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Effect of a Nigerian Type Diet on Indices of Cell Proliferation

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Abstract: The effect of a Nigerian type diet on indices of cell proliferation in albino rats administered cycads was examined. Rats of wister strain weighing between 80.0g and 79.7g were used in the experiment. These rats were assigned to four experimental groups and respectively exposed to a Nigerian type diet for a period of five weeks. The rats were sacrificed at the end of the experiment and the colon separated for analysis. A significantly low feed intake was observed in the Normal Diet (ND) fed rats compared to the other groups. No significant alteration ($p > 0.05$) was observed in weight gain in all the experimental groups. When compared with the controls, the ATPase activity (total ATPase, Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase) of both colonic mucosa and tissue were not significantly different ($p > 0.05$) in total lipid and total phospholipids of both the colonic mucosa and tissue in all the experimental groups. A Nigerian type diet may therefore have a protective role against colon cancer in rats fed with cycads at least within the first five weeks.

Key words: Cell proliferation, albino rats, Nigerian type diet

Introduction

Food can make or break our health and increasingly, factors related to food-its quality, its nutritional constituents even how it is grown and processed are considered primary agent for contributing to the initiation and promotion of cancer (www.nlm.nih.gov/medlineplus/ency/article/000262.htm). According to the National Academy of Sciences, 60% of all cancers in women and 40% of all cancers in man may be due to dietary and nutritional factors. One of the major factors accounting for the steady rise in the incidence of cancer and mortality rates is nutritionally imbalances (www.nlm.nih.gov/medlineplus/ency/article/000262.htm). Information about the role of diet has come from many observational studies around the world across cultures and geographical divides. It is now thought that up to a third of all cancers in westernized countries may be related to diet. Research has shown that a diet high in animal fat and low in fresh fruit and vegetables in other words the diet of industrialized western nations can increase overall risk of cancer (www.ehealthmd.com/library/colon_cancer/colon_cancer_causes). More specifically, cultures with the highest levels of fat consumption are also the ones with the highest death rates from breast and colon cancer (www.ehealthmd.com/library/colon_cancer/colon_cancer_causes).

The industrialized west primarily bases its diet on animal products. These contain very little fibre and are high in animal fat thought to be much more harmful than

vegetable fat. Accordingly, these countries have the highest rates of colon cancer (Abrahamse *et al.*, 1999). Tropical foods are rich in carbohydrate, low in fat and animal protein and contains large amount of fibre. Such foods produce large faecal outputs and protects against colon cancer and as such leads to low death rates from cancer (Abrahamse *et al.*, 1999).

In this present study, the effect of diet patterned after the typical Nigerian diet on colon cancer was investigated. This was carried out by estimating total lipid and phospholipids in the colon, ATPase activity in the colon and physical parameters including body weight and food intake.

Materials and Methods

Animals: Wister Albino rats were purchased from Nigerian Medical Research Institute (NIMAN) Yaba, Lagos State. The rats were weighed and assigned comparable weights in all groups ($\pm 0.3\text{g}$). The animals were acclimatized with their respective diets for a period of one week. The cages housing the rats were kept in an environment with free supply of air and light. Water and feed were changed daily.

Table A: Diet pattern for each group

Group	Diet type
I	High carbohydrate/high fiber diet+cycads
II	Normal diet
III	Normal diet+cycads
IV	High carbohydrate/high fibre

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Table 1: Mean Atpase activities (iu/l) in the tissue of rats following the administration of a Nigerian diet

Parameter	High Carbohydrate.	High carbohydrate.	Normal diet+cycads	Normal diet
	High fibre diet	High fibre diet+cycads		
Total ATPase	1.03x10 ⁻² ±1.02X10 ^{-2a}	3.25x10 ⁻⁵ ±7.50x10 ^{-6a}	5.75x10 ⁻⁵ ±1.11x10 ^{-5a}	5.50x10 ⁻⁵ ±5.00x10 ^{-6a}
Na ⁺ /K+ATPase	1.28x10 ⁻² ±7.89X10 ^{-3a}	4.10x10 ⁻² ±3.31x10 ^{-2a}	1.58x10 ⁻² ±6.18X10 ^{-3a}	1.35x10 ⁻² ±4.50X10 ^{-3a}
Mg ²⁺ ATPase	3.65x10 ⁻² ±2.90X10 ^{-3a}	3.08x10 ⁻² ±6.01X10 ^{-3a}	1.133x10 ⁻² ±8.60x10 ^{-2a}	4.55x10 ⁻² ±1.35x10 ^{-2a}
Ca ²⁺ +ATPase	4.30x10 ⁻² ±1.35X10 ^{-3a}	5.00x10 ⁻² ±1.56X10 ^{-2a}	5.75x10 ⁻² ±1.05X10 ^{-2a}	6.30x10 ⁻² ±5.00X10 ^{-3a}

