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Full Length Research Paper

Hypoglycemic indices of *Vernonia amygdalina* on postprandial blood glucose concentration of healthy humans

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This clinical study was aimed at investigating the effect of *Vernonia amygdalina* on postprandial blood glucose concentration of healthy human subjects. Proximate analysis indicated that this vegetable contained fiber, which is a non-soluble polysaccharide (NSP). The effect of the vegetable was compared with that of another indigenous vegetable also identified to be taken by diabetic patients, *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 and administered by squeeze-wash-drink and chew-raw to the subjects who also served as their own controls (*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 the other vegetable, *V.amygdalina* elicited significant reductions (P<0.05) in blood glucose levels at most postprandial time points and for area-under-curve (AUC) values: AUC reductions; *Vernonia*, 15%; *Gongronema*, 13%. The bioactive antioxidant substances which occur naturally in stems, roots, and leaves of these vegetables may possess insulin-like effect.

Key words: Diabetes dietary management, *Vernonia amygdalina*, carbohydrate metabolism, postprandial blood glucose.

INTRODUCTION

Diabetes mellitus is a metabolic disorder found in all nations of the world. It is one of the most prevalent epidemics of the 21st century (Mann, 2002). Recent global estimates indicate that the total number of patients affected by diabetes in 2004 was close to 190 million, a figure likely to have reached 325 million by 2005, that is, an increase of more than 70% (Lefebvre, 2005). World Health Organization (WHO) and African regional data estimates of 2007 showed that about 7 million people had diabetes as at the year 2000 and estimated that 18 million are expected to come down with the disease in 2030. This rapidly increasing prevalence is a significant cause for concern.

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.

Atawodi (2004) postulated that traditional medicinal

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plants have antioxidant properties. He stated that Africa is blessed with enormous biodiversity resources but plagued with several diseases including those with reactive oxygen species (ROS) as their etiological factor. Considerable evidence (Rahman and Zaman, 1989; Udosen and Ukpanab, 2003) has accumulated to implicate cellular damage arising from ROS at least in part, in the etiology and pathophysiology of human diseases such as diabetes, neurodegenerative disorders (examples include Alzeimer disease, Parkinson's disease, multiple sclerosis, Down syndrome, inflammation, viral infections, autoimmune pathologies and digestive system disorders). Free radicals are generated as part of the body's normal metabolic process and the free radical chain reactions usually produced in the mitochondrial respiratory chain; liver mixed function oxidases by bacterial leucocytes, through xanthine oxidase activity, atmospheric pollutants, drugs, and xenobiotics. In addition, chemical mobilization of fat stores under various conditions such as lactation, exercise, fever, infection, and even fasting, can result in increased radical activity and damage in particular, to the immune and nervous systems, while the stress hormones, adrenaline and nor-adrenaline, secreted by the adrenal glands under conditions of continuing and excessive emotional stress, are metabolized into simpler, albeit free radical molecules (Atawodi, 2004). 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).

Some indigenous vegetables, namely *Solanum incanum* (anara), *Vernonia amygdalina* (onugbu), *Gongronema latifolium* (utazi) and *Occimum gratissimum (nchanwu)* were identified as alternative treatments for diabetes by diabetic patients attending University of Nigeria Teaching Hospital (UNTH), Ituku Ozalla. These vegetables are locally available, affordable, acceptable and accessible and the patients believed that the bitterness of these vegetables would neutralize the "too much" sugar in their system. Giving their preponderance in this region, two of them, *V. amygdalina* (onugbu) and *G. latifolium* (utazi) were targeted for investigation to ascertain and document their therapeutic efficacy or otherwise, in treatment of non-insulin dependent diabetes mellitus (NIDDM).

MATERIALS AND METHODS

Subjects and ethical approval

This study was a clinical cross sectional work comprising sixteen subjects. Eight men and eight women were divided into two groups of eight. 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 to 125 mg/dL or 3.9 to 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 highy 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 study was approved by the University of Nigeria Teaching Hospital (UNTH) Research Ethics Committee.

Preparation and processing of the vegetables

Vernonia and *Gongronema* were 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

Vernonia and Gongronema (100 g each) were analyzed using the standard assay methods of the Association of Official Analytical Chemists (1995) for moisture, ash , fat (Soxhlet), protein (Kjeldal) and carbohydrate (Englyst) at Food Technology Department, International Institute of Tropical Agriculture (IITA) Ibadan. The samples were fresh and analyses were initiated within 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 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 $P<0.05$.

