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Full Length Research Paper

Biochemical observations in rats fed with cycads, Nigerian-like and western-like folic acid supplemented diets

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Alterations in antioxidant enzymes and lipid peroxidation was studied in 90 Wistar albino rats exposed to cycads and fed with Nigerian-like and western-like diets supplemented with folic acid. The animals were divided into three diet classes of 30 animals each. One group was fed with a wholly compounded Nigerian-like diet (NLD) which was low in protein and high in carbohydrate and fiber. Another group was fed with a western like diet (WLD) which was high in protein and fat, while the third group of animals was fed with a normal diet which served as the control class. The animals of each class were further divided into three subgroups of ten rats each. In each subclass, one group received the diet alone; another group received the diet and cycads, while the third group received the diet and folic acid. Low levels of catalase, feed intake and faecal output was observed with experimental animals fed NLD as compared to WLD and normal diet (ND) fed animals. High levels of antioxidant enzymes: super oxide dismutase, catalase and glutathione reductase as well as low levels of lipid peroxidation was observed with the addition of folic acid to the diets of experimental rats. These results suggest the role of folic acid in diet and colon carcinogenesis.

Key words: Antioxidant enzymes, cycads, diets, folic acid, lipid peroxidation.

INTRODUCTION

Mortality from colorectal cancer is a leading cause of death in North America, Oceania and western Europe (Greelee et al., 2001) and it is believed that the etiology of the disease is of both genetic and of dietary origin. Colon cancer which originates from environmental causes (sporadic colon cancer) represents about 95% of all cases of the disease and is mostly associated with dietary risk factors (Boutron-Ruault, 2002). A variety of studies support the earlier finding of Burkitt (1984) that the regular intake of carbohydrate; particularly of resistant starch and fiber, reduces the incidence of colon cancer (Cassidy and Cummings, 1994), while populations on a high fat and high protein diet such as those in western world are at a high risk of developing colon cancer (Hursting et al., 1990). However, inconsistent findings from observational studies have continued the controversy over the effects of dietary fiber on colorectal cancer. A study by Park et al. (2005) showed that fiber intake from cereals, fruits and vegetables were not associated with risk of colorectal cancer. Also, a study of colon cancer rates in South Africa showed that the low prevalence of colon cancer in black Africans cannot be explained by dietary "protective" factors, such as fiber, calcium, vitamins A and C, and folic acid, but may be influenced by the absence of "aggressive" factors, such as excess animal protein and fat, and differences in

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colonic bacterial fermentation (O'Keefe et al., 1999). On the other hand, a more recent paper reported that the risk of colorectal adenoma (the precursor of colorectal cancer) decreased by 41% for every additional 5% unit of fiber intake/day (Mathew et al., 2004). Red meat fat increased the risk by 20%, and white meat fat decreased the risk by 67% for every additional 5% unit of respective intake/day (Mathew et al., 2004). Experimental studies on the role of diets consumed by populations in colon carcinogenesis also agrees with some of these population studies, though most of them have been carried out using semi-purified or purified diets. Studies indicated that fiber, vitamins, especially the B vitamins, folic acid, minerals and other substances found in high fiber vegetables and grains may protect against colon cancer (Allison, 1995).

Epidemiological studies investigating associations between dietary intake of folate and colorectal cancer have also been inconsistent. A study by Cole and Baron (2007) reported that folic acid does not protect people who already suffer from colorectal cancer. While results from several other studies have demonstrated that a diet deficient in folic acid may be associated with an increased risk of colonic neoplasia, whereas dietary supplementation of this nutrient may be chemopreventive, may prevent aberrant DNA methylation, and thereby protect against colorectal cancer (Majumdar et al., 2004; de Vogel et al., 2008; Du et al., 2010). There is/are some evidences that suggest that relatively high dietary folate intake might be associated with reduced colorectal cancer risk, especially among individuals with low methionine intake (Terry et al., 2002). Folate is crucial for normal DNA methylation, synthesis and repair, and deficiency of this nutrient is hypothesized to lead to cancer through disruption of these processes. However, the mechanisms by which folic acid exerts its chemopreventive role (if any) in colorectal carcinogenesis remain to be fully elucidated. A 'dual-modulator' role for folate in colorectal carcinogenesis has been proposed, in which moderate dietary increases initiated before the establishment of neoplastic foci have a protective influence, whereas excessive intake or increased intake once early lesions are established, increases tumorigenesis (Hubner and Houlston, 2009; Mason, 2009).

