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DIETARY FIBRE POTENTIALS OF SOME SELECTED FLOURS AND THEIR EFFECT ON BLOOD GLUCOSE AND SERUM CHOLESTEROL REDUCTION

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ABSTRACT: *Three different flours were produced from unripe Plantain fruit, Okara (residue from soy milk production) and Detarium microcarpum and analysed for dietary fibre. The flours were incorporated in a standard diet which was fed albino rat for bioassay study. The flours were found to be very rich in dietary fibre with Detarium microcarpum showing the highest ($P < 0.05$) level of dietary fibre (78.6%) followed by Okara (24.4%) and then Plantain (6.6%). Diet formulated with Plantain flour supplemented with 5% Detarium microcarpum flour reduced blood glucose level from 356mg/dl to 113mg/dl while diet formulated with Plantain flour supplemented with 5% Okara flour reduced blood glucose level from 539mg/dl to 116mg/dl. The total cholesterol, low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol of the rats fed diet supplemented with 5% Detarium microcarpum flour and 5% Okara flour were lower than those of the normal control.*

KEYWORDS: Dietary fibre, Blood Glucose, Serum Cholesterol, Plantain flour, Okara flour, *Detarium microcarpum*

INTRODUCTION

Diabetes is a public health issue that needs quick attention. Diabetes mellitus is a heterogeneous metabolic syndrome with several different causes characterized by chronic hyperglycemia with partial or total lack of insulin secretion and a reduced sensitivity to the hormone in peripheral tissues. Diabetes mellitus can also be defined as a disease characterized by raised glucose concentration in the blood, as a result of deficiency or diminished effectiveness of insulin (Passmore and Eastwood, 1986).

Based on the chronic nature of diabetes and the sceptical attitude of people towards regular use of orthodox drug, the need for dietary management is called for. It has been ascertained that certain foods have dual functions - provide specific health benefits or physiological

effects as well as nutritional effects. Such foods are called functional foods due to their use in the prevention and management of chronic diseases. Therefore, diet therapy is an important means through which diabetes and its complications can be managed, reduced or prevented in order to prolong life expectancy. Nutrition is of utmost importance in intensive diabetes management and has been described as the keystone of care (Kalergis *et al*., 2005). The major aim of using dietary therapy to manage diabetes is to achieve better glycemic control by balancing food intake with endogenous and/or exogenous insulin levels. Glycemic index (GI) is an important consideration in the dietary management and prevention of obesity and chronic diseases (Jenkins *et al*., 1981; Brand-Miller, 2003). The consumption of low GI food is associated with a decrease of the risk of the progression of diabetes and glucose intolerance. These effects prompted the FAO/WHO (1998) consultation to endorse the use of GI in diet planning. The American Dietetic Association (ADA) reviewed the evidence of glycemic index as a nutrition therapy intervention for diabetics and acknowledged that low glycemic index foods may reduce postprandial blood glucose levels and asserted that there is sufficient evidence of long term benefit to recommend using low glycemic index diet as a primary strategy in meal planning (ADA, 2008). Glycemic index (GI) is a system developed by Jenkins *et al*. (1998) for classifying carbohydrates based on the effect that a food has on blood sugar levels when consumed. According to Brand-Miller (2003), GI and Glycemic Load (GL) are useful tools in predicting the blood glucose response to various foods. GI is a ratio of the blood glucose response to a given food compared to a standard (typically, glucose or white bread) while GL describes how different foods affect blood glucose (and insulin) level by taking into account the glycemic index and the amount of carbohydrate consumed. Glycemic response is referred to as the ability of foods to cause a rise in blood glucose level. Foods that have high glycemic index cause rapid and strong rise in blood sugar levels; diets rich in such foods have been linked to increased risk for both diabetes and heart disease (Kouassi *et al.*, 2009), While low glycemic index foods would cause slow release of glucose in the blood and in turn cause slow glucose absorption and decrease the risk of diabetes and chronic diseases. The glycemic index of starchy foods range from over 100 to as low as below 40 (with white bread as reference). Several potato and bread products have high values while unprocessed grains, pasta and legumes have lower values. Although, every component of a food (protein, fat, water etc) will influence the rate of carbohydrate absorption, fiber content is a major determinant in the postprandial glucose response (Wolever, 1990; Nishimune *et al*., 1991).

