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Investigation of the Effect of *Solanum incanum* on Postprandial Blood Glucose Concentration of Normoglycemic Nigerians

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Abstract: The effect of a Nigerian vegetable plant, *Solanum incanum* on postprandial blood glucose levels of normoglycemic Nigerians was investigated. Proximate analysis indicated that this vegetable contained significant amount of fiber, which is a Non-soluble Polysaccharide (NSP). The effect of the vegetable was compared with those of other indigenous vegetables also taken by diabetic patients, *Vernonia amygdalina* and *Gongronema latifolium*. A-50 g glucose was used for standard glucose tolerance test of each subject. The values were used to compare other test results. The blood glucose concentration of the subjects was determined (post absorptively) using Accu-chek active glucometer. The vegetables (50 g each) were processed according to the methods used by the patients; squeeze-wash-drink and chew-raw. They were administered to the different groups of subjects who also served as their own controls (*n* 8, *Solanum; n* 8, *Vernonia; n* 8, *Gongronema*) on separate days in randomized order. Blood glucose levels were checked at fasting (0 min) and postprandially at 30 min intervals for 2 h. Compared with other vegetables, *Solanum* elicited significant reductions ($p<0.05$) in blood glucose levels at most postprandial time points and for Area-under-curve (AUC) values. AUC reductions; *Solanum*, 18%; *Vernonia*, 15%; *Gongronema*, 13%). The bioactive antioxidant substances that occur naturally in stems, roots and leaves of African plants may possess insulin - like effect. The rich NSP content of *Solanum incanum* is also likely to be a reducing factor.

Key words: Type 2 diabetes, dietary practices, dietary fiber, nutrition education

INTRODUCTION

In recent decades, incidence of Type 2 diabetes mellitus (Non-insulin Dependent Diabetes Mellitus {NIDDM}) has increased to epidemic proportions in several traditional populations. On a global scale too, its rapidly increasing prevalence is a significant cause for concern (Lefebvre, 2005). The conventional medical approach of simply using insulin and oral drugs to control diabetes mellitus is inadequate, boring and lack compliance, thus, the patient's exposure to long term complications remains a risk. Some wild herbs and spices have been shown to be most effective, relatively non-toxic and have substantial scientific documentation to attest to their efficacy in diabetes management (Okeke, 1998). In many developing countries, larger parts of the population rely heavily on traditional medicinal plants to meet their primary health care needs and also to manage their diabetes mellitus. This is due to their perceived effectiveness, minimal side effects in clinical experience and relatively low cost. Some indigenous vegetables, namely; *Solanum incanum (anara),* Vernonia *amygdalina (onugbu)* and *Gongronema latifolium (utazi)* were identified as alternative treatments for diabetes by diabetic patients attending University of Nigeria Teaching Hospital (UNTH), Ituku Ozalla. These patients believed that the bitterness of these vegetables would neutralize high blood sugar levels. Given the preponderance, acceptability and affordability of these vegetables, Solanum incanum was targeted for investigation, to ascertain and document its therapeutic efficacy and otherwise in the treatment of NIDDM.

MATERIALS AND METHODS

Subjects and ethical approval: This study was a clinical work comprising twenty-four subjects. Twelve men and twelve women were divided into three groups of eight (8). These subjects served as their own control. Purposive sampling method was used to select only those who satisfied the pre-determined inclusion criteria which included individuals (men and women) who were normoglycemic (fasting blood sugar levels within 70-125 mg/dL or 3.9-6.9 mmol/L) after four blood glucose tests, performed at specific intervals (one week) to track glucose levels over time. The principle of randomization was highly observed in assigning the subjects to their experimental conditions; the ballot method was used. The subjects were recruited after thorough explanation of the details of the study protocol and their voluntary consent was obtained. The University of Nigeria Teaching Hospital (UNTH) Research Ethics Committee approved the study.

Preparation and processing of the vegetables: Freshly picked *Solanum* leaves were bought from a local market in Enugu and *Vernonia* and *Gongronema* plucked from a farm at the senior staff quarters at the University of Nigeria Nsukka. These vegetables were de-stalked, sorted and then washed in tap water Fifty grams each of these bitter leaves were processed according to the methods used by the diabetic patients who had been taking them for therapeutic purposes; squeeze-wash-drink and chewing raw. Squeeze-wash-drink involved squeezing the vegetables with the two hands until the juice started coming out. The vegetables were mashed completely and sieved to get out all the juice extracts. Chew raw involved eating the vegetables raw as soon as they were washed.