The aim of this study was to assess some early biochemical events in rats fed with cycads, Nigerian-like and western-like folic acid supplemented diets.

MATERIALS AND METHODS

Ninety (90) Wistar albino rats of comparable weights purchased from the Animal House, College of Medicine, Ambrose Alli University, Ekpoma, were used for this experiment. They were housed individually. The rats were weighed and grouped into three diet classes with 30 animals each, such that the weight difference between the groups was less than 0.2 g. One group was fed with a wholly compounded normal diet (ND) which served as the control class. Another class of animals was fed a wholly compounded Nigerian-like diet (NLD) which was low in protein and high in carbohydrate and fibre. The third class of rats was fed with a western like diet (WLD) which was high in protein and fat. Both the NLD and WLD served as test diets. The animals of each diet class were further divided into three subgroups of ten rats each. In each class, one group received the diet alone; another group received the diet and cycads, while the third group received the diet and folic acid. The animals were given food and water *ad libitum* and were force fed with folic acid and cycads such that they consumed 0.5 mg/kg body weight. The animals were acclimatized with their respective diets for one week before the commencement of the study which lasted for twelve weeks. During this period, food intake and faecal output were measured daily while weight gain was recorded weekly.

Feed preparation

The cycads leaves were washed and dried in the oven at 50°C and then blended into powder. The soya beans was cooked for about five hours, dried in the oven at 60°C and blended into powder. The other food components which were already in powder form were mixed in their various proportions. The normal diet (ND) was patterned after previously fed diets by Schuette and Richard (1986) in their study of the effects of diets high in fats and/or fiber on colonic absorption of dimethylhdrazine (DMH) in rats. The diet rich in carbohydrate and fiber were patterned after that of Anderson and Gustafson (1987) in their study of the hypolipidaemic effect of a high carbohydrate and high fat diet.

Body weight measurement

The weights of the experimental animals in each group were determined at the beginning and end of the experiment and shortly before the animals were sacrificed. From the values obtained, average weight gain and losses were estimated for each experimental group.

Determination of faecal output

The faeces of each rat was collected and weighed so as to ascertain the output per day. From values obtained, average faecal output was estimated for each experimental group.

Determination of feed intake

The amount of feed consumed per rat per day was estimated by determining the difference between weight of feed given and feed left over.

Isolation of the colon

At the end of the study period, the animals were fasted over night, sacrificed under ether anaesthesia and the colons were excised. The colon was flushed several times with ice cold normal saline solution until it was free of debris. The intestine was inverted and the mucosa was removed by scrapping with a glass slide. The tissue and mucosa were kept separately in plane bottles and stored in the freezer for analyses.

Sample preparation

One gram of colon tissue and mucosa of each rat was homogenized

separately in 5% trichloroacetic acid (TCA) for 10 s. The homogenate was subsequently centrifuged at 10,000 g for 15 min. The supernatant was used for lipid peroxidation and antioxidant enzyme assay.

Biochemical assay

Estimation of catalase was based on the method of Cohen et al. (1970). Glutathione reductase was measured by the method of Tietz (1969), lipid peroxidation was determined by the thiobarbituric acid (TBA) assay of (Mihara and Uchiyama 1978) and the method of Misra and Fridovich (1972) was used to estimate superoxide dismutase.

Statistical analysis

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

RESULTS

This study presents data on the role of wholly compounded diets on lipid peroxidation and antioxidant enzymes in early carcinogenesis. From the results obtained, NLD fed rats ate more and passed out more stool, while the WLD fed rats ate less and passed out less stool. The WLD fed rats gained weight, the WLD increased lipid peroxidation in the colon as compared with the NLD. NLD fed rats recorded low levels of superoxide dismutase, while the WLD fed rats recorded high level of superoxide dismutase. NLD fed rats recorded low levels of glutathione reductase, while WLD fed rats recorded high levels of the enzyme. NLD fed rats recorded high levels of catalase, while WLD fed rats recorded low levels of the enzyme.