Trowell (1975) reported the lowering effect on blood glucose level and blood cholesterol content by dietary fiber; while Pederson *et al*. (1980), reported that the supplementation of the diets of diabetic patients or those with impaired glucose tolerance with fiber in the form of bran, or guar gum or the use of naturally high fiber foods such as whole grain cereals or dried legumes resulted in an improvement in blood glucose profiles, reduction in urinary glucose and a decrease in the mean serum cholesterol level. Based on this health benefit, this research work was carried out to evaluate the potential of *okara* and *Detarium microcarpum* (rich in dietary fibre) supplemented plantain flour in reducing blood glucose and serum cholesterol.

METHODOLOGY

Sample Procurement/Preparation

The raw materials, mature unripe plantain (*Musa paradisiaca*) fruit, soybean seeds (*Glycine max*) and *Detarium microcarpum* seeds were obtained from Nsukka market. The raw materials were subjected to the following processing methods to get their respective flours.

Preparation of Plantain Flour

The green plantain fingers (30kg) were thoroughly washed with water, quickly peeled and sliced manually. The pulp was subjected to drying for 6 hours at a temperature of 80^{o}C using hot air dryer immediately after peeling. After drying, the dried slices were milled with hammer mill (Bentall Superb, Model 200L 09) and sieved through 0.25mm mesh sieve. The plantain flour produced was hermetically packaged and stored in the refrigerator at 4^{o}C until used for analyses and diet formulation.

Preparation of Okara flour

The soybean seeds were cleaned and washed by floatation to remove all the foreign materials, spoilt grains and debris. The cleaned beans were blanched in hot water for 25 minutes at 100^{o}C and dehulled. The dehulled cotyledons were washed with hot (100^{o}C) water twice and wet milled using 5litres of water to 1kg of beans. The slurry obtained was mixed and filtered through a muslin cloth to remove the milk and recover the residue called *okara*. The fresh *okara* was dried using hot-air dryer at a temperature of 70^{o}C, milled and sieved through 0.25mm pore sized sieve. *Okara* flour was then packaged hermetically and stored in the refrigerator at 4^{o}C until used for analyses and diet formulation.

Preparation of Detarium microcarpum seed flour

Detarium microcarpum seeds were cracked and soaked in clean water (25^{o}C) overnight. Following soaking, the seeds were dehulled manually and the dehulled seeds were dried in a hot air oven at 60^{o}C for 3 hours. The dried seeds were milled (Bentall Superb, Model 200L 09) and the flour sieved through 0.25mm pore sized sieve. The flour produced after sieving was packaged hermetically and stored in the refrigerator at 4^{o}C until used for analyses and diet formulation.

Dietary Fibre Determination

The method of AOAC (1995) was employed.

Experimental Design

Thirty five (35) female Wistar albino rats within the age of 3months weighing between 150 – 180g were used for this study. Diabetes was induced by slow intraperitoneal injection of 1% solution of alloxan (120mg/kg body weight) dissolved in normal saline and administered within few minutes of preparation. Diabetic state was confirmed after two days. Only rats with glucose concentration above 200mg/dl were considered to be diabetic. The rats were grouped and fed with different diets for three weeks after acclimatization period of one week.

A total of thirty-five (35) rats was used and divided into seven groups:

Group 1: Normal rats fed Control diet

Group 2: Diabetic rats fed control diet

Group 3: Diabetic rats fed unsupplemented plantain diet

Group 4: Diabetic rats fed plantain supplemented with 5% *Detarium microcarpum* flour diet
Group 5: Diabetic rats fed plantain supplemented with 5% *okara* flour diet
Group 6: Diabetic rats fed plantain supplemented with 5% guar gum diet
Group 7: Diabetic rats fed plantain supplemented with 2.5% guar gum diet.

Animal and housing

The rats were housed in stainless steel cages at room temperature (28±2^{o}C) and supplied with standard pellet food and water during the acclimatization period of one week. Afterwards, the rats were fed their respective diets for a period of three weeks. Daily food and water intake of the rats were recorded. The initial weight of the rats and their weight during the experiments were recorded.

Diet Formulation

Plantain flour was supplemented with either *okara* or *Detarium microcarpum* at 5% level.

