Cissus rotundifolia Soup Meal - It's Physiological Effect on the Postprandial Plasma Blood Glucose and Insulin Levels of Healthy Non Diabetic Subjects

Onyechi, U. A.
Department of Home Science Nutrition and Dietetics, University of Nigeria, Nsukka, Nigeria.
Email: uche.onyechi@yahoo.com **Phone:** +23408066794814

Abstract

The effect of Cissus soup meal, on postprandial plasma glucose and insulin levels of normal healthy individuals was investigated. The control meal was a traditional Nigerian soup meal containing meat, fish and vegetables as it is prepared in the South Eastern Nigeria. The test meal was the control meal with added Cissus flour. Chemical analysis showed that Cissus flour contains 28.3g/100g of soluble non-starch polysaccharide (Onyechi, 1995). 35g of Cissus flour was added to the control meal, to provide 10g fibre and served with rice to give a total carbohydrate content of 50 g. The meals were fed to healthy subjects after an overnight fast. The plasma glucose and insulin levels were measured at base line and at 30 minutes intervals for 150 minutes. The post-prandial rise in blood glucose levels were expressed as mean incremental blood glucose calculated relative to the fasting values. The difference between the effect of control soup meal and the Cissus soup meal on the postprandial blood glucose and insulin were analyzed by repeated measures analysis of variance, (ANOVA). The result showed a significant difference (P<0.05) on the postprandial glucose decrease of the test meal at all time intervals, compared to the control meal effect. There was apparent reduction in the postprandial insulin levels of the subjects fed the test meal compared to that of the subjects fed the control soup meal but there was no significant difference at P<0.05.

Keywords: Dietary fibre, *Cissus rotundifolia,* Postprandial glucose, Insulin levels, Healthy subjects

Introduction

Cissus rotundofolia is the starchy stem of a shrub; it is viscous and contains soluble non starch polysaccharides (s-NSP). Studies have shown that s-NSP such as guar gum when added to a carbohydrate meal elicited a reduction in the postprandial rise in glucose and insulin concentration in healthy and diabetic subjects (Jenkins et al. 1977, 1978, 1981; Ellis et al. 1988). The physiological effects of these polysaccharides were as a result of delay in absorption after a meal (Blackburn et al. 1984). The effect requires the presence of the viscous non-starch polysaccharide (NSP) source in the meal or ingested immediately before a meal, suggesting a direct influence on glucose availability (Vahouny, 1982). The other possible mechanism includes delayed gastric emptying due to reduced intraluminal diffusion of glucose to the absorptive surfaces in the small intestine. The more viscous the NSP source the greater the effectiveness in delaying absorption (Edward and Read, 1990). Studies have also shown that the glycaemic and insulin response of carbohydrate foods in healthy subjects was related to differences in the digestibility of the different starches (Crapo et al., 1977; Coulston et al., 1980). Other authors also indicated that amylase/amylopectin ratio is contributory to low glycaemic index (GI) in starchy foods (Goddard et al., 1984).

Nutrient analysis of cissus flour showed that it is high in starch and contains s-NSP (Onyechi et al 2007). Preliminary animal studies using cissus showed some positive therapeutic potential effect, as it reduced the plasma cholesterol level of rats (Onyechi, 2007). The result of this study suggested that further study be carried out to investigate the therapeutic effect of cissus on humans.

Cissus is locally known as 'ukoho'. The young shoots of Cissus are used as vegetables. The stem is sold as a food condiment in local markets in the Eastern and Northern parts of Nigeria. This study examined the effect of soup meals containing cissus flour on the post-prandial rise in plasma glucose and insulin levels of non-diabetic subjects. The processing method, nutrient composition, chemical and physical characteristic of cissus flour was also evaluated.

Materials and Method

Preparation and processing of plant food extracts: *Cissus rotundifolia* is a climbing or prostrate herb with glossy green, widely ovate to round blunt toothed margins. Cissus is found through out East Africa, Zimbabwe, Mozambique, South Africa, parts of central Africa (FAO, 1988). The young shoots of Cissus are used as vegetables. The minor economic importance lies in local medicinal uses (FAO, 1988). The stem is sold as food condiments in local markets in the Eastern and Northern parts of Nigeria. The stem was harvested fresh and wet, sun dried and transported to the UK for processing into flour.

The processing method involved scraping the bark of the stem. The stem was cut into small pieces, sun dried for one week until the pieces looked like toasted bread. The dried stem was crushed into smaller pieces and then ground in a coffee grinder into fine powder. The powered cissus is a yellowish brown powder (Onyechi, 1995). A 10kg quantity of the fine powder was processed which was stored in freezer bags at a temperature of -20^{o}C until required for use.