From the result, we can infer that the influence of folic acid on lipid peroxidation and antioxidant enzymes depend on diet type with the NLD lowering lipid peroxidation and increasing some antioxidant enzymes as compared to folic acid and the WLD. Though a direct extrapolation of the findings in experimental rats cannot be made to humans, a shift from locally consumed diets rich in fibre and low in fat may improve the incidence of colon cancer.

DISCUSSION

This study examined alterations in lipid peroxidation and anti oxidant enzyme levels in rats fed with cycads, Nigerian-like and western-like folic acid supplemented diets. Our findings showed that western-like diet (WLD) fed rats gained weight as compared to the NLD fed rats. The WLD is calorie rich, so it is not surprising that it caused an increase in weight, while the NLD which is low in calorie decreased weight. Studies by Ruggerri et al. (1987) showed that reduction in calorie intake and weight decreases colon cancer.

The data on feed intake by experimental animals presented in Table 3 showed that the NLD fed rats had increased feed intake, while the WLD fed rats had decreased feed intake. The large quantity of food consumed by the NLD fed rats may be attributed to the low calorie content of fiber. Since the NLD is high in fiber, animals in this group would meet their energy demand by eating more of the diet. The WLD contained less fiber but a high caloric density. Thus, to meet their energy requirements, the animals need not eat much.

Earlier reports indicated that high fiber diets are associated with increased faecal bulk (Burkit, 1971; Walker, 1978). The increased faecal output observed in the NLD fed rats could be the result of faecal bulking property of fiber. Potential mechanisms by which dietary fiber can protect against the development of colorectal cancer include increase in faecal bulk, dilution of potential carcinogens and decrease transit time. This study reveals that the NLD fed rats ate more and passed out more stool, while the WLD fed rats ate less and passed out less stool.

Also, it has been suggested from earlier studies that a diet high in carbohydrate and fiber may protect against colon cancer (Burkit, 1984; Eriyamremu et al., 1995). Data available from this study showed that the NLD caused high lipid peroxidation and therefore may be predispose to colon cancer. The observed effect of NLD on colonic mucosa lipid peroxidation is not surprising. Though, the NLD is rich in fiber and is expected to protect against colon cancer, it is however not a perfect diet and may have added to the stress in the colon. If it can be recalled, a typical NLD is low in protein and such diet has been shown to induce stress in animals (Tatli et al., 2000). Increase stress increases lipid peroxidation.

Data from this study also showed that WLD increased further the level of lipid peroxidation in the colon. This may not be unconnected with the level of amines in the protein and the level of lipid in the diet. This is in consonance with previous studies which shows that a diet high in fat and protein may increase susceptibility to colon cancer (Burkit, 1984).

Addition of cycads to the diet caused increase in lipid peroxidation. This could be the result of increased stress on the colon by cycads as it is modified in the colon into dimethylhydrazine, a potent carcinogen by gut microfloral.

Folic acid reduced lipid peroxidation in the animals. This implies that folic acid may prevent colon cancer. This result corroborates those of previous epidemiological studies which reported a protective effect of the prenatal and perinatal maternal use of folic acid against the incidence of brain tumors in offspring (French et al., 2003). Therefore a nutritional shift from Nigerian-like diet to western-like diet is likely to induce more stress.

A similar trend was observed in the colon tissue of experimental animals. However, the level of lipid

Table 1. Mean lipid peroxidation and antioxidant enzyme level in the colon mucosa of rats fed with cycads, Nigerian-like and western-like folic acid supplemented diets.

