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GLYCAEMIC VARIATIONS AFTER ADMINISTRATION OF *IRVINGIA GABONENSIS* SEEDS FRACTIONS IN NORMOGLYCEMIC RATS

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Abstract

The action of *Irvingia gabonensis* seed fractions in reducing or slowing down the intestinal absorption of glucose was evaluated in normoglycaemic rats. The crude seeds (CS), the defatted seeds (DS) and the protein fraction (PF) were administered at dose of 400mg/ kg body weight to normoglycemic rats submitted to oral glucose test (OGTT) with glucose (2g/kg body weight). The results obtained show a significant reduction of the postprandial glucose level after a glucose load of (2g/kg body weight) as well as fasting blood glucose levels with the three fractions.

Key words: antihyperglycaemic, blood glucose, solubles fibres, *Irvingia* seeds fractions

Introduction

Diabetes mellitus is a chronic metabolic disorder affecting approximately 5% of the world population. Hyperglycaemia is the principal cause of complications and effective blood glucose control is the key to improving the quality of life in patients with diabetes (DeFronzo,1992). Currently available therapy for diabetes include insulin, and various oral anti diabetic drugs are used to achieve better glycaemic control, but their use is associated to serious adverse effects (Doi et al. 1979). Many traditional plant treatments for diabetes are used throughout the world. Plant drugs (Bailey and Day, 1989) are frequently considered to be less toxic and more free from side effects than synthetic ones. Based on the WHO (1980) recommendations hypoglycemic agents of plant origin used in traditional medicine are important. The attributed antihyperglycemic effects of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes.

High fiber diet has been shown to work better in controlling diabetes (Sharma et al. 1990; Vuskan et al.2000), and may control blood glucose levels as well as oral diabetes drugs. *Irvingia gabonensis* (Aubry -Lecomte ex O'Rouke) Baill, or bush

mango is a medium sized tree. It belongs to the family Irvingiaceae (order-Rutales) a small tropical family containing two other genera (*Klaiedoxa*, *Desbordesia*) and the genus *Irvingia* which contains three species all occurring in West and Central Africa. Two varieties of *Irvingia gabonensis* have been identified based on long phenological and reproductive phenological observations (Okafor 1975 and Ladipo et. al. 1996). There is not much difference between the two species except for fruit sweetness and some tree morphological differences. *Irvingia gabonensis* I. and *wombolu*. The seeds are used for soup thickening (White and Albernethy, 1996) and the leaves are used in traditional medicine in the treatment of dysentery. Kengni (2003) has reported the composition of seeds from Cameroon to be 68.5% fat, 6.1% total carbohydrate, 2.7% ash, 6.2% crude protein, 6.9% soluble fibre, 17.3% insoluble fibre, 0.1% phenolic compounds. We have shown the hypoglycaemic effect of the methanol extract of *I gabonensis* seeds in rats (Ngondi et al., 2006). The present study was designed to evaluate the action of *Irvingia gabonensis* seeds fractions in reducing or slowing down the intestinal absorption of glucose in normoglycemic rats, and to evaluate their effects on fasting blood glucose levels.

Materials and Methods

Animals

Thirty male albino Wistar rats were maintained on standard laboratory diet and tap water *ad libitum* at the Animal house of the Department of Biochemistry, Faculty of Science, University of Yaounde I, Cameroon. Prior to the experiment, the animals were subjected to fasting for 12 h but allowed free access to water. The experimental protocol was approved by the Animal Studies Committees of University of yaounde I

Collection and preparation of plant material

Fruits of *Irvingia gabonensis* were collected in the month of august 2001 in a village around Ebolowa, in the South province of Cameroon. Botanical identification was performed at the National Herbarium, Yaounde, Cameroon in comparison with the voucher specimen n°314522/HNC. The seeds were removed and carefully washed with water and dried in an oven (50°C) for 3 days.

The crude seeds (CS)

Two-hundred grams of dried seeds were ground and stored in well closed container.

The defatted seeds (DFS)

Eight -hundred grams of dried seeds were extracted with hexane in a Soxhlet apparatus for 2 days. The seeds were then dried at room temperature for 5 days and ground into powder. The resulting material yielded 308 g (38.50 % w/w).