Chemical and physical methods of analysis of the plant food extract: The test food was analysed using standard methods (Kirk and Sawyer, 1991) for moisture (104^{o}C for 16h); ash (Total minerals; 525^{o}C for 12h); fat (Soxhlet; light petroleum-diethyl ether extraction) and protein (micro-Kjeldahl method; N x 5.7). The starch content of the flour was determined by an enzymic method (Englyst et al., 1992a). The Englyst method (Englyst et al., 1992b) was used to determine total NSP and the water-insoluble fraction of the NSP; the water-soluble fraction of the NSP was determined as the difference.

Subjects: The biodata of five healthy non-diabetic subjects who participated in the study is shown in Table 1. Detailed written information was given to each subject after a discussion on the aims of the study and consent forms were signed. The subjects doctors were written to ascertain whether there was any reason the subjects should not participate in the study. The protocol for the study was approved by the research and ethical committee of King's College, University of London.

Table 1: Biodata of the subjects that participated in the study

Subjects (No)	Sex	Age (yrs)	Height (m)	Weight (kg) Before study	Weight (kg) After study	Body mass index
1	M	38	1.80	91	91	28
2	F	27	1.64	57	56	21
3	M	24	1.27	67	67	22
4	F	33	1.71	96	95	33
5	F	32	1.54	54	52	23

Note: *Body mass index = wt/ht^{2}, Yrs=years, M=meters, Kg=kilograms*

Preparation of experimental diets: The control meal was a traditional soup as prepared by the Ibos. The food ingredients were spinach, palm oil, red pepper, dried ground prawns, onions, fresh tomatoes, chicken breast (without skin) smoked codfish, oxo cubes and salt. The chicken and the smoked fish were cut into small piece and boiled for 10 minutes with salt and some of the onions. The tomatoes, the rest of the onions and the red peppers were homogenized in a Moulinex blender and added to the boiling chicken. The ground prawns, palm oil and beef extract were added and continued boiling for another 10 minutes. The spinach was sliced thinly and added to the soup and boiled for few minutes. The soup was prepared in batches sufficient for each of the two meals for each individual and frozen at -20^{o}C until required. The test diet was essentially similar in content to the control meal; the only difference was the addition of the cissus flour. Cissus was calculated to provide 10g of total NSP which was 35g of cissus flour. The cissus flour was added to the defrosted soup and mixed well before heating in the microwave oven.

The soup was served with long grain Tilda rice (Tilda Rainham, Essex, UK). About 200g of the rice was prepared each day by adding 600g of cold water and boiled for 10 minutes. The amount of rice in each meal was adjusted so that the available carbohydrate consumed by each subject was 50g. Subjects were served 600g of water with the meal. The nutrient composition of the soup meal (Table 2) was calculated using McCance and Widdowson's composition of foods (1988) and data from the analysis of cissus flour (Onyechi, 1995).

Table 2: Composition of the control soup meal and test meal containing Cissus flour fed to healthy non-diabetic subjects (Ingredients in g/portion)

Ingredients	Control soup meal	Cissus soup meal
Chicken without breast	100	100
Smoked fish	50	50
Ground dried shrimps	5	5
Red palm oil	15	15
Salt	7	7
Onions	40	40
Fresh tomatoes	50	50
Spinach	40	40
Oxo cubes	6	6
Water	400	400
Rice, tilda	100	60
Cissus flour	-	35

Note: *The amount of rice in the test meals was calculated based on the available CHO content of Cissus to obtain a 50g CHO meal. 35g of Cissus flour contains 20.0g available CHO, 60g of rice flour contains 30.00g available CHO, A total of 50 g available CHO*

Feeding of the subjects: The subjects attended once a week for two weeks the metabolic kitchen of the Department of Nutrition and Dietetics, King's College London, having fasted overnight. One soup meal was consumed on each visit. In order to minimize any carry-over effect, the test days were separated by one week. The served meal consisted of vegetable soup and boiled rice which was adjusted to allow for the carbohydrate content of cissus and fed with sufficient water. The subjects were asked to consume the meals within 15minutes on each visit.

Blood sampling: On arrival subjects were allowed to rest for 10 minutes to enable the blood volume to stabilize and the veins to warm up. It was ascertained that the subjects had nothing to eat for the last 12 hours prior to the blood sampling. Fasting venous blood samples (10ml) was taken from the subjects using EDTA exetainer. Then 10 ml blood samples were taken at 30 minutes interval for 21/2 hours. The first postprandial blood sample was taken 30 minutes after commencing the meal. Six samples totalling 60mls of blood were collected from each subject. All blood sample was placed in wet ice and deproteinized by addition of 1 ml uranyl acetate (URAC), deproteinizing solution. The samples were centrifuged at 4^{o}C, the plasma was separated from the cell and stored at -20^{o}C for further analysis.

Glucose analysis: The plasma glucose was measured by standard glucose oxidase method (Werner et al., 1970) using a Boehringer Mannhein kit (Boehringer Mannheim House, Bell Lane, Lewes BN7 1LG). 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 bath at 20-25^{o}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.