Table 1: Diet Composition (%)

Ingredient(g)	Control Diet	Plantain diet	Plantain supplemented with Detarium diet	Plantain supplemented with Okara diet	Plantain supplemented with Guar gum (5%) diet	Plantain supplemented with Guar gum (2.5%) diet
Casein	15	15	12	12	12	12
Corn starch	59.7		5.7	5.7	5.7	5.7
Corn oil	10	5	5	5	5	5
Sucrose	10					
Vit-mineral Mix[1]	5	5	5	5	5	5
L-methionine	0.3	0.3	0.3	0.3	0.3	0.3
*Plantain flour		74.7				
*95%Plantain+5%Detarium flour			68.4 + 3.6			
*95%Plantain+5%Okara				68.4 + 3.6		
*95%Plantain+5%Guar gum[2]					68.4 + 3.6	
*95%Plantain+2.5%Guar gum[2]						70.1 + 1.8

[1] Vit-Mineral mix was composed of the following: Vitamin A -8,000,000iu, Vitamin D3 – 1875000, Vitamin E – 20,000iu, Niacin – 25000mg, Vitamin K3 – 5500mg, Vitamin B1 – 3000mg, Vitamin B6 – 4000mg, Calcium – 3050mg, Folic – 7500mg, Vitamin B12 – 1250mg, Biotin – 2000mg, Choline Chloride – 1500mg, Copper – 400,000mg, Iron – 6500mg, Zinc – 70200mg, Manganese – 85,500mg, Iodine – 75,000mg, Selenium – 400mg, Cobalt – 80mg, Antioxidant – 200mg.
[2] Food-grade guar gum.

Blood glucose determination

Blood sample was collected after overnight fasting (8 hours) from the rats and used immediately to test for blood glucose concentration. The concentration of glucose/sugar in the blood sample was determined using the one touch glucometer and test strips (life scan inc. Johnson-Johnson Company, Mulpiter California, USA). A drop of whole blood was placed on a strip connected to the glucometer. The glucometer automatically displayed concentration of the blood glucose.

Serum cholesterol determination

At the end of the experiment, the animals were allowed to undergo overnight fasting (8 hours) and blood was collected by ocular method until they were weak. The blood sample was collected in non- EDTA tubes. The serum collected were separated by centrifugation at 2500rpm for 15minutes and the supernatant utilized to determine the total cholesterol, total triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) using the Randox commercial kit (manufactured in UK). The procedure as described in the kit's operation manual was followed.

Food and water intake measurement and body weight of the rat

Food and water intake were measured by weighing serving dishes before and after meals. The body weight of the rat was measured in grams using weighing balance.

Statistical analysis

Data generated were analysed using one-way analysis of variance (ANOVA) (Gomez and Gomez, 1984) while mean separation was done using Duncan new multiple range test (DNMRT) (Gomez and Gomez, 1984) least significant different at $p<0.05$. Significance was accepted at $p<0.05$.

RESULTS

The results of the dietary fiber composition of the flour samples are shown in Table 2, while the results of the feed intake, water intake and mean blood glucose concentration of the rats and are shown in Figure 1, Figure 2 and Figure 3 respectively. Table 3 shows the result of the effect of the diet on the lipoprotein profile of the rats while Figure 4 depicts the effect on the body weight of the rats with time.

Table 2: Dietary Fiber Compositions of *Okara, Detarium microcarpum* and Plantain flour

Samples*	Dietary Fiber (%)
Okara	24.4 ± 0.22^{c}
Detarium microcarpum	78.6 ± 0.02^{d}
Plantain	6.6 ± 0.19^{a}
95%Plantain+5%*Detarium*	10.6 ± 0.26^{b}
95%Plantain+5%*Okara*	9.7 ± 0.05^{b}

*Values are means of triplicate determination ± SD. Means on the same column with different superscripts differed significantly ($p<0.05$).