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 amygdalina	8	98.38 ± 5.2	109.13± 4.1	92.56± 6.2	111.8 (15)
Gongronema latifolium	8	95.75 ± 5.5	106.28 ± 5.6	92.50± 5.4	112.5 (13)

Table 2. Mean fasting blood glucose (FBG), glucose tolerance tests (GTT) and postprandial blood glucose (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 (mg/dl)	Area under curve (m^2) (%)
Vernonia amygdalina	8	98.38 ± 5.0	108.0 ± 3.9	87.50± 5.1	106.7 (19)
Gongronema latifolium	8	94.50± 5.6	106.75± 5.4	87.66± 6.0	107.4(18)

RESULTS

One hundred grams of *Vernonia* (wet weight) contained 14.5% protein, 2.1% fat, 1.2% ash, 6.4% fiber, 59.1% moisture and 18.5% 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 *Vernonia* 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 *Vernonia* caused 15% and *Gongronema*, 13% reduction for squeeze-wash and 19 and 18% reduction, respectively, for chew raw.

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 showed 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 comparison test was used to ascertain the statistical significant difference in the reduction effect of the two

Table 3. Mean postprandial blood glucose levels following administration of vegetables using squeeze wash method over time intervals (values in mg/dl: SD in parentheses) n=16.

Time (min)	*V. amygdalina*	*G. latifolium*
0	98.38 (5.01)	95.75 (5.45)
30	96.25 (5.15)	94.75 (5.45)
60	93.75 (5.11)	92.75 (5.45)
90	95.25 (6.94)	93.75 (4.45)
120	96.41 (6.87)	94.05 (6.34)

Table 4. Mean postprandial blood glucose levels following administration of the vegetables using chew raw method (values in mg/dl: SD in parentheses) n=16.

Time (min)	*V. amygdalina*	*G. latifolium*
0	98.38 (5.01)	94.50 (5.63)
30	94.63 (4.75)	91.50 (5.95)
60	88.13 (5.22)	88.38 (6.07)
90	90.02 (5.22)	89.34 (6.07)
120	92.13 (5.22)	91.02 (6.07)

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

Squeeze wash reduction (%)	Number	Mean rank
Vernonia amygdalina	8	11.50
Gongronema latifolium	8	5.50
Total	16	
x^2	18.264	
P	.000	

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

Squeeze wash reduction (%)	Treatment	Number	Mean rank	*Sig.
	V. amygdalina	8	11.50	.012
	G. latifolium	8	5.50	

*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	160.66	1	80.333	61.204	.000
Within Groups	5	15	1.313		
Total	27.563	16			

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

(1) treatm (j) treatm	Mean difference (I-J)	Std. Error	Sig.	95% confidence Interval	
				Lower bound	Upper bound
V. amygdalina G. latifolium	3.8031*	.57283	.000.	2.3593	5.2470

The mean difference is significant at the .05 level.

vegetables chewed raw and is presented on Table 8. It compared *Vernonia* with *Gongronema* and 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 two vegetables were statistically significant even with the chewing raw method.

DISCUSSION

The postprandial time points of administration of *V. amygdalina* have shown that it has antihyperglycemic effect. The study by Gyang et al. (2004) supports the finding that *V. amygdalina* has hypoglycemic effect in normoglycemic rats. These studies lend support to the claim by herbalists in Plateau and Nassarawa States that *V. amygdalina* may have an antidiabetic effect (Gyang et al., 2004). This activity could be attributed to certain compounds of different nature presents 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 (Scaterzzini 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 *V. amygdalina* showed a significant dose dependent reduction of blood glucose levels in normal rats (Gupta et al., 2005)

In the study on the chemicals detected in plants used for folk medicine in South Eastern Nigeria by 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, ultraviolent 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 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 *V. amygdalina* were Vernonioiside B and Myricetin (flavonol); and in *Gongronema* are Gonioanthelma and Gonolobus (Manach et al., 2004).

The nutrient composition revealed that *Vernonia* contained moisture and fiber 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 fiber does not raise blood glucose levels, but has a benefit of adding bulk to help make one feel full was reported by 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). The author opined that if a poor diet can help usher in Type 2 diabetes, a healthy high fiber diet can keep it under control.

Conclusion

This study revealed the cause and effect relationships between the two vegetables studied and postprandial blood glucose levels. The findings served as a means to unveil their constituent compositions and their relevance to Type 2 diabetes management. The findings would also update scientists in the field of nutrition and all other concerned individuals about the effect of diet, nutrients and natural products in diabetes management. Diabetic patients from this study would understand what the studied vegetables do exactly to the blood glucose and as such are encouraged to take them for therapeutic purposes

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