Proximate analysis of the vegetable extracts: *Solanum, Vernonia and Gongronema* (100 gms each) were analyzed using the standard assay methods of the Association of Official Analytical Chemists (AOAC, 1995) for moisture, ash, fat (Soxhlet), protein (Kjeldal) and carbohydrate (Englyst) at Food Technology Department, IITA Ibadan. The samples were fresh and analyses were initiated within twenty-four (24) h of procurement.

Administration of test items: On the first experimental day, using an Accu-chek Active glucometer, the Blood Glucose Concentration (BGC) of the subjects were determined through a Fasting Blood Glucose (FBG) after an overnight fast of 10-12 h and standard physical activity. A glucose tolerance test with 50 g of glucose was performed on each subject to serve as the standard with which other test results were compared. The blood glucose concentrations of the subjects were determined at 0, 30, 60, 90 and 120 min, post treatment. On the next day of experiment, the subjects' FBG were determined after an overnight fast, after which the processed vegetable samples (juice extracts) were administered to them using squeeze-wash-drink method. The BGC of all samples were determined at the same time intervals as the first experiment. This same procedure was repeated another day with another 50 g of the vegetable samples chewed raw by the subjects. There was a two-day interval between each study.

Statistical analysis: Integrated blood glucose increments were estimated by calculation of the Area under the Curve (AUC). Blood glucose values below the baseline (fasting) were treated as zero. Differences between the effects of the test items on the blood glucose incremental values were analyzed by repeated measures ANOVA with the statistical package for Social Sciences (SPSS Version 11). Post-hoc analysis (Tukey HSD) for multiple comparisons of data was carried out for parametric tests. Kruskal Wallis and Mann-Whitney tests were used for non parametric data. Data were assessed for normality with one-sample Kolmogorov-Smirnov and homogeneity of variances was tested with Levene tests. Values for the various experimental categories were compared. Significant differences were accepted at $p<0.05$.

RESULTS

One hundred grams of *Solanum* (wet weight) contained 9.4 protein, 1.8 fat, 2.5 ash, 6.5 fiber, 71.3 moisture and 2.5 carbohydrate. The results of the Fasting Blood Glucose (FBG), Glucose Tolerance Tests (GTT), Postprandial Blood Glucose (PPG) levels following the administration of the vegetables using squeeze- wash-drink method were shown in Table 1 and using chew raw method shown in Table 2. They both showed that Solanum caused reduction in blood glucose levels. Following administration at various time intervals, the peak reduction of all the vegetables occurred at 60 min, after which they stabilized at normal values using squeeze-wash (Table 3) and chew raw (Table 4) methods. Area-under-curve (AUC) values indicated that *Solanum* caused 21%; *Vernonia*, 19%; *Gongronema*, 18% reductions (Fig. 1).

To ascertain whether the rate of reduction of these vegetables were statistically significant, Kruskal Wallis test was carried out and results were presented in Table 5. Data revealed that $p = 0.000$ showing a statistical significance ($p<0.05$). This indicated that at least one of the vegetables differed from the others in the way it reduced blood sugar.

The vegetables were then paired to compare whether the differences between them in terms of reduction rate was statistically significant. For this, Mann Whitney test of multiple comparison was used and presented in Table 6. The reducing effects the vegetables had were statistically significant ($p<0.05$) using squeeze-wash-drink of administration. ANOVA testing the mean values of blood sugar reduction as a result of the three vegetables chewed raw is shown in Table 7. It tests statistical significant difference in the rate of reduction of the independent variables. The computed data showed that $p = 0.000$ ($p<0.05$) showing that the reducing effect of at least one of the vegetables differed significantly from others. Tukey HSD multiple comparison test was used ascertain the statistical significant difference in the reduction effect of the three vegetables chewed raw and is presented on Table 8. It first compared *Vernonia* with *Gongronema* and *Solanum*, compared *Gongronema* with *Vernonia* and *Solanum* and finally compared *Solanum* with *Vernonia* and *Gongronema*. It showed that the mean difference in the reducing power of the vegetables is significant at 0.05 levels. This demonstrated that the differences between the reducing effects of the three vegetables were statistically significant even with the chewing raw method.

DISCUSSION

Administration of Solanum incanum has showed that it has antihyperglycemic effect. This activity could be

Table 1: Mean Fasting Blood Glucose (FBG), Glucose Tolerance Tests (GTT), Postprandial Blood Glucose (PPG) levels of the subjects and areas under the curve with administration of the vegetables using squeeze wash method

Treatment	Number	FBG mean ±SD (mg/dl)	GTT mean ±SD (mg/dl)	PPG mean (mg/dl)	Area under curve (m^2) (%)
Vernonia A.	8	98.38±5.2	109.13±4.1	92.56±6.2	111.8 (15)
Gongronema L.	8	95.75±5.5	106.28±5.6	92.50±5.4	112.5 (13)
Solanum I.	8	96.88±4.6	106.72±4.4	87.06±3.5	106.7 (18)
Total	24	96.04±5.3	107.38±4.7	90.7083 ±5.6	110.3 (16)