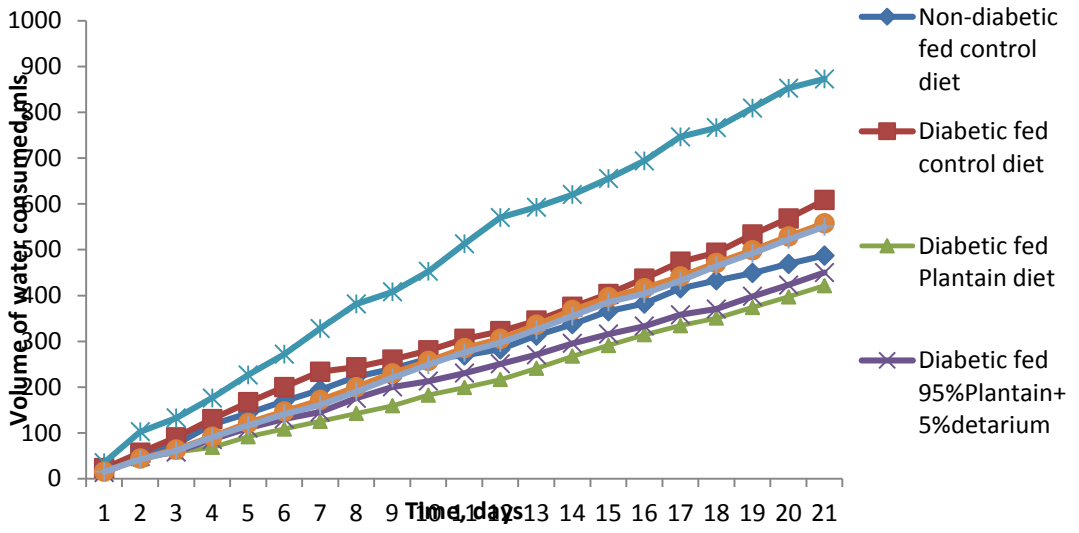

Figure 1: Cumulative water intake of the rats.

