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Original Article

Improvement of glucose tolerance in rats fed with diets containing *Vernonia amygdalina* leaves

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ABSTRACT: The effect of 5% and 10% dietary incorporation of leaves of *Vernonia amygdalina* (VA) on oral glucose tolerance was studied in normoglycemic male albino rats. The feeding of the vegetable incorporated diets (5% VA and 10% VA) resulted in marked improvement in oral glucose tolerance in rats. After one week of diet administration, following an oral glucose load (3g/kg body weight), blood glucose concentration (BGC) (mg/dL) in rats fed the 5% VA peaked at 15 minutes (147.24 ± 18.46), while the BGC in rats fed the control diet and 10% VA peaked at 30 mins (180.00 ± 28.57 and 159.48 ± 16.07 respectively). After two weeks of feeding of the vegetable incorporated diets, post administration BGC peaked at 15 minutes in the test groups [5% VA diet (152.64 ± 33.46) and 10% VA diet group (121.95 ± 24.78)] while the peak remained at 30 minutes in the control group. After 3 weeks of diet administration BGC remained peaked at 15 minutes in the 5% and 10% VA diet groups (137.16 ± 61.29 and 132.75 ± 8.86 respectively). Rats in both test groups, after 3 weeks on the test diets, had their glycaemic indices reduced by 15–18%. These findings indicate that feeding on diets containing *Vernonia amygdalina* could positively modulate oral glucose tolerance. The vegetable could be useful in dietary management of conditions associated with oral glucose tolerance, and by extension, hyperglycemia.

KEYWORDS: Diabetes mellitus; Dietary incorporation; Oral glucose tolerance; *Vernonia amygdalina*

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INTRODUCTION

The oral glucose tolerance test (OGTT) measures an individual organism's ability to utilize ingested glucose, the body's main source of energy, over a given period of time. OGTT is a good marker of the diabetic state and a test of immense value and when used in combination with fasting plasma glucose concentration, it facilitates the diagnosis of diabetes, as against using the latter alone. In fact, for a long time, it was the mainstay for the diagnosis of diabetes (Bartoli *et al.*, 2011).

Under normal physiological conditions, high blood glucose concentration promotes insulin release from the β -cells of the islets of Langerhans of the pancreas. Insulin stimulates the uptake of glucose by peripheral tissues especially skeletal muscle [by up-regulating the expression of glucose

transporter-4 (GLUT-4) and by stimulating the exocytosis of stored GLUT-4], and promotes the storage of glucose in the liver (as glycogen) through the stimulation of glycogen synthase activity (Zunino, 2009; Zaid *et al.*, 2008). Loss of responsiveness to insulin by insulin-responsive tissues results in the sustained elevation of blood glucose concentration (hyperglycemia), and ultimately to type 2 diabetes mellitus, a metabolic condition that affects the metabolism of carbohydrates, lipids and proteins (Pareek *et al.*, 2009). Besides hyperglycemia, type 2 diabetes mellitus is characterized by insulin resistance in peripheral tissues, and the eventual destruction of the β -cells of the islets of Langerhans (Guillausseau *et al.*, 2008). It affects 285 million people (6.4%) globally, and is estimated to affect 438 million people by the year 2030, most of who would reside in developing countries (IDF, 2010). In fact, currently, diabetes

mellitus is thought to be the most prevalent endocrine disorder in Nigeria (Eseyin *et al.*, 2010).

A significant percentage of the population in sub-Saharan Africa and many countries in the developing and under-developed regions of the world live in rural communities and have limited access to conventional medical treatment. Conventionally the management of diabetes mellitus involves non-pharmacological (diet control and exercise) and pharmacological (administration of insulin and hypoglycaemic drugs) approaches. Administration of exogenous insulin and oral hypoglycaemic agents such as biguanides and sulfonylureas are the available orthodox therapies for the management of diabetes. These agents however do not revert the course of diabetic complications and often come with some toxicity (Pareek *et al.*, 2009). The recommendation of the WHO supporting the search for plants that are effective in managing diabetes mellitus (WHO, 1980) and the thinking that botanicals are largely free from the toxicity associated with orthodox pharmaceuticals have emboldened the search for phytotherapeutics with anti-diabetic potentials. One plant that has been variously studied for usefulness in managing a wide array of medical conditions is *Vernonia amygdalina* Del.

