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Influence of dietary fiber from coconut kernel (*Cocos nucifera*) on the 1,2-dimethylhydrazine-induced lipid peroxidation in rats

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The influence of dietary fiber from coconut kernel isolated by the neutral detergent fiber method on the antioxidant status in rats treated with the colon specific carcinogen 1,2-dimethylhydrazine (DMH) was studied in rats fed a high-fat diet for 15 weeks. The DMH-treated fiber group showed higher levels of lipid peroxides than the control group treated with DMH at the preneoplastic and neoplastic stages. Free fatty acid levels were found to decrease significantly in the DMH-treated control group, whereas it was near normal in the fiber groups. Superoxide dismutase and catalase activity also were found to be increased in the liver, intestine, proximal colon, and distal colon. Glutathione levels in all the tissues studied showed significant decreases in the fiber group. The results suggest that coconut kernel fiber can protect cells from loss of oxidative capacity with the administration of the procarcinogen DMH. (J. Nutr. Biochem. 10:555–560, 1999)

Keywords: dietary fiber; 1,2-dimethylhydrazine; lipid peroxidation

Introduction

Experimental, clinical, and metabolic studies have consistently shown that certain types of fiber can decrease carcinogenic metabolites in the gut and reduce or delay chemically-induced carcinogenesis.[1,2] The active metabolites of most carcinogens are thought to evolve from the formation of oxygen-derived free radicals such as superoxide and hydroxyl radicals and intermediates of oxygen reduction products such as hydrogen peroxide. Oxidant damage to DNA, protein, and other macromolecules appears to be the major contributing factor to aging and many degenerative processes (e.g., cancer, heart disease, cataracts, and cognitive dysfunction).[3–6] The evidence for radial mediation of many events in carcinogenesis is very strong.[7,8] Oxygen-derived free radicals, which are generated in fecal material next to colonic epithelium, may play a significant role in the etiology of colon cancer. Studies have shown that the colon-specific carcinogen 1,2-dimethylhydrazine (DMH) possesses an acute oxidative stress to both liver and colon tissue and may cause diminished activity of antioxidant enzymes in the colon.[9,10]

Carcinogenisis studies in rodents and epidemiologic studies in humans suggest a potential role of dietary fiber in the prevention of colorectal cancer.[2] Previous studies from our laboratory demonstrated that coconut fiber brings remarkable modification in the activity of the antioxidant enzymes and also in the levels of lipid peroxides.[11] In addition, people in the South India (particularly in Kerala) also consume large amounts of coconut kernel and coconut oil compared with people from other geographical regions in India, but the incidence of cardiovascular complications is comparable. We studied the effect of dietary fiber from coconut kernel isolated as neutral detergent fiber (NDF) on the antioxidant status in rats treated with the colon-specific carcinogen DMH. The results of this work are reported in this article.

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Table 1 Diet composition

	Fiber free (%)	NDF (%)
Group A		
Dextrin	13	13
Corn starch	30	—
Casein (Vitamin and fat free)	20	20
Fiber	—	30
Ground nut oil	20	20
Group B		
Salt mixture	4	4
Sucrose	10	10
Yeast	1	1
Choline chloride	1	1
Vitamin mixture	1	1

NDF–neutral detergent fiber.

Materials and methods

Animals and diets

Male albino rats of the Sprague-Dawley strain weighing 100 to 120 g were randomly divided into four groups and fed the following diets. Group 1 was fed an isocaloric fiber free diet; Group 2 an isocaloric fiber free diet + DMH injection; Group 3 a coconut fiber diet; and Group 4 a coconut fiber diet + DMH injection. The diet composition is given in *Table 1*.

The composition of the salt mixture was reported earlier;[11] 16.4 g of A (see *Table 1*) of the NDF diet supplied the same number of calories as 12 g of fiber-free diet. The caloric intake of the fiber-free and NDF diet groups were kept the same by adjusting the intake of A. The intakes of salt mixture and vitamin mixture were kept the same in various groups by giving the same amount of B (see *Table 1*; 0.97 g/rat) mixed into the required, weighed quantity of A. Food and water were given ad libitum. The rats were weighed weekly at the same time of day and prior to the DMH injection. The duration of the experiment was 30 weeks. The diets were given during the entire initiation period: 4 weeks of acclimatization, 15 weeks of DMH administration, and an additional 1 week. During the last 10 weeks of the experiment, the rats were given standard rat pellets.

Preparation of fiber

NDF was isolated from the coconut kernel according to the procedure of Goering and Van Soest.[11] The NDF was subjected to digestion with α-amylase to remove residual starch.