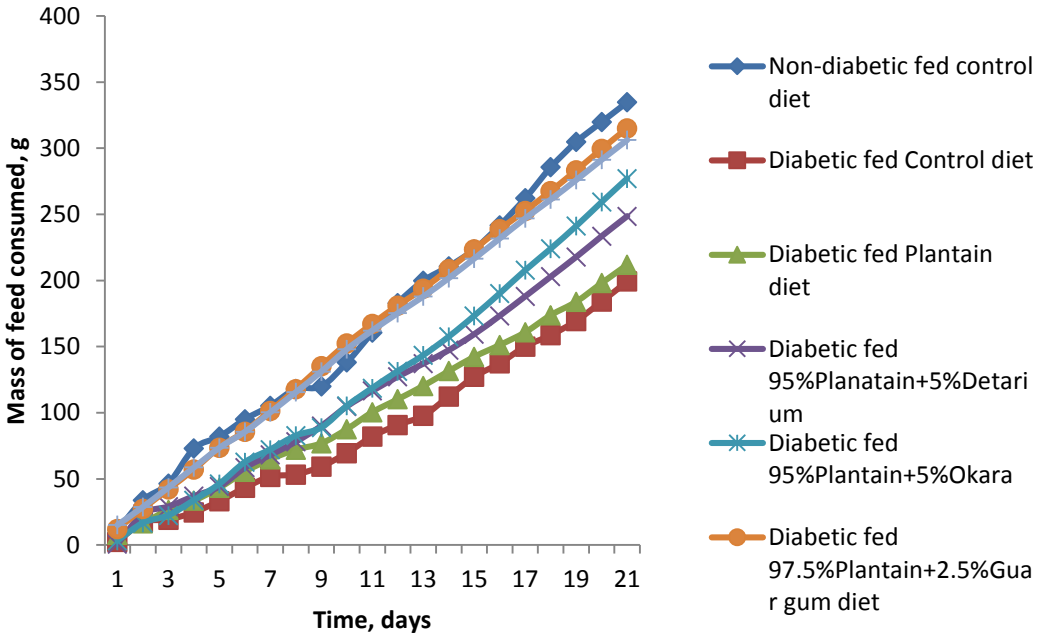

Figure 2: Cumulative feed intake of the rats

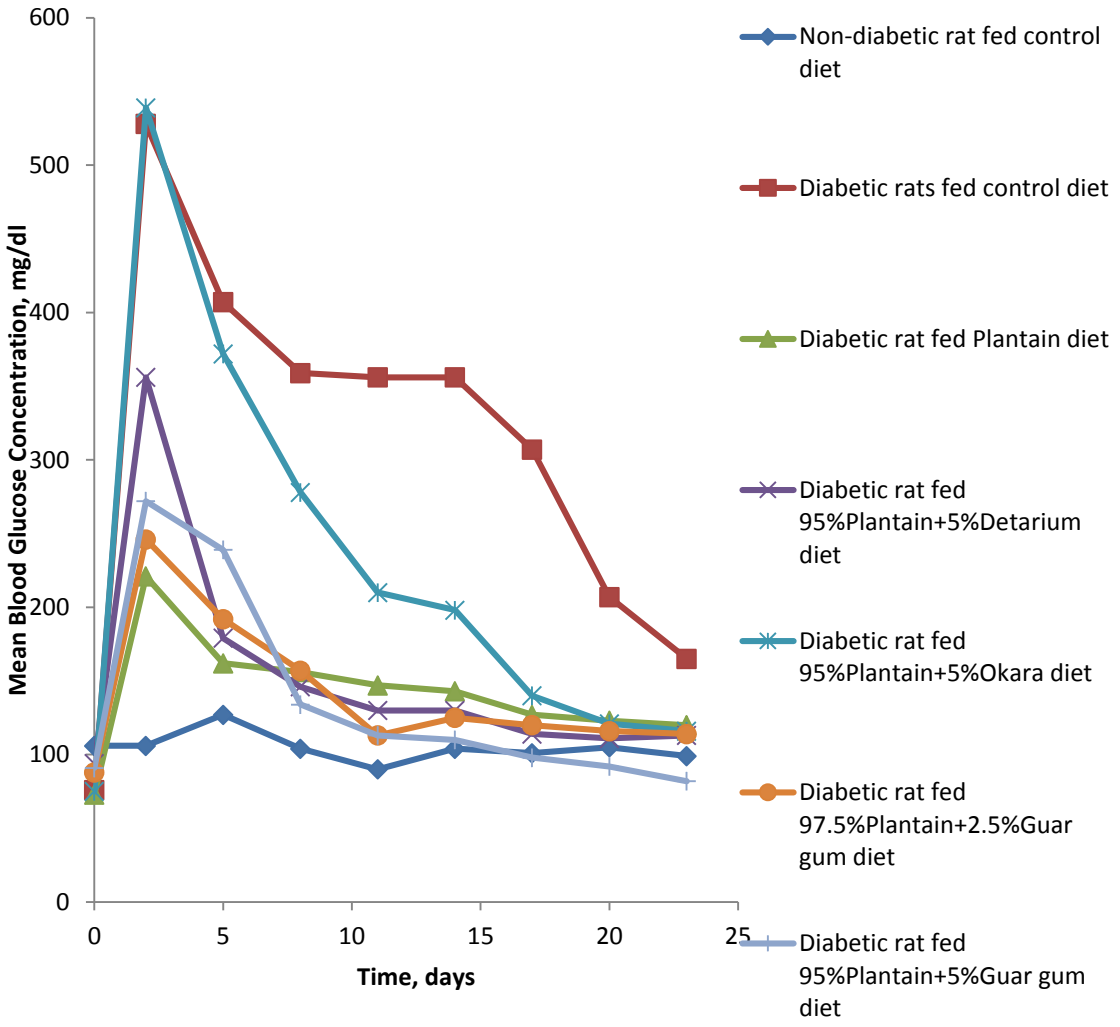

Figure 3: Mean blood glucose concentration

Table 3: Effect of the Diets on Serum Lipoproteins of the rats

Groups*	Experimental Diet	Total Cholesterol (mmol/l)	Total Triglyceride (mmol/l)	Low density lipoprotein (LDL) (mmol/l)	High density Lipoprotein (HDL) (mmol/l)	Very low density lipoprotein (VLDL) (mmol/l)
Non-diabetic rats	Control Diet	$1.60^{abc}\pm0.49$	$0.16^{a}\pm0.069$	$0.33^{a}\pm0.29$	$1.2367^{ab}\pm0.27$	$0.03^{a}\pm0.017$
Diabetic rats	Control Diet	$2.143^{c}\pm0.71$	$1.3433^{b}\pm0.68$	$0.1733^{a}\pm0.12$	$1.7^{b}\pm0.96$	$0.27^{b}\pm0.14$
Diabetic rats	Plantain Diet	$2.05^{bc}\pm0.86$	$0.313^{a}\pm0.13$	$0.2833^{a}\pm0.197$	$1.7^{b}\pm0.96$	$0.063^{a}\pm0.04$
Diabetic rats	95%Plantain+5 %Detarium Diet	$1.31^{ab}\pm0.37$	$0.3567^{a}\pm0.22$	$0.31^{a}\pm0.36$	$0.93^{ab}\pm0.0$	$0.07^{a}\pm0.04$
Diabetic rats	95%Plantain+5 %Okara Diet	$1.47^{abc}\pm0.0$	$0.2833^{a}\pm0.189$	$0.333^{a}\pm0.46$	$1.09^{ab}\pm0.54$	$0.0567^{a}\pm0.04$
Diabetic rats	97.5%Plantain+ 2.5%Guar Gum Diet	$0.72^{a}\pm0.0$	$0.2567^{a}\pm0.064$	$0.18^{a}\pm0.184$	$0.4833^{a}\pm0.17$	$0.05^{a}\pm0.017$
Diabetic rats	95%Plantain+5 %Guar Gum Diet	$0.75^{a}\pm0.3$	$0.6233^{a}\pm0.168$	$0.0533^{a}\pm0.045$	$0.58^{ab}+0.29$	$0.12^{a}+0.03$