Vernonia amygdalina Del (Asteraceae) is a perennial shrub that grows to 2-5 m in height, throughout tropical Africa, and is commonly called bitter leaf. Its leaves are used traditionally in the preparation of soups and porridges. The plant reportedly finds application in the management of a myriad of ailments (Ijeh and Ejike, 2011). The hypoglycemic potentials of *V. amygdalina* had been reported as early as two decades ago (Akah and Okafor, 1992; Ogbuokiri and Ekpechi, 1989). Other researchers have used different preparations/extracts of the plant leaves, and have reported significant blood glucose lowering properties of the plant (Ijeh and Ejike, 2011). In this report, we describe our investigation of the response of rats, fed a *V. amygdalina* incorporated diet, to an oral glucose challenge.

MATERIALS AND METHODS

Processing of plant materials and composition of test diets

Fresh leaves of *Vernonia amygdalina* Del were harvested from a local farm in Owerri, Imo State, Nigeria and were botanically identified by Dr GGE Osuagwu of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The leaves were sorted to remove dead and wilted leaves and rinsed in clean tap water to remove dust and dirt. They were then air-dried at room temperature under a flowing fan until a constant dry weight was obtained. The leaves were milled to coarse fine powder, using a steel laboratory mill.

The test diet was compounded by mixing milled standard growers feed (Vital Feeds Nigeria Limited) with the dried milled *Vernonia amygdalina* leaves in ratios of 95:5 and

90:10 (feed/vegetable) to constitute the 5% VA and 10% VA diets respectively. The unmixed growers feed (100%) served as the control diet. The diets were then converted to pellets by extrusion through an improvised device made by neatly slicing the end of a 5 ml syringe.

Rats

Twenty four adult male Wistar rats (weight range 118–142 g) were obtained from the Animal Breeding Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. On arrival, the rats were acclimatized to the animal house for one week before being randomly (while controlling for weight) assigned to six plastic cages (2 cages for each group of eight rats). The rats were exposed to approximately 12 hour light/dark cycles under humid tropical conditions, and given tap water and feed *ad libitum* throughout the duration of the study.

Oral glucose tolerance test

Oral glucose tolerance test was carried out using a modification of the method described by Taiwo *et al.* (2009). Rats were fasted overnight and given an oral glucose load of 3 g/kg body weight *per os*. Following the oral glucose load, blood was obtained at 0, 15, 30, 45 and 60 minutes from the tail vein of the rat and analysed for glucose using a glucometer (Accu-check Advantage, Roche Diagnostics, Mannheim). The procedure was repeated after one, two and three weeks of feeding the test rats on the test diets. From the data generated from the OGTT, the positive incremental area under the curve (AUC) was calculated for each group using the equation:

$$AUC = \{[(t_1 - t_0) \div 2] \times (C_0 + C_1)\} + \{[(t_2 - t_1) \div 2] \times (C_1 + C_2)\} + \{[(t_3 - t_2) \div 2] \times (C_2 + C_3)\} \dots$$

(where t = time and C = concentration of glucose) (Brouns *et al.*, 2005).

The glycaemic index was calculated as:

$$GI = AUC_{\text{test}} / AUC_{\text{control}}$$

Statistical analysis

The data generated were subjected to descriptive statistical analysis, and a comparison of differences between means using One Way ANOVA. A significant threshold of $P < 0.05$ was employed for the analysis. Data analysis was carried out using SPSS version 18.0 (SPSS Inc, Chicago, IL). The results are presented as line graphs generated using Microsoft Excel (Microsoft Corp., Redmond, WA).

RESULTS AND DISCUSSION

The oral glucose tolerance test is a good and cheap measure of insulin secretion, sensitivity and glucose uptake (Rhee *et al.*, 2010; Matsuda and DeFronzo, 1999) and since an impaired glucose tolerance is an indication of a deranged glucose metabolism and a pointer to subsequent diabetes

(Eseyin *et al.*, 2010), the test is good for studying agents that improve glucose tolerance and, by extension, are useful in the management of diabetes (and diabetic complications). Its main advantage is in its ability to detect stages of pre-diabetes more accurately than other methods, and its ability to investigate postprandial glucose levels in a physiological way (Luijck *et al.*, 2011).