Parameter	Normal diet (ND)	Normal diet + cycads (NDC)	Normal diet + folic acid (NDF)	High carbohydrate high fibre diet (NLD)	High carbohydrate high fibre diet + cycads (NLDC)	High carbohydrate high fibre diet + folic acid (NLDF)	High protein high fat diet (WLD)	High protein high fat diet + cycads (WLDC)	High protein high fat diet + folic acid (WLDF)
Catalase	2.59±0.012	2.00±0.043	2.90±0.031	5.00±0.004	1.70±0.043	5.40±0.014	1.50±0.041	1.20±0.012	3.20±0.028
Glutathione reductase	2.00±0.01	1.60±0.02	1.70±0.02	1.20±0.03	1.05±0.03	2.00±0.06	2.60±0.01	1.20±0.03	2.80±0.04
Super oxide dismutase	2.80±0.03	2.50±0.11	3.00±0.04	2.00±0.02	1.50±0.02	3.50±0.01	4.80±0.14	2.00±0.07	4.40±0.12
Lipid peroxidation	1.50 ±0.03	1.70±0.01	1.40±0.02	2.20±0.01	2.50±0.03	0.70±0.04	3.00±0.06	2.90±0.01	1.00±0.02

Table 2. Mean lipid peroxidation and anti oxidant enzyme levels in the colon tissue of rats fed with cycads, Nigerian-like and western-like folic acid supplemented diets.

Parameter	Normal diet (ND)	Normal diet + cycads (NDC)	Normal diet + folic acid (NDF)	High carbohydrate high fibre diet (NLD)	High carbohydrate high fibre diet + cycads (NLDC)	High carbohydrate high fibre diet + folic acid (NLDF)	High protein high fat diet (WLD)	High protein high fat diet + cycads (WLDC)	High protein high fat diet + folic acid (WLDF)
Catalase	3.40± 0.02	2 .50±0.028	3.57±0.02	5.26±0.13	1.90±0.08	5.69±0.07	2.50±0.018	1.40±0.026	4.24±0.11
Glutathione reductase	8.90±0.30	8.10±0.20	10.90±0.10	4.30±0.02	6.00±0.18	12.70±0.34	16.50±0.30	7.00±0.12	19.50±0.15
Super oxide dismutase	3.80± 0.13	3.60±0.13	4.20±0.15	3.00±0.02	2.00±0.02	4.60±0.03	6.70±0.13	2.20±0.08	7.20±0.20
Lipid peroxidation	1.50±0.05	2.20±0.02	1.20±0.06	2.50±0.06	3.10±0.01	0.60±0.02	3.30±0.05	3.60±0.02	1.00±0.03

peroxidation in the colon tissue was higher than that of the mucosa. The mucosa is less complex in structure than the tissue and it is the colon's first line of defense against luminal agents. Therefore, it is resistant to reactive oxygen species breakdown (Brownlee et al., 2007).

We measured some antioxidant enzymes in the animals amongst which are superoxide dismutase, glutathione reductase and catalase. We observed low levels of superoxide dismutase in rats fed NLD which also recorded high levels of lipid peroxidation. This may be attributable to the diet either affecting the synthesis of the enzyme or the activity of the enzyme. As high lipid peroxidation is suggestive of high membrane lipid peroxidation and therefore tissue damage, it may result in cell destruction and loss of enzyme action.

We observed high superoxide dismutase level in the rats fed WLD as compared to NLD fed rats. Western like diet contains high protein. Dietary proteins are necessary for enzyme synthesis. Consequently, the observed increase in super oxide dismutase level of rats fed WLD could be the result of high availability of dietary proteins (Tatli et al., 2000).

The inclusion of cycads to the diet caused high lipid peroxidation. High lipid peroxidation may have resulted to tissue damage and therefore leakage of enzyme and may have accounted for

Table 3. Mean weight of feed intake (g), faecal output (g) and weight gain (g) of rats fed with cycads, Nigerian-like and western-like folic acid supplemented diets.