Table 2: Mean FBG, GTT and PPG levels of the subjects and areas under the curve following administration of the vegetables using chew raw method

Treatment	Number	FBG mean ±SD (mg/dl)	GTT mean ±SD (mg/dl)	PPG mean ±SD (mg/dl)	Area under curve (m^2) (%)
Vernonia A.	8	98.38±5.0	108.0±3.9	87.50±5.1	106.7 (19)
Gongronema L.	8	94.50±5.6	106.75±5.4	87.66± 6.0	107.4 (18)
Solanum I.	8	97.63±5.8	106.16±5.6	84.34±2.5	104.1 (21)
Total	24	96.50±5.2	106.97±4.8	86.50±4.8	105.8 (19)

Table 3: Mean post prandial blood glucose levels following administration of vegetables using squeeze wash method over time intervals (Values in mg/dl: SD in parentheses) n = 24

Time in minutes	*Vernonia A.*	*Gongronema L*	*Solanum I.*
0	98.38 (5.01)	95.75 (5.45)	96.88 (4.61)
30	96.25 (5.15)	94.75 (5.45)	94.13 (4.16)
60	93.75 (5.11)	92.75 (5.45)	87.75 (3.88)
90	95.25 (6.94)	93.75 (4.45)	88.50 (3.90)
120	96.41 (6.87)	94.05 (6.34)	89.88 (4.02)

Table 4: Mean post prandial blood glucose levels following administration of the vegetables using chew raw method (Values in mg/dl: SD in parentheses) n = 24

Time in minutes	*Vernonia A*	*Gongronema L.*	*Solanum I.*
0	98.38 (5.01)	94.50 (5.63)	97.63 (5.82)
30	94.63 (4.75)	91.50 (5.95)	93.63 (3.93)
60	88.13 (5.22)	88.38 (6.07)	84.50 (2.78)
90	90.02 (5.22)	89.34 (6.07)	86.88 (2.78)
120	92.13 (5.22)	91.02 (6.07)	88.38 (3.04)

attributed to certain compounds of different nature present in it. Over the decades, an expanding body of evidence from epidemiological and laboratory studies have demonstrated that some edible plants as a whole or their identified ingredients with antioxidant properties have substantial protective effects on diabetes (Sabu and Kuttan, 1982) cardiovascular and renal disorders (Anderson *et al*., 2000; Miller *et al*., 1998) and several other human ailments (Scarterzzini and Speroni, 2000; Lampe, 2003) Bioactive molecules present in indigenous vegetables may possibly possess insulin-like effect or stimulate the pancreatic beta cells to produce insulin which in turn lowers the blood glucose level (Atawodi, 2005). Similar observations have been reported by other researchers (Akah and Okafor, 1992; Fuentes *et al*., 2004; Sepici *et al*., 2004). Findings also revealed that administration of *Solanum incanum* showed a significant dose dependent reduction of blood glucose levels in normal rats (Gupta *et al*., 2005).

Table 5: Kruskal Wallis test for multiple comparison of data (squeeze wash)

Treatment	Number	Mean Rank
% squeeze wash reduction		
Vernonia A.	12	11.50
Gongronema L.	12	5.50
Solanum I.	12	20.50
Total	36	
x^2	18.264	
P	0.000	

Table 6: Mann Whitney test of multiple comparison of data (squeeze wash)

% squeeze wash reduction			
Treatment	Number	Mean Rank	*Sig.
Vernonia	12	4.50	0.001
Solanum	12	12.50	
Vernonia	12	11.50	0.012
Gongronema	12	5.50	
Solanum	12	12.54	0.001
Gongronema	12	4.50	

* significant ($p<0.05$)

Table 7: ANOVA showing mean values of reduction by the vegetables using chew raw method

% chew raw reduction	Sum of squares	df	Mean square	F	Sig.
Between groups	160.665	2	80.333	61.204	0.000
Within Groups	27.563	33	1.313		
Total	188.229	35			

In the study on the chemicals detected in plants used for folk medicine in South Eastern Nigeria (Obute and Adubor, 2005) it was discovered that there are substances called flavonoids and phenolic compounds which occur naturally in stems, roots and leaves of plants. They are ubiquitous in occurrence and protect plants against external pathogens, ultraviolet light and heat. They possess anti-inflammatory properties and act as modulators of the immune system in a number of biological systems. This stems from the fact that they are powerful antioxidants protecting biosystems against damaging effects of free radicals. Most flavonoids