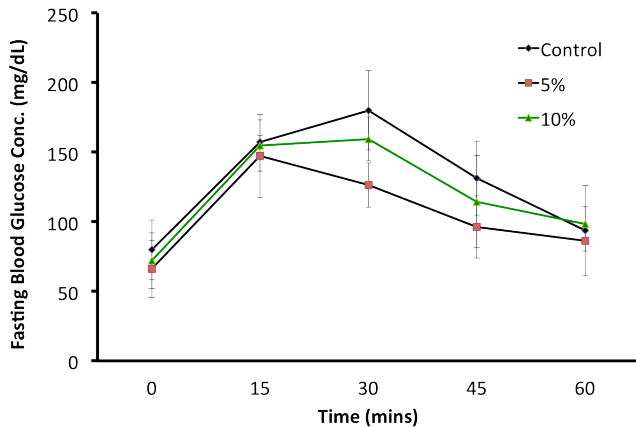


Figure 1: OGTT after one week of feeding on the 5 and 10% *Vernonia amygdalina* incorporated diets.

The results (Figures 1-3) revealed that after the first week of feeding on the test diets blood glucose concentration (BGC) peaked at 15 minutes in the group fed 5% VA incorporated diet while the group fed the control diet and the 10% VA diet had peak BGC at 30 minutes, following an oral glucose load. Blood glucose concentration however peaked at 15 minutes in the 5% VA diet and 10% VA diet groups after 3 weeks of feeding on the vegetable diet but the BGC peak remained at 30 minutes in the control group throughout the study. Also after two weeks of feeding on the test diet, BGC was significantly ($P < 0.05$) lower in the group fed 10% VA incorporated diet relative to the control group 30 minutes after the glucose load. After 3 weeks of feeding on the test diet there was a significant ($P < 0.05$) reduction in BGC at 30 minutes in both test groups, relative to the control group. Peak BGC was lower in the test groups throughout the period of the study indicating that VA had hypoglycaemic properties. Furthermore, by the end of the third week, both test diets considerably lowered the AUC's for the OGTT curves, giving glycaemic indices that had shrunk by more than 15% each (Table 1).

These findings show that dietary incorporation of *Vernonia amygdalina* improved oral glucose tolerance as indicated by the lower glucose concentrations at peak BGC in the test groups, the reduction in the time it took to reach the peak BGC and for the decline to begin, and the reduction in the AUC for the OGTT curves and the glycaemic indices of the test diets. There are two mechanisms that could explain the

glycaemic effects observed here. The active principles in the test diets could (1) slow down glucose transport into the blood from the lumen of the gastro-intestinal tract, or (2) improve insulin secretion and/or sensitivity, and thus glucose transport from the blood to the other tissues, and its utilization there. Though these are speculations (as further studies are required to establish the exact mechanism(s) of action of the agents), it is obvious that agents that affect glucose metabolism employ the mentioned mechanisms. While the first mechanism ensures that glucose does not get over-loaded in the blood, the second ensures that the glucose is utilized rapidly by the cell (Kalsbeek *et al.*, 2010). The finding of lower glycaemic indices in the test groups at the end of the study supports the former mechanism, but does not preclude the latter.

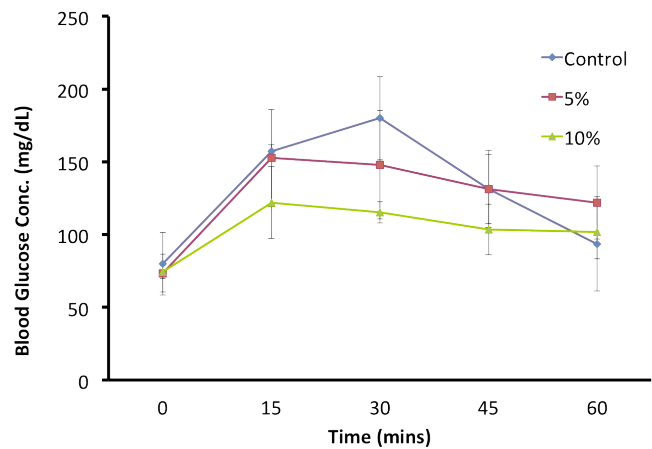


Figure 2: OGTT after two weeks of feeding on the 5 and 10% *Vernonia amygdalina* incorporated diets.

