The Effect Of Detarium Microcarpium and Vernonia Amygdalina on Glycaemic Response Of Normal Healthy Non-Diabetic Subjects

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Abstract-- Background: Some locally available plant foods in Nigeria have received attention as dietary agents that modulate gastrointestinal functions and carbohydrate metabolism. Objective: The effect of some of these plant foods like Detarium microcarpium (legume) and Vernonia amygdalina (vegetable) on postprandial glucose response of healthy non-diabetic adults was investigated. These two studies were conducted separately to collate data on foods in Nigeria that will improve glycemic control.

Method: Detarium microcarpium bread and control bread from wheat flour were fed to ten non-diabetics. Vernonia amygdalina was processed and administered by squeeze-wash-drink (VASWD) and chew-raw (VACR) to eight non-diabetic subjects with 50g glucose tolerance test as the control. Postprandial glucose levels were determined at 30 min intervals over 2½ hours.

Results: ANOVA showed a statistically significant reduction (p<0.05) in the incremental blood glucose level of Detarium and post prandial glucose of Vernonia at most postprandial times. Area-under-curve of glucose was 62% for Detarium and 15% and 19% for Vernonia squeeze-wash-drink and chew-raw, respectively.

Conclusion: Detarium microcarpium and Vernonia amygdalina maybe useful in the prevention and management of diabetes.

Index Term-- African foods, glucose-response, diabetes mellitus.

I. INTRODUCTION

The place of diet in the treatment of diabetes mellitus has been recognized throughout the history of nutrition. The recommended diets for diabetics have been revolutionized in the past from low carbohydrate to high carbohydrate diets with varying amounts of protein and fat [1]. However, the recommendation for people with diabetes is a diet high in complex carbohydrate and low in saturated fat [2]. Diabetes is one of non-communicable chronic diseases (NCDs) that have emerged in the 21st century and is now seen as global epidemics that pose a great public health challenge in sub-Saharan Africa [3]. Globally it is estimated that a total of 190 million are afflicted with diabetes in 2004. This figure may have increased by 70% in 2005, to 325 million [4]. In 2000, 7 million people were afflicted with diabetes in African regions of the world of which Nigeria is one. It estimated that by the year 2030 this figure will double to 18 million people [5]. A more worrying fact is that 80% of people living with diabetes in Africa are undiagnosed. For every person known to have diabetes there is at least one unidentified case and death due to diabetes is expected to double over the next ten years [6].

The material resources for diabetic care are either scarce or unavailable in most African countries. Where the resources are available they are often unaffordable. Essential supplies such as insulin, syringes and needles, oral hypoglycaemic drugs and equipment for monitoring blood sugar are expensive and or scarce [7]. Dietary treatment has become even more important than before. The main thrust of dietary counseling in Nigeria has always been to urge the avoidance of refined carbohydrate and greater substitution of legumes, fruits and vegetables. Studies have shown that there are a lot of unexploited, underutilized Nigerian foods that have great potentials [8]. These authors postulated that these foods are eaten less frequently by urban dwellers in Nigeria. Traditional food system, play a significant role in maintaining the well-
being and health of indigenous people [9]. The authors showed that different varieties of legumes, nuts, seeds, wild fruits and lesser known vegetables are in abundance in Nigeria and could be of health benefit in the prevention and management of non-insulin dependent diabetes mellitus (NIDDM). Some of these foods are high in dietary fiber, high in non-starch polysaccharide (NSP), high in water soluble NSP (S-NSP), anti oxidants and have low glycemic index. These functional properties have been implicated in improved glycemic control [10, 11].

Leguminous foods such as guar gum have received attention as dietary agents that modulate gastrointestinal function as well as carbohydrate metabolism. Studies have shown that foods such as guar gum that contain S-NSP when incorporated into starchy foods and glucose drink, attenuate the postprandial rise in glucose and insulin concentration in healthy and diabetic subjects [12, 13,14]. Animal studies have shown that the postprandial effects of S-NSP depend mainly on their ability to increase the viscosity of digesta in the upper part of the gastrointestinal tract [15, 16]. The increase in intraluminal viscosity of the digesta is major factor that inhibit the rate of digestion and absorption of available carbohydrate [17, 15].