*Values are means of triplicate determination ± SD. Means on the same column with different superscripts differed significantly ($p<0.05$).

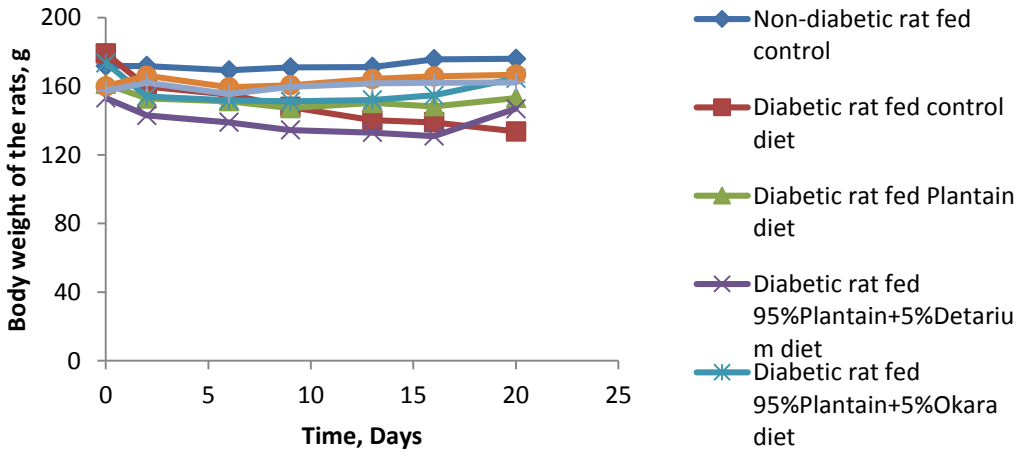

Figure 4: Body weight of the rats with time

DISCUSSION

Dietary Fiber Composition

Detarium microcarpum showed the highest value of dietary fiber (78.6% ±0.02), followed by *okara* (24.4 4% ±0.22) and then plantain (6.6%±0.19). The dietary fiber observed in this study for *okara* is lower than the value (52.3%) reported by Rinaldi *et al*. (2000). *Detarium microcarpum* is very rich in dietary fiber (78.6%), thus, can be used as dietary fiber supplement. This is attributed to its molecular structure which is rich in xyloglucan. *Okara* would also be a good source of dietary fiber. Based on the findings of Basman and Koksel (1999), increasing the percentage of supplementation does not increase the level of B-glucan significantly but badly affects the aesthetic properties of the supplemented flour. Thus, the supplementation was limited to 5%. More so, Table 2 shows that there was not much difference between the dietary fiber value of 5% *Detarium microcarpum* supplemented flour (10.6%±0.26) and 5% *okara* supplemented flour (9.7%±0.05) compared to the wide difference between the values of *Detarium microcarpum* single flour (78.6%± 0.02) and *okara* single flour (24.4% ±0.22).

Water and Feed Intake

The diabetic rats fed diet formulated with 95% plantain and 5% *okara* took the highest volume of water 872.83mls followed by the diabetic rats fed control diet (CD). All the rats showed increased water consumption with time. The high water intake could be attributed to high passage of urine which is a symptom of Diabetes mellitus. Figure 2 shows cumulative feed intake of the rats. The diabetic rats fed control diet did not feed well compared to the other groups. From figure 2 the non-diabetic rats fed control diet showed the highest feed intake unlike the diabetic groups which had low feed intake. Despite increased food consumption shown by other groups, they exhibited decreased body weight due probably to their polyphagic condition.