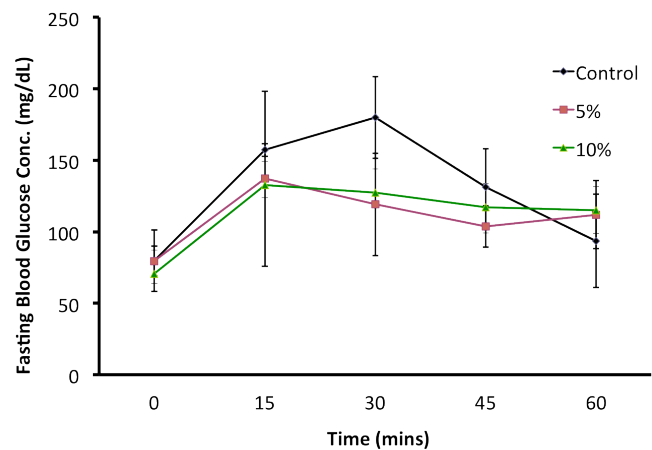


Figure 3: OGTT after three weeks of feeding on the 5 and 10% *Vernonia amygdalina* incorporated diets.

Table 1: Areas under the curve (AUC) for the effects of dietary incorporation of *Vernonia amygdalina* on the OGTT curves. VA represents *Vernonia amygdalina* incorporated diet.

	Week 1	Week 2	Week 3
Incremental areas under the OGTT curve			
Control	8329.5	8329.5	8329.5
5% VA	6690.2	7944.9	6838.3
10% VA	7709.3	6429.8	7052.3
Glycemic index (%)			
Control	1.00 (100)	1.00 (100)	1.00 (100)
5% VA	0.80 (80.3)	0.95 (95.4)	0.82 (82.1)
10% VA	0.93 (92.6)	0.77 (77.2)	0.85 (84.7)

These findings also show that the improvement in blood glucose tolerance was time dependent as seen in Figure 3 which shows that there was a decrease in peak blood glucose concentration after three weeks of feeding when compared with the peak observed after the first and second weeks of feeding the vegetable diet. This implies that the consumption of the test diet had a cumulative advantage. The improvement of glucose tolerance with time suggests that when consumed habitually, the test diet could be useful

in the prevention of diabetes; and for those already diabetic, it may be useful in regulating glucose utilization after a carbohydrate-rich meal. This is particularly useful, as the bulk of the challenge of diabetics is keeping the blood glucose levels within the physiological threshold. Pharmacologic agents used to achieve such regulation often result in hypoglycemia which could be very dangerous (Brod *et al.*, 2011). Going by the data presented here, the consumption of VA as parts of the regular diet could be useful for diabetics as it could help regulate their blood glucose levels, thereby preventing hyperglycemia and in turn reduce their dependence on pharmacologic agents.

Several reports indicate that the extracts of VA have hypoglycaemic potentials (Ijeh and Ejike, 2011). However, little has been investigated about the use of diets incorporated with the plant leaves in the management of diabetes and related metabolic disorders. Because adherence to drug/dietary regimens by diabetic subjects is often poor (Davies *et al.*, 2013; Mann *et al.*, 2009), the use of VA in diets may be very useful especially in the South Eastern parts of Nigeria where the plant leaves are used in the preparation of a variety of soups. This allows subjects take the preparations without the psychological feeling that they are taking medications, a factor that helps in ensuring compliance. Such use would maximize the utilization of all the pharmacologic principles in the plant leaves that might be important for the observed biochemical effects.

This study may be limited by its scope, as it did not investigate the mechanism of improvement in oral glucose

tolerance, and the effects of the diet on metabolic parameters downstream of impaired glucose metabolism. Those are subjects of our on-going research. However, this is to our knowledge the first report of the usefulness of diets incorporated with the leaves of VA in the improvement of oral glucose tolerance. The inherent advantages of this approach are at the core of the strength of this study.

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