In Nigeria there are plant food preparation used traditionally as thickening agent in soups. These foods increase the viscosity of liquid foods when processed as flour. Preliminary analysis of one of these powdered plant extract show they contain significant amounts of NSP and the major fraction was S-NSP [18, 19]. One of such food is Detarium microcarpium, a legume locally known as ‘orofor’.

Some indigenous vegetables have been identified as alternative treatment for diabetes. One such vegetable is Vernonia amygdalina locally known as ‘onugbu’. This vegetable is bitter and is eaten by diabetic patients attending University of Nigeria Teaching Hospital (UNTH), Ituku Ozalla, Nigeria. These diabetics believe that this vegetable will neutralize the ‘too much sugar’ in their system. As postulated by Atawodi, traditional medicinal plants have antioxidant properties [20]. However, Africa is plagued with several diseases including those with reactive oxygen species (ROS) as their ecological factor. Udosen and Ukpanab [21] implicated cellular damage from ROS in the etiology and pathophysiology of human diseases such as diabetes. Gupta [22] showed that vegetables like vernonia 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.

Based on these documentary evidence Detarium and Vernonia were therefore investigated in two different studies to ascertain their potential as dietary supplement for improving glycaemic control in healthy adults. This was preliminary work in collating data on indigenous Nigerian foods that could be exploited for the treatment of diabetes in Nigeria.

II. MATERIALS AND METHOD

Detarium microcarpium study

Preparation of detarium flour

Detarium is a leguminous plant belonging to the subdivision Caesalpinioideae [23]. Each pod contains one seed which is rounded, oval or flattened, about 40mm in diameter [24]. The flour of detarium was processed by boiling the seeds for 45-60min until the deep brown purple seed coats peeled off easily when touched. The cotyledons were soaked in water for 60min, washed with cold water three times, the water changed each time, soaked overnight to wash away the gummy material. They were sundried for 24 hours and ground into a fine powder with a coffee grinder (Moulinex blender/mill) to pass through a 1mm screen sieve and air-dried at room temperature for 24 hours until no lumps were formed. A yellowish-white powder with a strong characteristic odour was obtained [25].

Subjects and ethical approval

Ten healthy non-diabetic male subjects from King’s College, London, participated in the study. These subjects had normal blood glucose level of 3.9-6.9mmol/L. The basal metabolic index (BMI) showed that all the subjects were within normal weight. Written information was given to each subject and consent forms were signed. The General practitioners (GP) of the participants were written to ascertain their health status with respect to the study. The protocol was approved by the King’s College research and ethical committee.

Preparation of Detarium microcarpium bread (DMB)

The quantity of the food ingredients used in the preparation of the bread is shown in Table 1. Chorleywood bread process [26] was used for the processing. A recipe consisting of brown wheat flour (Ploughman’s, Allied Mills, London, UK), salt, yeast (fresh compressed), fat (hydrogenated vegetable fat Flora Van den Bergh Foods Ltd. Crawley Sussex, UK) and 750g and 900g water/kg for the control and DMB respectively. Detarium flour was incorporated into the bread as a replacement for the wheat flour. Each bread roll contained 2.5-2.6g S-NSP (water soluble fibre). Two hours after baking, DMB rolls were frozen in self sealed freezer bags at -20$^\circ$ until required for use.

The test meal consisted of 2 small bread rolls (weight 164g), 38g apricot jam (Robertsons UK) and water to make to make meal weight of 400g. The weight of the dough was calculated such that a total of 50g CHO was contained in the two bread rolls. Each DMB roll contained 2.5g S-NSP. The total available carbohydrate (CHO) of the meal was 70g, the DMB rolls supplied 46g available CHO and 5g S-NSP.
Food ingredients used in the preparation of Control bread (CB) and Detarium microcarpum bread (DMB) rolls.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>CB</th>
<th>DMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown flour</td>
<td>1000</td>
<td>850</td>
</tr>
<tr>
<td>Salt</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Detarium flour</td>
<td>0</td>
<td>150*</td>
</tr>
<tr>
<td>Fat (hydrogenated)</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Improver</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fresh yeast</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Water</td>
<td>675</td>
<td>900</td>
</tr>
</tbody>
</table>

* Equivalent to 63g soluble fiber (Onyechi, 1995)

Analysis of the DMB rolls

The proximate analysis of the DMB meal was calculated using standard assay methods of the [27] for moisture, fat (Soxhlet), protein (Kjeldal) fibre, available carbohydrate, total energy (kcal/100g).