Values are mean ± SEM from sixteen rats. Means of the same row followed by the same letters are means that are not significantly different (p>0.05)

Table 2: Mean Atpase activity (iu/l) in the mucosa of rats following the administration of a nigerian diet

Parameter	High Carbohydrate.	High carbohydrate.	Normal diet+cycads	Normal diet
	High fibre diet	High fibre diet+cycads		
Total ATPase	0.64±1.026 ^a	0.50±0.14 ^a	0.70±0.44 ^a	1.12±0.0.34 ^a
Na ⁺ /K+ATPase	0.19±7.38x10 ^{-2a}	0.16±0.13 ^a	0.13±0.11 ^a	0.15±9.35x10 ^{-2a}
Mg ²⁺ ATPase	0.44±0.18 ^a	0.46±0.13 ^a	0.57±0.31 ^a	0.98±0.43 ^a
Ca ²⁺ +ATPase	0.40±0.18 ^a	0.31±0.11 ^a	0.40±0.23 ^a	0.57±0.20 ^a

Values are mean ± SEM from sixteen rats. Mean of the same row followed by the same letter are means that are not significantly different (p>0.05)

Feed: The cycads were washed and dried in the oven at 50°C and blended into powder. The soya bean was cooked for about 5 hours, dried in the oven at 60°C and blended into powder. The other food components which were already in powdered form were mixed in their various proportions. The Normal Diet (ND) was patterned after previously fed diets by Schuette and Richard (1986) with slight modification in their study of the effect of diet high in fat and /or fibre on colonic absorption of DMH in rats. The diet rich in fibre was patterned after that of Anderson and Gustafson (1987) with slight modification in their study of the hypolipidaemic effect of a high carbohydrate and high fat diet.

Body weight measurement: The weights of the rats in each group were determined at the beginning of the experiment and shortly before the animals were sacrificed. From the values, weight gain or losses were estimated for each experimental group.

Determination of feed intake: Feed intake was the difference between the weight (g) of feed given and the amount remaining per day.

Isolation of colon: At the end of the five weeks of feeding, all the rats were weighed and starved overnight. Subsequently, they were sacrificed after sedating with chloroform and then dissected accordingly. The colon was removed, washed in normal saline (0.9% NaCl solution) to remove the debris and cut open. The mucosa was scrapped into a plane bottle (10 mL). The samples were stored in the freezer for analyses.

Preparation of sample nuclei membrane homogenate
Atpase assay: 1g of colon tissue and mucosa of each rat was weighed and homogenized separately with acid washed sand in pre-chilled mortar using 9ml of 0.9%

NaCl solution. The homogenate was subsequently centrifuged at 10,000g for 15mins. The clear supernatant obtained was used for ATPase assay. ATPase activity was assayed by the method of Adam-Vizi and seregi (1982).

Lipid assay: 1g of colon tissue and mucosa of each rat was weighed and homogenized separately in a mixture of chloroform, methanol and water (2:1:0:8 V/V/V). The homogenates were centrifuged at 10,000 rpm for 10mins and the supernatants were used as source of lipid. Colon total lipid was estimated based on the phosphovanillin reaction according to Zother and Krisch (1962) method. Colon phospholipid was quantified by a combination of the methods of Barlett (1959) and Cuzner and Davidson (1967).

Statistical analysis: The results were expressed as mean±SEM. Analysis of variance was used to test for differences between all the groups. Duncans's multiple range test was used to test for significant differences between the means (Sokal and Rohlf, 1969).

Results

The results are as presented in the Table 1-5. Results showed that tissue and mucosa ATPase activity were not significant (p>0.05) when compared with control. (Table 1 and 2). Total lipids/ phospholipids in the tissue and Mucosa were not significant (p>0.05) when compared with control (Table 3 and 4). However, significantly lower (p<0.05) feed intake was obtained in the Normal Diet (ND) fed rats (Table 5).