Insulin analysis: The Boehringer Mannhein diagnostic based on enzyme immunological reactions was used for the quantitative determination of human insulin in-vitro. The ES 22 combi step analyzer program B auto machine was used. Precipath IM was the quality control serum used to run each analysis and values were within the stipulated range. Five standards were used which ran in duplicates along with duplicate samples of the control serum and test samples. The machine automatically dispensed and washed out the tubes with reaction solutions. The tubes were automatically read after the incubation period by passing along a conveyer into the spectrophotometer, where the solution is aspirated out and absorbance plotted. A computer program was used to read the blank, standard, control serum and the test sample for each run. The absorbance readings were calculated in the calculation mode and the standard curve plotted. The concentration of plasma insulin in the test sample was calculated from the standard curve.

Statistical analysis: The glucose and insulin increments (changes relative to fasting values) were determined at 30, 60, 90, 120, and 150 minutes. The difference between the effects of the control and cissus soup meals on the blood glucose and insulin were analysed by repeated measure of analysis of variance, ANOVA, SAS Statistical package, (SAS Institute Inc., 1985). Significance difference between the control and the cissus soup meals were accepted at $p<0.05$.

Results and Discussion

Table 3 shows the nutrient composition of Cissus flour. Table 4 shows the nutrient composition of the soup meals.

The effect of the soup meal on postprandial glucose level: The fasting blood glucose levels of the subjects were within the normal range for non-diabetic subjects. The post-prandial rise in blood glucose levels are expressed as mean incremental blood glucose calculated relative to the fasting values as shown in Table 5. Repeated measures analysis of variance of the incremental plasma glucose levels showed that there was a significant meal effect. The postprandial plasma glucose level was significantly lower after the consumption of Cissus soup meal compared to the control at all postprandial intervals.

Effect of the soup meal on the plasma insulin levels: The fasting plasma insulin levels of subjects were within the normal range for non-diabetic subjects. The mean incremental plasma insulin levels of the subjects are shown in Table 6. ANOVA showed that there was apparent reduction on the incremental insulin levels after the consumption of Cissus soup meal compared to the control. However the result was not significant.

Table 3: Nutrient Constituents of Cissus /100g

Parameter (g/100g)	Cissus
Moisture	5.9
Fat	0.9
Protein	4.9
Ash	3.4
Total CHO	84.7
Available CHO	56.4
Dietary fibre (by difference)	28.3
Total NSP	**26.3**
Soluble NSP	**15.5**
Kcal (by bombing)	366.0
Available energy	257.0
Starch	45.3
Viscosity (cps)	2000-20,000

Onyechi (1995)

Table 4: Nutrient composition (g/100g) of Control soup and Cissus soup meal portion by calculation

Nutrients	Control soup meal	Cissus soup meal
Protein (g)	14.0	14.4
Fat	7.0	7.1
Carbohydrate (g)	52.0	52.0
Dietary fibre (g)	0.5	0.5
Added total fibre by food Analysis (g)	-	10.0
Added NSP by Englyst Analysis (g)	-	8.0

Note *carbohydrate value is calculated from the following, Control soup +100g boiled rice = 52g CHO, Control soup + Cissus (35g) +60g boiled rice = 52g CHO*

Table 5: Mean incremental plasma glucose levels (mmol/L) subjects fed control soup and test meal containing cissus flour

Time (min)	Control soup meal	Cissus soup meal
30	2.65 ±0.16[a]	1.10 ±0.21[a]
60	3.13 ±0.25[a]	1.36 ±0.23[a]
90	2.92 ±0.29[a]	1.59 ±0.22[a]
120	2.46 ±0.28[a]	1.01 ±0.15[a]
150	1.65 ±0.22[a]	0.60 ±0.17[a]

Values in rows with same superscript are significantly different from each other at p<0.05.

Table 6: Mean incremental plasma insulin levels (mU/L) of subjects fed control soup meal and test meal containing cissus flour

Time (min)	Control soup meal	Cissus soup meal
30	37.30 ±6.42	27.65 ±7.24
60	26.65 ±5.37	20.91 ±4.67
90	19.76 ±4.46	14.31 ±2.92
120	14.12 ± 3.22	11.63 ±2.95
150	10.18 ±3.42	8.56 ±2.42

Post-prandial plasma glucose and insulin levels: Area under the curve (AUC) of the subjects fed the control soup meal and Cissus soup meal is shown in Table 7.

The results showed that the AUC for glucose was significantly reduced (P<0.0005) after the consumption of ciccus soup meal compared to the control meal. The AUC for insulin was significantly lowered after the cissus meal compared to the control (P<0.0005).