The non purified protein fraction (PF)

Two-hundred grams of ground defatted seeds underwent another extraction with ethanol (95%) in soxlet apparatus for three days. The residue obtained was dried at room temperature for 5 days. The resulting material yielded 96 g (48 % w/w)

Aqueous solution of *Irvingia gabonensis* fraction administration

The seeds fractions were suspended in distilled water and administered orally through intragastric tube at the dose of 400mg/kg body weight.

Oral Glucose Tolerance Test

An oral glucose tolerance test (OGTT) was performed on normal rats (230-250g body weight) deprived of food for 16 h before and during the experiment with free access to water. The different seed fractions were administered orally at dose of (400mg/kg body weight) to three groups of 8 rats each, 30 min before oral administration of glucose (2g/kg body weight). Two groups of 8 rats each served as controls. Animals in positive controls group received distilled water (4 ml/kg), 30 min before oral administration of glucose (2g/kg body weight) while those in negative control group received 4ml/kg of distilled water twice. Blood samples were taken before administration of the test material and glucose (baseline) and 1h, 1.5h, 2h and 2.5h thereafter.

Effects of *Irvingia gabonensis* seeds fraction on fasting blood glucose.

Forty five rats weighing approximately 130g \pm 2.5g were fed on standard diet and divided into five groups of nine rats each. Rats in groups (I, II, III and IV) received oral administration of shea butter oil (*Vitellaria paradoxa* Gaertn. syn. *Butyrospermum paradoxum*) at dose of 4ml/kg b.w for five weeks. Another group of nine rats (V) received oral administration of distilled water (4ml/kg b.w) at the same time. The standard diet which was non-purified diet supplied 67% of total energy as carbohydrate, 25% as protein and 8% as fat. It consisted of fish meal, cornmeal, wheat bran, groundnut cake, and vitamin-mineral mixture and contains ~10 % (wt/wt) dietary fiber. The same diet was used through the whole experiment. After five weeks of treatment, blood glucose concentrations were measured and animals in groups I, II, III and IV with higher blood glucose levels were treated as follows. Group I: (positive control): Shea butter oil (4ml/kg b.w) and distilled water (4ml/kg b.w), GroupII: Shea butter oil (4ml/kg b.w) and (400mg/kg b.w) of crude seeds solution. GroupIII: Shea butter oil (4ml/kg body weight) and (400mg/kg b.w) of defatted seeds solution. Group IV : Shea butter oil (4ml/kg body weight) and (400mg/kg b.w) of protein fraction solution. Animals in group V continued receiving distilled water (8ml/kg b.w) and was used as negative controls. The test materials were administered daily using an intragastric tube for 21 days . Blood samples were taken before administration of the test material (day 0) and at end of the experiment (21 days).

Collection of blood and measurement of blood glucose

Blood samples for glucose determination were obtained from the tips of tails of the rats. 0.5ml of blood was dropped on the reagent pad of the one touch strip (Life Scan Inc. Milpitas, California, USA). The strip was inserted into a One-Touch brand meter and the reading noted (WHO, 1980).

Statistical analysis.

All values are expressed as mean blood glucose levels \pm SEM (standard error of mean). Data were analyzed by one-way ANOVA, and then differences among means were analyzed using the Fisher's protected LSD test. Differences were considered significant at $P < 0.05$.

Results

Hypoglycaemic effect on oral glucose tolerance test

In control group, glucose loading led to a rapid increase of blood glucose after 1h whereas the blood glucose response was considerably flattened with the administration of the crude seeds solution ($p < 0.0001$; $p < 0.02$), the defatted seeds solution ($p < 0.0001$; $p < 0.05$), and the protein fraction ($p < 0.0001$; $p < 0.01$) at 1h and 2.5h respectively (Table 1).

Effect on fasting blood glucose concentrations and body weight after 21 days of treatment

As shown in Table 2, rats receiving oral administration of shea butter oil had significantly higher fasting blood glucose concentrations than negative controls on day 0. On day 21, blood glucose concentrations decreased significantly in rats treated with shea butter oil and 400mg/kg defatted seeds ($P < 0.01$) or protein fraction ($P < 0.05$) compared with rats receiving shea butter and distilled water (positive control). The blood glucose concentrations were not affected significantly in response to treatment with the crude seeds solution. The average body weight of rats receiving shea butter oil was higher compared with negative controls after five weeks of treatment. However, the administration Irvingia seeds fractions and shea butter oil, induced a weight loss of 7.01%, 17.89% and 8.65% respectively with crude seeds, defatted seeds and the protein fraction. There was no significant difference in daily food intake between groups.

