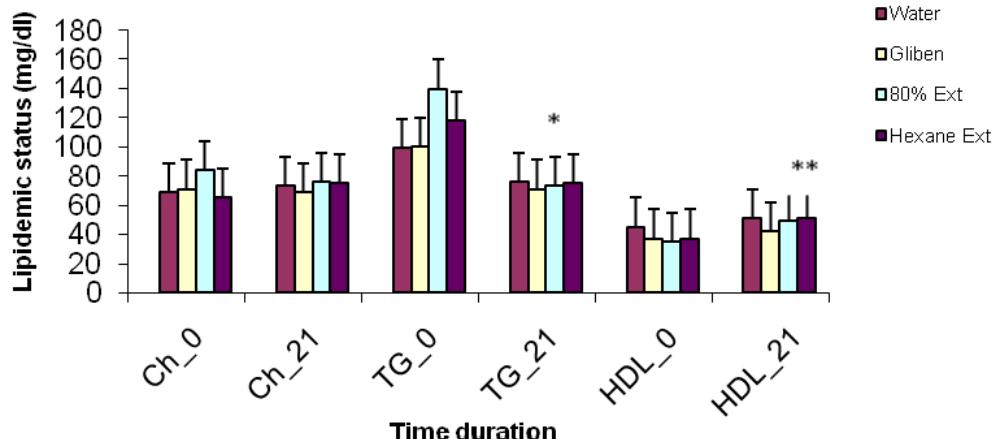


Figure 2. Chronic effect of *I. gabonensis* seed extracts on lipidemic status of Type 2 diabetic rats. \*  $p < 0.03$ , \*\* $p < 0.004$ .

**Table 3. Fatty acid composition of *I. gabonensis* seed.**

Name of the fatty acids	Retention time of standard acids	Retention time of experimental acids	Relative percentage of fatty acids
Capric	6.37	6.29	1.56
Lauric	11.13	11.14	42.52
Myristic	16.39	16.42	48.78
Palmitic	21.51	21.41	4.55
Oleic	25.64	25.67	0.09
Stearic	26.16	26.27	0.41
Linolanic	25.48	25.54	2.11

Table 3 shows the list of different fatty acids identified from the GC chromatogram of n-hexane extract (fat) of *I. gabonensis* seeds. Lauric acid and myristic acids were found to be the major constituents of the oil while minor constituents are palmitic, linolanic, capric, stearic and oleic acids.

The current investigation was undertaken to assess the effect of *I. gabonensis* seeds extracts on streptozotocin induced type 2 diabetic model rats which serve as a model of hypoinsulinemia i.e.  $\beta$ -cell secretory defect. Treatment with 80% ethanol extract of dfr and n-hexane extract of *I. gabonensis* seeds significantly lowered mean values for fasting glucose in type 2 diabetic rats. These results are in accordance with other investigators.<sup>24</sup> The hypoglycemic effect of *I. gabonensis* extracts in type 2 diabetic rats may be attributed to potential

enhancement of the serum insulin effect through either increased release of bound insulin or increased pancreatic production of insulin from existing  $\beta$ -cells. It is evident that n-hexane extract significantly increase serum insulin concentration by 133% in comparison to WC rats. Since 80% ethanol extract of dfr did not increase serum insulin level at the end of the trial period, so it might exert hypoglycemic effect by extrapancreatic mechanism. It is established that soluble dietary fibers may cause increased fecal excretion of bile acids<sup>25</sup> and the size of the bile acid pool<sup>26</sup> and also increase steroid losses.<sup>27</sup> It is also established that soluble dietary fibre may delay the absorption of carbohydrates in the gut. The delayed stomach emptying brought on by *I. gabonensis* seeds causes dietary sugar to be absorbed more gradually, which can lessen the typical elevation in blood sugar

levels after a meal.<sup>28</sup> This may be the probable mechanism by which *I. gabonensis* lowered serum glucose level and decreased total cholesterol and TG level. Studies have shown that individuals with type 2 diabetes who consume large amounts of dietary fiber, especially soluble fiber, have better glycemic control and lower plasma lipid concentrations.<sup>29</sup> In our study, HDL level was significantly ( $p < 0.05$ ) increased in both 80% ethanol and n-hexane treated group after 21 days of the study period. LDL cholesterol level showed no significant change over the course of the study. Decrease in serum cholesterol and TG level could have been due to the improvement in glycemic control. *I. gabonensis* seed fiber, like other soluble fibers, has the ability to bind to bile acids in the gut and transmit them through the face, requiring the body to produce more bile acids from cholesterol.<sup>30</sup> This effect may be responsible in lowering blood cholesterol and others lipids by *I. gabonensis* seeds.

Recent research indicates that myristic acid, when taken orally over time, may help manage type 2 diabetes. It has been observed to lower high blood sugar levels by improving the body's response to insulin and by reducing body weight. This suggests myristic acid could be a promising agent for both preventing and treating type 2 diabetes and its associated health problems.<sup>31</sup> Another study found that a combination of lauric acid and the amino acid tryptophan, when administered into the small intestine of healthy men, led to a modest decrease in their fasting blood sugar.<sup>32</sup> Since n-hexane extract of *I. gabonensis* seeds contains more than 91% of these two acids, in light of these observed glucose-lowering effect of myristic and lauric acid, it could offer a new, diet-based approach to control high blood sugar in people with type 2 diabetes.

The n-hexane extract of *I. gabonensis* seeds is primarily composed of the seed's beneficial fats. Scientific studies, particularly acute toxicity tests, consistently indicate a very low level of toxicity for this extract, with LD<sub>50</sub> values often exceeding 5000 mg/kg, classifying it as practically non-toxic.<sup>33</sup> While comprehensive long-term toxicity studies specifically on the isolated n-hexane extract are not as abundant

as acute studies, the traditional widespread use of the seeds and the nature of the compounds extracted by n-hexane suggest a high safety margin for this extract.<sup>33</sup> While the ethanolic extract of *I. gabonensis* root barks (EEIGRB) appeared safe for immediate use, a study revealed that sustained daily oral administration over 28 days led to mild toxicity. Specifically, when given at a dose of 400 mg/kg, the extract caused damage to both the liver and kidneys, evidenced by abnormal tissue changes in these organs.<sup>34</sup> Therefore, due to the risk of liver and kidney damage, prolonged use of EEIGRB should be avoided.

In this study, we observed beneficial glycemic and lipidemic effects of the extracts but we failed to investigate the biological pathways or molecular interactions responsible for these improvements, such as how insulin production is stimulated or lipid metabolism is altered. Furthermore, we did not do the histopathological examination that limits understanding of organ protection, and insufficient characterization of the extracts, particularly from the 80% ethanol extract we failed to identify any active compound.

## CONCLUSION

The results obtained indicate that the glycemic and lipidemic condition of type 2 diabetes model rats are improved by n-hexane extract and 80% ethanol extract of dfr of *I. gabonensis*. Apart from its action similar to that of dietary fiber, n-hexane extract appears to have an insulinomimetic and/or insulin sensitizing effect. More chemical and biological research on the plant is necessary to determine its active principle(s) and mode of action.

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