Parameter	Normal diet (ND)	Normal diet + cycads (NDC)	Normal diet + folic acid (NDF)	High carbohydrate high fibre diet (NLD)	High carbohydrate high fibre diet + cycads (NLDC)	High carbohydrate high fibre diet + folic acid (NLDF)	High protein high fat diet (WLD)	High protein high fat diet + cycads (WLDC)	High protein high fat diet + folic acid (WLDF)
Feed intake	63.62±0.08	59.69±0.12	61.15±0.07	69.12±0.02	59.69±0.03	61.15±0.11	62.17±0.18	59.69±0.03	61.15±0.08
Faecal output	6.58±0.13	7.08±0.02	6.57±0.05	13.58±0.03	7.08±0.10	6.27±0.11	4.58±0.04	7.00±0.06	6.00±0.11
Weight gain	20.00±1.006	18.00±0.461	16.00±0.641	12.00±0.947	18.00±0.139	16.00±0.823	37.00±0.761	18.00±0.142	16.00±0.631

the low super oxide dismutase level observed. High super oxide dismutase level was observed in the animals fed folic acid. Folic acid is believed to be involved in the synthesis, repair and functioning of DNA (Jennings, 1995) and may have caused increase synthesis of the enzyme super oxide dismutase. So, the combined effect of folic acid on lipid peroxidation and super oxide dismutase suggest that it will reduce lipid peroxidation and increase antioxidant enzymes, thereby slowing the process of carcinogenesis.

Low glutathione reductase level was observed in the colon mucosa of rats fed NLD as compared to those fed WLD. High level of lipid peroxidation was also observed with NLD fed rats which suggests that the colon of rats suffered oxidative stress. Cellular glutathione is highly sensitive to oxidative stress (Kayode et al., 2009), thus, exposure to the reactive oxygen species and free radicals generated may result in rapid depletion of glutathione levels in the colon. Moreover, glutathione reductase is a protein that is mostly synthesized endogenously. Its production will likely reduce in malnutrition. Glutathione is also necessary for amino acid transport. Less protein in the NLD will not only affect the antioxidant property of glutathione but also its transport of amino acid and this will potentiate colon carcinogenesis. Low glutathione reductase level was observed in the colon mucosa of rats fed cycads. Low super oxide dismutase level was also observed in this group of experimental rats. This would diminish the ability of the mucosa to scavenge free radicals and would have accounted for the increase in membrane lipid peroxidation also observed with the rats fed the diet. Addition of folic acid to the diet caused increase in glutathione reductase level. Being involved in DNA synthesis, folic acid may have caused increase synthesis of the enzyme. When we measured colonic tissue glutathione level of experimental animals, we observed the same trend. However, the level of glutathione reductase in the mucosa was higher than that of the tissue.

The NLD fed rats recorded high catalase level as compared to the rats fed WLD. Increase in catalase level by the NLD fed rats represents an attempt made by the colon mucosa to overcome reactive oxygen species and thus oxidative stress. It appeared that the NLD caused increase synthesis of the enzyme and therefore may protect against colon cancer. But the high lipid peroxidation observed with this group of rats indicates that catalase failed to prevent reactive oxygen species damage, bearing in mind, the low levels of super oxide dismutase and glutathione reductase also observed with this group of animals.

Decreased catalase level was observed with WLD fed rats. Catalase is a metalloenzyme which is dependent on the availability of copper and iron. Studies have shown that very low levels of activity of catalase are raised prac-tically by the feeding of minute amounts of copper. High fiber vegetables are good sources of copper and the WLD fed rats' decreased feed intake. This would decrea-se the level of catalase.

The inclusion of folic acid to the diets increased catalase activity. Folic acid enhances iron absorption and utilization (Turnland et al., 1985), thereby increasing the activity of catalase since it is a hemoprotein.

When we measured the level of catalase in the colon tissue of experimental rats, we observed a similar trend. High levels of catalase, feed intake and faecal output were observed with experimental animals fed NLD when compared with WLD and ND fed animals. High levels of antioxidant enzymes, super oxide dismutase, catalase and glutathione reductase as well as low levels of lipid peroxidation were observed with the inclusion of folic acid to the diets of experimental

rats. These results suggest the role of folic acid in the diet and colon carcinogenesis. In conclusion, it is quite evident from our results that the Nigerian-like diet and folic acid may help prevent colon cancer. The mechanisms by which this occurs are still not clear and therefore needs intensive investigation.

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