Feeding of the subjects

The subjects were asked to fast over night for 2 hours and came to the metabolic kitchen of the Department of Nutrition and Dietetics, King’s College London on two separate times to consume one control bread meal and DTM meal. In order to control any carry over effect, the test days were separated by one week. The subjects were supervised to ensure that the meals were consumed within 15 mins.

Blood sampling and glucose assay

Fasting venous blood sample of 10ml volume was taken from each subject on the test day and collected in EDTA extainers. A further five 10ml blood samples were taken from the subjects postprandially at 30mins intervals for 2.5 hours after the consumption of the meal. Six samples of 60ml blood were centrifuged and the plasma decanted and stored at -20°C until required for analysis.

Plasma glucose was analysed by standard glucose oxidase method [28] using the Boehringer Mannheim kit method (GOD-Perid method; Boehringer Mannheim, Lewes, East Sussex, UK) after deproteinsation. The frozen deproteinized plasma was allowed to thaw and mixed in a rotamixer for 2 minutes. A 100 ul of the supernatant was mixed with 5 ml of the reagent which contains buffer, enzymes and chromogen. The sample was mixed in a rotamixer and incubated in a water bath at 20-25°C for 40 minutes avoiding direct exposure to sunlight. The absorbance of the sample and the standard were measured against a blank in a spectrophotometer at 610 nm.

The Glycemic index (GI)

Areas under the curve for postprandial plasma glucose concentration (0-150min) in healthy human who consumed DMB and CB meals were calculated.

Statistical analysis

The blood glucose increments (changes relative to fasting values) were determined at 30, 60, 90,120 and 150mins. Integrated glucose was estimated by calculation of area under the curve (AUC; trapezoid rule). Glucose values below the base line were treated as zero. The difference between the effects of the DMB meal on the blood glucose was analyzed by repeated measure of analysis of variance, ANOVA, SAS Statistical package [29]. Significance difference was accepted at p<0.05.

Vernonia amygdalina study

Preparation and processing of Vernonia amygdalina extract

Vernonia amygdalina belongs to the family Compositae and it is locally known as “onugbu” and called bitter leaf. It is green leafy vegetable that contains bitter pigment necessitating squeezing and washing before cooking and consumption. It is cherished in Nigeria for the distinctive flavor.

Vernonia vegetable was plucked from farm at the senior quarters of University of Nigeria Nsukka. The vegetable were de-stalked, sorted and then washed in tap water. Fifty grams of Vernonia was processed using squeeze-wash-drink (VASWD) method used by the diabetic patients. This involved squeezing the vegetables with two hands until the juice is produced, the vegetables was mashed and sieved to extract all the juice. The subjects also used the chew raw (VACR) method, that is chewing the vegetables in their raw state after washing with clean cold water.

Subjects

Eight non-diabetic subjects were purposively selected. The subjects had normal blood glucose level and were within normal BMI. The details of the study were explained and consent was obtained. The study was approved by University of Nigeria Teaching Hospital (UNTH) ethical committee.

Proximate analysis of vernonia extracts

Vernonia (100g) was analyzed using the standard assay methods of the Association of Official Analytical chemist [23] for moisture, ash, fat (Soxhlet) protein (Kjeldal) and carbohydrate (Englyst) at Food Technology Department, International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. The sample was fresh and analyses were initiated within 24h of procurement.

Administration of test meal

Three types of meal treatment were given. On the day of the experiments, the subjects came in after an overnight fast (10-12h). Using an Accu-chek Active glucometer, the fasting blood glucose (FBG) was taken. A glucose tolerance test with 50g of glucose was performed on each subject to serve as a control for the test meal. The blood glucose concentrations of the subjects were determined at 30, 60, 90, and 120min postprandially. The test meal was a 20ml of processed vegetable juice extract (VASWD) was served to the subjects. Postprandial blood samples were taken at the same time interval. The last of the experiment was 50g of the vegetable sample was chewed raw (VACR) by the subjects. The same experimental procedure was carried out. There was one week interval between the experimental days.
Statistical analysis
Difference between the effects of the test meals on the blood glucose incremental values were analyzed by repeated measures of analysis ANOVA (SPSS Version 11). The effect of the test meals on the postprandial blood glucose level was compared. Significant difference was accepted at p<0.05.