Discussion

High fat and low fibre intake are major features of the Western diet where as high fibre low fat diets prevail in Africa, Asia and other areas of the world where the risk of colon cancer is low. Burkitt hypothesized that dietary

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Table 3: Mean concentration of tissue total lipid (mg/ml) and phospholipids (mg/dl) in rats following the administration of a nigerian diet

Parameter	High Carbohydrate. High fibre diet	High carbohydrate. High fibre diet+cycads	Normal diet+cycads	Normal diet
Total Lipid(mg/ml)	126.70±28.50 ^a	72.30±3.11 ^a	120.60±41.23 ^a	127.70±49.20 ^a
Total phospholipids (mg/dl)	4.25x10 ⁻⁴ ±1.32x10 ^{-4a}	7.25x10 ⁻⁴ ±3.43x10 ^{-4a}	1.08x10 ⁻³ ±2.96x10 ^{-4a}	1.55x10 ⁻⁵ ±3.50x10 ^{-4a}

Values are mean ± SEM from sixteen rats. Mean of the same row followed by the same letter are means that are not significantly different (p>0.05)

Table 4: Mean concentration of total lipid (mg/ml) and phospholipids (mg/dl) in the mucosa of rats following the administration of a nigerian diet

Parameter	High Carbohydrate High fibre diet	High carbohydrate High fibre diet+cycads	Normal diet+cycads	Normal diet
Total lipids (mg/ml)	52.88±8.41 ^a	82.33±26.34 ^a	103.45±30.81 ^a	101.15±55.75 ^a
Total Phospholipids (mg/dl)	4.10x10 ⁻⁴ ±1.97x10 ^{-4a}	8.50x10 ⁻⁴ ±4.52x10 ^{-4a}	2.74x10 ⁻⁴ ±1.11x10 ^{-4a}	1.85x10 ⁻⁴ ±1.15x10 ^{-4a}

Values are mean±SEM from sixteen rats, Mean of the same row followed by the same letter are means that are not significantly different (p>0.05)

Table 5: Mean weight (g) of feed intake and weight gain (g) by rats following the administration of a nigerian diet

Parameter	High Carbohydrate High fibre diet	High carbohydrate High fibre diet+cycads	Normal diet+cycads	Normal diet
Feed Intake (g)	85.63±8.56 ^a	85.75±11.15 ^a	100.00±0.00 ^a	19.75±4.59 ^b
Weight gain (g)	15.8750±8.3025 ^a	5.6250±3.9915 ^a	7.1250±2.3649 ^a	42.000±0.5000 ^a

Values are mean±SEM from sixteen rats, Means of the same row followed by the same letter are means that are not significantly different (p>0.05)

fibre acted to protect against colorectal carcinogenesis by increasing stool bulk and so diluting the carcinogen in the colonic lumen, speeding the rate of transit of the gut contents and so decreasing the time available for carcinogen action on the colon mucosa. And thus modifies the gut flora to one less prone to produce carcinogens (Burkitt, 1971).

Studies by Murray *et al.* (1980) has shown that high fibre diet increased the activity of intestinal mucosa Na⁺/k⁺ATPase.

This present study examined the effect of a Nigerian based diet on colon membrane lipid and phospholipids as well as colon membrane ATPase activity. The data presented in this work have shown that Nigerian diet has no significant difference on the total phospholipids, total lipid and ATPase activity of the colon membrane. This supports the observation by Adamson and Mbajorgu (1985) that colonic Mucosa, ATPase activities are not altered when rabbits were fed with cassava or plantain. Observation by Eriyamremu (1992) showed that high carbohydrate high fat diet did not significantly alter the total phospholipids level in the colon or the liver. The data presented in Table 5 give the results of feed consumption which was significantly lower in rats fed normal diet when compared to the other groups. Normal diet contained a lower percentage of fibre thus a high colonic density. Therefore not much of the normal diet would be consumed and this could explain the low level of feed intake observed in the rats fed with the normal diet.

From this study, it could be concluded that a Nigerian type diet may have a protective role against colon cancer in rats fed with cycads at least within the first five weeks.

However, more study is needed on the long term effect of a Nigerian type diet on cell proliferation in albino rats.

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