Discussion

Diets high in fat, especially saturated fatty acid induced body weight gain, glucose tolerance and increased the risk of type 2 diabetes (Feskens et al. 1995; Marshall et al. 1994; Uusitupa et al. 1994). In this study we observed a significant increase in body weight and blood glucose after administration of shea butter oil. When *Irvingia gabonensis* seed fractions and shea butter oil were administered simultaneously, a reduction in body weight was observed. A high fiber diet has been shown to be effective in controlling diabetes and may control blood glucose levels as well as oral diabetes drugs (Astrup et al. 2000).

Table 1: Effect of the aqueous solution of *Irvingia gabonensis* seed fractions on blood glucose level after a glucose load (2 g/Kg b.w) in normoglycemic rats

Treatment groups	Blood glucose at different time after treatment (mmol/l)				
	0h	1h	1.5h	2h	2.5h
Crude seeds 400mg/kg + glucose 2g/kg b.w	3.25±0.13	4.70±0.42 **** (32.56%)	5.10±0.22 (-18.05%)	3.27±0.20 (13.26%)	2.85±0.15 * (16.17%)
Defatted seeds 400mg/ Kg + glucose 2g/kg b.w	3.00±0.17	3.77±0.23 **** (45.91%)	4.17±0.36 (3.44%)	3.27±0.21 (13.26%)	2.90±0.14 ** (14.7%)
Protein fraction 400mg/ Kg + glucose 2g/kg b.w	3.15±0.17	4.00±0.23 **** (42.60%)	3.77±0.22 (12.7%)	3.45±0.12 (8.48%)	2.80±0.20 *** (17.6%)
Distilled water 4ml/ Kg + glucose 2g/kg b.w	3.27±0.36	6.97±0.71	4.32±0.10	3.77±0.17	3.40±0.16
Distilled water (Negative control) 2x 4ml/kg b.w	3.17±0.11	3.15±0.18	3.19±0.24	3.19±0.24	3.14±0.21

The values are mean ± s.e.m (standard error of mean); The values given in parentheses represent the percentage reduction in blood glucose compared with the control.

Number of rats per group = 8

* P < 0.05; ** P < 0.02; *** P < 0.01; **** P < 0.0001 compared with the positive control (glucose)

Table 2: Effect of the aqueous solution of *Irvingia gabonensis* seed fractions on body weight, fasting blood glucose levels and food intake in normoglycemic rats after 21 days of treatment.

Treatment groups	Body weight and Blood glucose before and after treatment				Food Intake (g/rat/day)
	Body weight (g)		Blood glucose (mmol/l)		
	Initial	21 days after	Initial	21 days after	
Crude seeds 400mg/Kg + Shea butter 4ml/kg b.w	228,00±3.82	212,00±6.24 ** (-7.01%)	5.66±0.22 ⁺	5.36±0.20 (-5.3%)	25.02±1.25
Defatted seeds 400mg/Kg + Shea butter 4ml/kg b.w	228,80±3.05	188.01 ±7.71 ** (-17.89%)	6.08±0.10 ⁺	4.36±0.37** (-28.28%)	23,56±1.32
Protein fraction 400mg/Kg + Shea butter 4ml/kg b.w	231,20±3.40	211,20±5.39* (-8.65%)	5.27±0.16 ⁺	4.70±0.47* (-10.81%)	30.42±1.06
Distilled water (Positive control) 4ml/ Kg+ Shea butter 4ml/kg b.w	230,40±3.69	256,00±6.54* (11.11%)	5.62±0.22 ⁺	5.86±0.95 (3.58%)	33,52±1.17
Distilled water (Negative control) 8ml/ Kg b.w	195,00±3.59	204,60±2.83 (4.92%)	3.04±0.09	3.05±0.11	35.23±0.99

The values are mean ± s.e.m (standard error of mean) for 6 rats

The values given in parentheses represent the percentage reduction of body weight or blood glucose after 21 days of treatment.

*P < 0.05; **P < 0.01; compared with the initial blood glucose level or body weight of the rats (day-0) in the respective groups. ⁺P < 0.0001; compared with the negative control.