Blood Glucose Concetration

There was an increase in the mean blood glucose concentration after alloxan administration. The diabetic rats fed control diet still recorded blood sugar concentration that was higher than 200mg/dl on the 20th day while other diabetic rats fed the treatment diets showed quick recovery from high blood sugar concentration (Figure 3) to below 200mg/dl level of concentration.

Alloxan caused an increase in the blood glucose level of the experimental rats when compared with the control ($p<0.05$). The blood glucose concentration was significantly reduced after the 21days of treatment in all animals except diabetic group fed control diet (negative control).

Lipoprotein Profile

Serum total cholesterol levels of rats fed plantain diet supplemented with 5% guar gum and 2.5% guar gum did not differ significantly ($p>0.05$) and they compared well with those fed plantain diet supplemented with 5% *Detarium microcarpum* flour. Total cholesterol

(1.47mmol/l) of rats fed plantain diet supplemented with 5% *okara* flour showed no significant difference ($p>0.05$) when compared with that of normal control (1.60mmol/l). Total cholesterol of diabetic control rats (diabetic rats fed control diet) and that of diabetic rats fed unsupplemented plantain diet was higher than that of normal control rats (non-diabetic fed control diet), showing that alloxan administration increased the total cholesterol level of the rats. Rats fed unsupplemented plantain diet (PD), showed total cholesterol (2.05mmol/l) level higher than normal control (1.60mmol/l) which signifies that plantain flour needs to be supplemented with high fiber flours to achieve high efficacy of total cholesterol reduction. There was no significant difference ($p>0.05$) in the total triglyceride level of the normal rat fed control diet and the diabetic rats fed supplemented diets. The LDL of the rats in all the groups showed no significant difference ($p>0.05$). It was evident from the study that the supplemented diet given to the rats lowered the HDL cholesterol except those subjected to unsupplemented plantain diet (PD). There was no significant difference ($p>0.05$) in the VLDL cholesterol level of normal control rats and rats in other groups except the diabetic control group (group 2). There was significant reduction in the total cholesterol, LDL cholesterol of rats fed diet supplemented with *Detarium microcarpum* flour, and guar gum; when compared with the total cholesterol, LDL cholesterol and HDL cholesterol of the normal rats fed control diet. This was attributed to their very high dietary fiber content.

Body Weight during the experimental study

The non-diabetic rats fed control diet, did not show reduction in body weight but rather had increased body weight. Diabetic rat fed control diet showed reduction in weight till the end of the experiment. Diabetic rat fed plantain diet showed a reduction in weight which increased marginally at the end of the experiment. Diabetic rats fed 95% Plantain diet supplemented with 5% *Detarium* and *okara* showed a reduction in weight which was observed to increase abruptly from day 16 more than the group fed 100% Plantain diet. The group fed diet containing 2.5% guar gum and 5% guar gum only showed a slight reduction on the 6^{th} day (immediately after alloxan administration) and then, increased subsequently. Dehydration and body weight loss have been associated with Diabetes mellitus (Pupim *et al*., 2005). In diabetic rats, increased food and water consumption and decreased body weight were observed. This indicates the polyphagic condition and loss of weight due to excessive breakdown of tissue proteins (Kamalakannan and Prince, 2006).

IMPLICATION TO RESEARCH AND PRACTICE

This research was focused to provide diabetic subjects with diet rich in dietary fibre. From the analyses conducted, the flours are rich in dietary fibre and thus can be utilized in the formulation of diabetic diets which requires flour incorporation in order to increase the dietary fiber composition of the final product. Flour incorporation of not more than 5% of *Detarium microcarpum* or 5% Okara (soybean residue) in other flours will increase their dietary fiber level without negatively affecting their aesthetic property.

CONCLUSION

The flour samples were good sources of dietary fiber with *Detarium microcarpum* being the richest in dietary fiber (78.6%) followed by *okara* (24.4%) and plantain (6.6%) which could physiologically slow down sugar and cholesterol absorption in the blood. From the study, the two dietary fiber rich flours- *Detarium microcarpum* and *okara* were shown to reduce blood glucose concentration and serum total cholesterol concentration, and therefore, would be utilized in the formulation of diabetic foods.

FUTURE RESEARCH

Glycemic index determination of confectionaries produced with composite flour from these selected flours is recommended for future research.

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