III. RESULTS

Detarium microcarpium study
The nutrient composition of the raw dough is shown in Table 2. The result indicated that Detarium microcarpium raw bread dough had more moisture (46g) and fat (0.8g), while the CB dough had more protein (7.8g) and carbohydrate (68.6g). The weight and nutrient composition of the bread meals, baked CB rolls and baked DMB rolls is shown in Table 3.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Control (CB)</th>
<th>Detarium microcarpium bread (DMB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>39.1</td>
<td>46.1</td>
</tr>
<tr>
<td>Protein</td>
<td>7.8</td>
<td>6.8</td>
</tr>
<tr>
<td>Fat</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Fibre</td>
<td>4.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Available CHO</td>
<td>68.6</td>
<td>60.9</td>
</tr>
<tr>
<td>Total energy</td>
<td>309.6</td>
<td>276.3</td>
</tr>
</tbody>
</table>

Weight of the raw dough used to provide 50g carbohydrate was 160g.

Effect of the DMB on the blood glucose levels of the subjects

The result showed that fasting blood glucose levels were found to be within the normal range for non-diabetic subjects. The pooled mean of the fasting blood glucose level for the subjects was 4.30mmol/L. The post-prandial rise in blood glucose levels was expressed as incremental blood glucose levels which were calculated relative to the fasting values are shown in Table 4. Analysis of the data using ANOVA showed a significant bread meal effect (Wilks’ Lambda 11.1; df 6 and 18; (p=0.0049) and a significant time effect at (p=0.0129). Comparison of the mean incremental blood glucose rise after the consumption of the two bread meals showed that DMB meal showed a significant difference when compared to the CB meals at (p=0.0008). When the difference between the bread meals was analyzed at each time interval, the result showed that there was a significant difference for the DMB meal at 90, 120 and 150 minutes when compared with the control bread. The area under the curve for glucose (Table 5) was significantly reduced (p<0.0005) after the DMB meal compared to the CB meal.

Vernonia amygdalina study
The proximate composition of Vernonia 100g wet weight is shown in Table 6. The fiber content of Vernonia leaf was high 6.4% per 100g. The protein also was high 14.5%.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>CBM</th>
<th>DMB</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>1.30 ±0.18</td>
<td>0.64 ±0.11</td>
</tr>
<tr>
<td>60</td>
<td>1.62 ±0.61</td>
<td>0.94 ±0.12</td>
</tr>
<tr>
<td>90</td>
<td>1.61 ±0.15</td>
<td>0.58 ±0.08</td>
</tr>
<tr>
<td>120</td>
<td>0.31 ±0.25</td>
<td>0.13 ±0.07</td>
</tr>
<tr>
<td>150</td>
<td>0.94 ±0.16</td>
<td>0.12 ±0.06</td>
</tr>
</tbody>
</table>

Values in rows with the same superscript are significantly different

Effect of the DMB on the blood glucose levels of the subjects
The physico-chemical property of *detarium* xyloglucan and the result of other S-NSP studies indicated that the xyloglucan fraction was probably a major factor in determining the biological active nature of *detarium* flour. Thus viscosity effect [33] reduction in insulin response by viscous NSP [34, 35, 36, 37, 38] slow absorption [39], gastric emptying/small intestine absorption [36, 40] all contributed to *detarium* bread meal significant lowering of blood glucose compared to the CB meal.

The anti nutrient factor such as tannin [41], lectin [42] and phytic acid [43] may not be excluded. It was possible that these anti nutrients may have contributed to the biological active role of *detarium* in reducing postprandial blood glucose. However DMB rolls were subjected to heat treatment during processing. Therefore, the effect of these anti nutrients may have been inactive [44].

*Detarium microcarpium* a previously uncharacterized plant food indigenous to Nigeria could have potential as dietary adjunct in improving glycaemic response and may provide a variety to the diet of the diabetics that reside in the urban areas.