Adequate amounts of dietary fiber are believed to be important for people wishing to lose weight. Results obtained in this study showed that *Irvingia gabonensis* seed fractions reduced the postprandial glucose load with the maximum effect observed with the defatted seeds material. The defatted seed material which has more dietary fiber and protein than the crude seeds may delay stomach emptying, leading to a more gradual absorption of dietary sugar and this can reduce the elevation of blood glucose. In addition, fiber adds bulk to the diet and tends to reduce food intake, energy intake and body weight in long-term (Burton-Freeman, 2000). A reduction of blood glucose levels has been reported with other's solubles fibres, such as psyllium (Rodríguez-Morán et al. 1998), guar gum (Landin et al. 1992), pectin (Schwartz et al. 1988), oat bran (Hallfrisch et al. 1995), and glucomannan (Doi et al. 1978; Vuksan et al. 2000), fenugreek seeds (Sharma et al. 1990).

Body weight and blood glucose were also reduced with the non- purified protein fraction. Like other's plant proteins, *Irvingia* proteins may have anti amylase activity. Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starch from being digested by pancreatic amylase. Starches are complex carbohydrates that cannot be absorbed unless they are first broken down by the digestive enzyme amylase and other, secondary, enzymes (Marshall and Lauda, 1975; Choudhury et al. 1996). Layer et al. (1985), Boivin et al. (1987), and Brugge and Rosenfeld. (1987) suggested that plant protein extract from *Phaseolus vulgaris* has been found to prevent the absorption of carbohydrate. Animal and human studies have suggested that when soy is used as a source of dietary protein, it may have several biological effects on the body that might help with weight loss (Bhathena and Velasquez, 2002). In a preliminary study, Allison et al. (2003) found that people trying to lose weight using a meal-replacement formula containing soy protein lost more weight than a group not using any formula. On the other hand, the stimulation of insulin secretion may be another possible action site for *Irvingia gabonensis* proteins. In this study we observed the lowest variation of the post prandial load with the protein fraction. These findings are in agreement with previous studies with soy protein (Forsythe, 1988).

References

1. Allison, D.B., Gadbury, G., Schwartz, L.G, et al.(2003). A novel soy-based meal replacement formula for weight loss among obese individuals: a randomized controlled clinical trial. *Eur J Clin Nutr.***57**:514–22.
2. Astrup, A., Grunwald, G.K., Melanson, E.L., et al. (2000). The role of low-fat diets in body weight control: a meta-analysis of ad libitum dietary intervention studies. *Int J Obes Relat Metab Disord* .**24**:1545–52 [review].
3. Bailey, C.J., Day, C. (1989). Traditional treatments for diabetes. *Diabetes Care* **12**:553-564.
4. Bhathena, S.J, Velasquez, M.T. (2002). Beneficial role of dietary phytoestrogens in obesity and diabetes. *Am J Clin Nutr.***76**:1191–201[review].
5. Boivin, M., Zinsmeister, A.R., Go, V.L., DiMagno, E.P. (1987). Effect of a purified amylase inhibitor on carbohydrate metabolism after a mixed meal in healthy humans. *Mayo Clin Proc* .**62**:249–55.