Table 7: Postprandial plasma glucose and insulin concentration (0-150mins) of healthy human subjects consuming control soup meal and Cissus soup meal

Soup meal	Glucose (mmol/L. min)	Insulin (Mu/L. min)
Control soup meal	360 ± 29	3135 ± 423
Cissus soup meal	162 ± 24[8*]	2363 ± 548

** Mean value of Cissus soup meal was significantly different from that of the control P<0.001.*

The result of this study showed that when normal healthy subjects were fed cissus soup meal it resulted in significant decrease in postprandial glucose levels at p<0.05 compared to the control soup meal. The result also showed reduction in the insulin levels though it was not significant.

Analysis of the nutrient content of cissus flour showed that it is high in carbohydrate which is mostly starch (45.3g/100g) (Onyechi *et al.*, 2007). Studies have shown that the glycaemic and insulin response of carbohydrate foods in normal and glucose tolerant volunteers was not only related to the fibre content of the foods. These authors showed that the effect was also due to the digestibility of the different starches (Crapo *et al.* 1977; Coulston et al. 1980). Goddard *et al.* (1984) showed in their study that amylose/amylopectin ratio is a contributory factor to low glycemic index. Foods that are known to have high levels of amylose in their starch granules are more slowly digested compared to carbohydrate foods that contain less amylose and more amylopectin (Wolfrom and Khaden, 1965; Schoch and Maywald, 1976). Starches high in amylopectin content are digested more quickly than those high in amylose content (Borchers, 1961; Sandstedt, 1962). This is due to the fact that amylopectin has a larger molecule than amylose and has a much larger surface area per molecule than amylose. Furthermore, amylose units are more bound to each other by hydrogen bonds making them less available for amylolitic attack than amylopectin. Therefore the difference in rate of digestibility between amylose and amylopectin starches may be due to the larger surface area of amylopectin (Foster, 1965; Wolfrom and Khaden, 1965; Schoch and Maywald, 1976). However, in the current study no investigation was carried on the structure of the starch in cissus. It is therefore not possible to ascertain the amylose/amylopectin ratio. It is possible that the starch is in a form which is poorly digested. In addition there was no heat application in the processing of cissus flour (Onyechi et al. 2007). Though the 10 minutes warming in the microwave oven of the soup meal may have resulted in some gelatinisation of the starch, incomplete gelatinisation may also have rendered it less digestible.

Cissus is not a legume but the starchy stem of a shrub and little has been done to examine such materials. A material which may have some similarity is konjac mannan, which is prepared from fresh roots and has shown similar results as other viscous polysaccharides (Ebihara *et al.* 1981). These authors fed young men 80g glucose solution with or without 5g konjac added. The result showed that the addition of konjac mannan reduced plasma glucose and insulin concentration during 30-120minutes time interval. The authors indicated that the beneficial effects of konjac mannan which is a viscous polysaccharide are as a result of delayed stomach emptying, modified response in gastrointestinal hormones and delayed glucose diffusion. Cissus has some similarities with konjac mannan in that although one is described as a root and the other a stem, they are both "woody" parts of plants which contain viscous and soluble NSPs although not as storage NSP as in legumes.

The result obtained from this study showed a trend towards a reduction in postprandial insulin levels compared to the control soup meal though the result was not significant. This result is in contrast to the result by Jenkins et al. (1977) which showed a significant reduction in the postprandial plasma insulin levels with the addition of 5 or 10g guar gum to soup meals containing 75g carbohydrate. The reduction of postprandial plasma insulin levels by incorporation of soluble NSP into meals may be due to slower glucose absorption. Another possibility may be that the release of gastrointestinal hormones which stimulate insulin release might be modified by NSP such as guar gum or pectin in the gut content (Morgan *et al.,* 1979; Jenkins *et al.,* 1980). These studies showed a modification of gastric inhibitory polypeptides (GIP) release by these two soluble NSPs. Carbohydrate and fat are the most important stimuli of GIP release which occurs from the proximal and mid–small gut. Maybe slowing of gastric emptying and the slower transit through the GI increase the thickness of the unstirred layer which operates to decrease the stimulus for GIP secretion. The reduction in the GIP level may partly account for the lower postprandial serum insulin levels occurring after meals containing guar gum and pectin. However, this is possibly more likely to be a mechanism on long term feeding. Data obtained from this study did show a trend towards a reduction in postprandial insulin levels compared to the control meal even though the result was not significant. The reason could be due to the small sample size of 5 subjects that participated in the study. The variability in age and sex of the subjects could also be another reason.

Conclusion: The result of this study showed that cissus may contains some biological active ingredients that can have positive effect in modulating blood glucose and insulin levels in diabetic subjects. The non-significant effect of cissus on the plasma insulin levels was contradictory to some of the results in the literature. This may be due to non heat application in the processing method of the cissus flour.

A more controlled study was planned where there was heat application with food that is more widely accepted in the urban areas.

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