### IV. DISCUSSION

**Detarium study**

The result of this study showed that when healthy subjects were fed bread meals containing 75g CHO and 6g S-NSP from DM flour, there was a significant main meal effect (p=0.0049) compared to the low fibre CB. When the incremental blood glucose level of DMB was compared to the CB, a significant lowering effect was found after the consumption of DMB (p=0.0008) at 90,120 and 150 minutes post-prandially. The area-under-the-curve of DMB showed a significant reduction (62%). This was lower than the glycomic indices of guar containing foods in studies where subjects consumed S-NSP at doses higher than those used in the current study [30]. It seemed likely that the S-NSP fraction of *detarium* seed [25] was in part responsible for the physiological effect of DMB meal.

Chemical analysis had shown that the S-NSP fraction which was about 600g/kg dry weight of *detarium* flour consist mainly of xyloglucan. Analysis of *detarium* xyloglucan showed it has an intrinsic viscosity of 8.9dl/g [31]. This was indicative that *detarium* is a high molecular weight polymer which explained why it generated high viscosity in aqueous solution. An important determinant of the biological activity of S-NSP is their ability to generate viscosity in the lumen of the stomach and small intestine. It is now considered to be of great importance in reducing the rate of digestion and absorption of available carbohydrate [15]. An increase in the viscosity of stomach content could impair gastric function and acted as a physical barrier to amylase-starch interaction in the lumen of the small intestine [32].

The anti nutrient factor such as tannin [41], lectin [42] and phytic acid [43] may not be excluded. It was possible that these anti nutrients may have contributed to the biological active role of *detarium* in reducing postprandial blood glucose. However DMB rolls were subjected to heat treatment during processing. Therefore, the effect of these anti nutrients may have been inactive [44].

*Detarium microcarpium* a previously uncharacterized plant food indigenous to Nigeria could have potential as dietary adjunct in improving glycaemic response and may provide a variety to the diet of the diabetics that reside in the urban areas.

### Vernononia study

*Vernonia* extract showed a reduction at all time intervals but the peak reduction was at 60 and 90min. Area-under-curve (AUC) values indicated that *Vernonia* caused 15% and 19% with squeeze-wash and chew-raw method respectively, *Vernonia* extracts showed significant reduction (p<0.05) at 30,60, and 90mins post-prandially.

The post-prandial time points of the administration of *Vernonia* extract have shown that it had hypoglycemic effect. Gyang *et al.*, [45] showed in their studies that *Vernonia* had hypoglycemic effect in normoglycemic rats. This study supported the claim by herbalist in Plateau and Nassarawa States, Nigeria (oral interview), that *Vernonia* may have antidiabetic effect. The possible reason by which *Vernonia* exhibited hypoglycemic effect could be due to certain compounds it contained. Edible plants with identified antioxidant properties have protective effects on diabetics [46]. Studies have also shown that *Vernonia* showed a significant dose dependent blood glucose reduction in rats [47]. Obute and Adubor [48] in their study of the chemicals in plant showed that stems, roots and leaves of plants contain flavonoids and phenolic compounds. These are antioxidants that protect biosystems against damaging effects of free radicals and they are medicinal. The medicinal property of *Vernonia* may be attributed to the active ingredients *Vernonoinoiside B* and *Myricetin* (flavonol) which it contained [49].

The nutrient composition showed that *Vernonia* contained fiber. Studies have shown that indigenous African plant foods that are rich in non-starch polysaccharides (NSP) have the potential to modulate postprandial blood glucose and insulin concentrations in humans [8, 49, 50]. Fiber rich meal produced a marked flattening of the postprandial glycaemia. Other studies in the literature have observed similar effects with high NSP meals [35, 37, 38].
V. CONCLUSION
The result of the study showed a significant lowering effect on the post-prandial glucose levels of healthy non-diabetic subjects fed DMB meal and VA extract. The findings indicated that both Detarium and Vernonia contained fiber that has been shown to reduce postprandial blood glucose levels. Vernonia also contained active ingredients Vernonioidside B and Myricetin which are flavonol, that are antioxidants and medicinal. These have been linked to the prevention and treatment of non-communicable chronic diseases (NCDs) [49]. On the basis of this result it can be concluded that Detarium microcarpum and Vernonia amygdalina could be useful dietary supplement in the prevention and management non-insulin dependent diabetes.
Other similar plant foods should be investigated for their anti-diabetic properties for prevention and management of diabetes in Nigeria. These plants foods are cheap, locally available and consumed in Nigeria. The information collated will provide a data base on potential indigenous plant foods that could be used in the prevention and management of diabetes in Nigeria.

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