6. Brugge, W.R., Rosenfeld, M.S. (1987). Impairment of starch absorption by a potent amylase inhibitor. *Am J Gastroenterol.* **82**:718–22.
7. Burton-Freeman, B. (2000). Dietary fiber and energy regulation. *J Nutr.* **130**(2S **Suppl**):272S-275S [review].
8. Choudhury, A., Maeda, K., Murayama, R., DiMagno, E.P. (1996). Character of a wheat amylase inhibitor preparation and effects on fasting human pancreaticobiliary secretions and hormones. *Gastroenterology* . 111:1313-20.
9. DeFronzo, R.A., Bonadonna, R.C., Ferrannini, E. (1992). Pathogenesis of NIDDM. A balance overview. *Diabetes care* . **15**: 318-368.
10. Doi K, Matsuura M, Kawara A, Baba S. (1979). Treatment of diabetes with glucomannan (konjac mannan). *Lancet.* **1**:987–8 [letter].
11. Feskens, E.J., Virtanen, S.M., Rasanen, L., et al. (1995). Dietary factors determining diabetes and impaired glucose tolerance. A 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. *Diabetes Care.* **18**:1104–12.
12. Forsythe, W.A. (1986). Comparison of dietary casein or soy protein effects on plasma lipids and hormone concentrations in Gerbil (*Meriones Unguiculatus*). *J Nutr.* **116** :1165-71.
13. Hallfrisch J, Scholfield DJ, Behall KM. (1995). Diets containing soluble oat extracts improve glucose and insulin responses of moderately hypercholesterolemic men and women. *Am J Clin Nutr.* **61**:379–84.
14. Jenkins, D.J.A. (1981). Slow release carbohydrate and the treatment of diabetes. *Proc. Nutr. Soc. London.* **40**: 227-235.
15. Kengni, E. (2003). Food value of fruits from indigenous fruit trees in the lowland humid tropics of west and Central Africa: Case of *Irvingia gabonensis* and *Dacryodes edulis* in Cameroon. Doctorate PhD thesis. University of Yaounde I.
16. Ladipo D.O., Fondoun, J. M., Ganga, N. (1996). Domestication of the bush mango (*Irvingia* spp): some exploitable intra specific variations in West and Central Africa. In proceedings of an FAO ICRAF-IUFRO proceedings (Non-wood Forest Products FAO NO 9.
17. Landin, K., Holm, G., Tengborn, L., Smith, U. (1992). Guar gum improves insulin sensitivity, blood lipids, blood pressure, and fibrinolysis in healthy men. *Am J Clin Nutr.* **56**:1061–5.
18. Laver, P., Carlson, G.L., DiMagno, E.P. (1985). Partially purified white bean amylase inhibitor reduces starch digestion in vitro and inactivates intraduodenal amylase in humans. *Gastroenterology.* **88**:1895–902.
19. Marshall, J.J., Lauda, C.M. (1975). Purification and properties of phaseolamin, an inhibitor of alpha-amylase, from the kidney bean, *Phaseolus vulgaris*. *J Biol Chem.* **250**:8030-7.
20. Marshall, J.A., Hoag, S., Shetterly, S., et al. (1994). Dietary fat predicts conversion from impaired glucose tolerance to NIDDM. The San Luis Valley Diabetes Study. *Diabetes Care.* **17**:50–6.
21. Ngondi Judith Laure, Emile Joachim Djiotsa, Zephyrin Fossouo, Julius Oben (2006). Hypoglycaemic effect of the methanol extract of *Irvingia gabonensis* seeds on streptozotocin diabetic rats. *Afr. J. Trad. CAM* (2006) **3** (4): 74 – 77

22. Okafor, J. and Okolo, H.C. (1974). Potentials of some indigenous fruit trees of Nigeria. Paper presented at the 5th Annual Conference of the Forestry association of Nigeria Jos (1974) pp 60–71.
23. Rodríguez-Morán, M., Guerrero-Romero, F., Lazcano-Burciaga, G. (1998). Lipid and glucose lowering efficacy of plantago psyllium in type II diabetes. *Diabetes Its Complications*. **12**:273–8.
24. Sharma, R..D., Raghuram, T.C., Rao, N.S. (1990). Effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes. *Eur J Clin Nutr* . **44**(4): 301-6.
25. Schwartz SE, Levine RA, Weinstock RS, et al. (1988). Sustained pectin ingestion: effect on gastric emptying and glucose tolerance in non-insulin-dependent diabetic patients. *Am J Clin Nutr*. **48**:1413–7.
26. Uusitupa, M., Schwab, U., Makimattila, S., et al. (1994). Effects of two high-fat diets with different fatty acid compositions on glucose and lipid metabolism in healthy young women. *Am J Clin Nutr*. **59**:1310–6.
27. Vuksan, V., Sievenpiper, J.L., Owen, R., et al. (2000). Beneficial effects of viscous dietary fiber from Konjac-mannan in subjects with the insulin resistance syndrome: results of a controlled metabolic trial. *Diabetes Care*. **23**:9–14.
28. White, L., Albernethy, K. (1996) *Guide de la végétation de la réserve de la Lopé, Gabon*; ECOFAC Gabon.
29. World Health Organization (WHO), (1980). Expert committee on Diabetes Mellitus: Second Report. Technical Report Series Number 646, World Health Organization, Geneva, P. 61.