Table 8: Tukey HSD Post Hoc Tests for multiple comparisons Dependent variable: percentage chew-raw reduction

					95% confidence Interval	
					Lower Bound	Upper Bound
Verno	*Gong*	3.8031*	0.57283	0.000	2.3593	5.2470
	Sola	-2.4890*	0.57283	0.001	-3.9329	-1.0451
Gong	*Verno*	-3.8031*	0.57283	0.000	-5.2470	-2.3593
	Sola	-6.2921*	0.57283	0.000	-7.7360	-4.8483
Sola	*Verno*	2.4890*	0.57283	0.001	1.0451	3.9329
	Gong	6.2921*	0.57283	0.000	4.8483	7.7360

The mean difference is significant at the 0.05 level

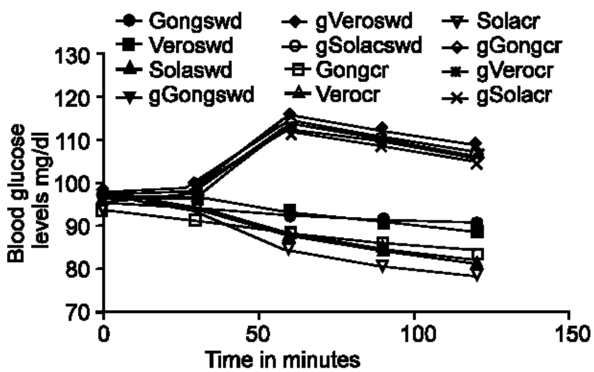

Fig. 1: Mean Glucose Tolerance Test (GTT) and postprandial blood glucose levels of the subjects in the three vegetable groups using squeeze-wash-drink and chew-raw methods of test administration

Key:

Gongswd: Gongronema squeeze-wash-drink
Veroswd: Vernonia squeeze-wash-drink
Solawsd: Solanum squeeze-wash-drink
gGongswd: Glucose tolerance test (GTT) of Gongronema squeeze-wash-drink
gVeroswd: GTT of Vernonia squeeze-wash-drink
gSolaswd: GTT of Solanum squeeze-wash-drink
Gongcr: Gongronema chew raw
Verocr: Vernonia chew raw
Solacr: Solanum chew raw
gGongcr: GTT of Gongronema chew raw
gVerocr: GTT of Vernonia chew raw
gSolacr: GTT of Solanum chew raw

belong to a group of chemicals called polyphenols and their antioxidant properties are dependent on this polyphenolic chemical structure. It is the variation in the chemical constituents in plants that will make some more medicinal than others. It is also worthy of note that no medicinal plant is functional without the active ingredients. The active ingredients identified in *Vernonia amygdalina* were Vernonioiside B and *Myricetin (flavonol); Solanum* is *Solasodine* and *Gongronema* are *Gonioanthelma* and *Gonolobus* (Manach *et al*., 2004).

It is postulated that plants accumulate antioxidant chemicals as secondary metabolites through evolution as a natural means of surviving in a hostile environment (Onyechi *et al*., 1998). These vegetables therefore act either by directly scavenging the reactive oxygen metabolites due to the presence of various antioxidant compounds, or by increasing the synthesis of antioxidant molecules (Gupta *et al*., 2005).

The nutrient composition revealed that *Solanum* was mostly moisture and fiber and contains minimal calories and so contributes less sugar to the blood sugar pool. The findings of this study are in support with those that African plants rich in non-starch polysaccharides reduce postprandial blood glucose and insulin concentrations in humans (Manach *et al*., 2004). It is also in consonance with the study which also revealed that foods rich in fiber contents induce lesser blood glucose responses (Oli *et al*., 1982). A similar finding; that is, that fiber does not raise blood glucose levels, but has a benefit of adding bulk to help make one feel full was discovered by other researcher (Henry, 2004). Other findings (Ylonen *et al*., 2003) also showed evidence that a high intake of dietary fiber is associated with enhanced insulin sensitivity and therefore may have a role in the prevention and control of Type 2 diabetes. Roughage lowers the need for insulin by slowing the absorption of carbohydrates and preventing surges in blood sugar, because it is not digested or absorbed by the body (Henry, 2004). He opined that if a poor diet can help usher in Type 2 diabetes, a healthy high fiber diet can keep it under control.

Conclusion: The findings of this research work are important contributions towards the attempt to find realistic dietary recommendations such that diabetic patients can better their blood sugar and possibly avoid the need for additional diabetes medications Compliance will help to prevent the onset of microvacular and macrovascular complications of diabetes. This is in view of the accumulated evidence that suggest that Reactive Oxygen Species (ROS) can be scavenged through chemoprevention utilizing natural antioxidant compounds present in foods and plants (Atawodi, 2005).

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