Carcinogen administration

DMH hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO USA). DMH was dissolved in 1 mM/L EDTA just prior to subcutaneous (SC) injection and the pH was adjusted to 6.5 with 1 mM/L NaOH to ensure the stability of the chemical. The animals in groups 2 and 4 were given weekly SC injections of DMH in the groin at a dose of 20 mg/kg body weight for 15 weeks.

The animals in the control groups were injected with an equal volume of EDTA (1 mM/L). Rats were sacrificed at two time periods. An initial period was conducted 1 week after the 15 consecutive weeks of DMH injection. The final sacrifice period was undertaken at the 30th week of the experiment. Each rat was subjected to complete autopsy. The tissues were removed to ice-cold containers for various estimations.

Thiobarbituric acid reactive substances (TBARS) were estimated by the TBA assay method of Nichans and Sammulsson.[12] Hydroperoxides, conjugated dienes, and free fatty acids (FFAs) were also monitored,[11] and glutathione was estimated.[11] The catalase activity was determined by the method of Maehly and Chance[13] and superoxide dismutase by the method of Kakkar et al.[14] Statistical significance was calculated by Student's *t*-test.[15]

Results

The effects of coconut fiber supplementation on the FFA levels in various tissues are given in *Table 2*. There was a significant decrease in the levels of free fatty acids in all tissues at the 15th and 30th weeks in the DMH-treated control rats (Group 2) compared with the control rats. There was an apparent reduction in the levels of FFA in all tissues of Group 3 (coconut fiber) group compared with the control group (Group 1). The DMH-treated coconut fiber group showed a significant reduction in the FFA levels in liver when compared with Group 2 and Group 3. There was no significant difference in the intestine, but there was an

Table 2 Concentration of free fatty acids in liver, intestine, proximal colon, and distal colon

	Week	Group 1	Group 2	Group 3	Group 4
Liver (mg/100 g tissue)	15th	698.0 ± 35.82	632.56 ± 34.79*	363 ± 15.37*	530 ± 19.61*
	30th	745.26 ± 288	628 ± 27.27*	559.1 ± 25.71*	473.25 ± 25.15*
Intestine (mg/100 g tissue)	15th	759.5 ± 29.90	638 ± 17.86*	523.5 ± 28.27*	490 ± 16.79*
	30th	859.4 ± 44.68	663 ± 24.91*	685 ± 16.38*	703 ± 30.2*
Proximal colon (mg/100 g tissue)	15th	864 ± 31.84	742.6 ± 31.18*	574 ± 26.15*	800 ± 26.66
	30th	807.75 ± 30.69	650.56 ± 29.92*	617.75 ± 26.6*	714 ± 23.85
Distal colon (mg/100 g tissue)	15th	828 ± 31.57	694 ± 30.82*	547.9 ± 24.33*	723 ± 30.12
	30th	717.25 ± 27.85	640.5 ± 23.24*	659.46 ± 24.2*	671.3 ± 32.3

Note: Values are mean ±SE of six rats in each group.
Group-1-Isocaloric Fiber Free Diet
Group-2-Isocaloric Fiber Free Diet + Dimethylhydrazine
Group-3-Coconut Fiber Diet
Group-4-Coconut Fiber Diet + Dimethylhydrazine
*$P \leq 0.05$.

Table 3 Levels of thiobarbituric acid reducing substances in liver, intestine, proximal colon, and distal colon

	Week	Group 1	Group 2	Group 3	Group 4
Liver (mmol/ 100 g tissue)	15th	0.9 ± 0.225	0.62 ± 0.0217*	0.87 ± 0.0278*	0.99 ± 0.0346*
	30th	0.396 ± 0.012	0.123 ± 0.06	0.384 ± 0.0103*	0.365 ± 0.05*
Intestine (mmol/100 g tissue)	15th	0.954 ± 0.026	0.667 ± 0.072*	0.66 ± 0.024*	0.454 ± 0.0104*
	30th	0.491 ± 0.017	0.101 ± 0.003*	0.41 ± 0.15*	0.463 ± 0.015*
Proximal colon (mmol/100 g tissue)	15th	0.37 ± 0.129	0.282 ± 0.0116*	0.47 ± 0.0146*	0.641 ± 0.019*
	30th	0.328 ± 0.008	0.15 ± 0.004*	0.398 ± 0.017	0.2943 ± 0.012*
Distal colon (mmol/100 g tissue)	15th	0.51 ± 0.0117	0.326 ± 0.008*	0.4473 ± 0.009*	0.4196 ± 0.017*
	30th	0.433 ± 0.018	0.1129 ± 0.004*	0.4536 ± 0.016*	0.4068 ± 0.019*

Note: Values are mean ±SE of six rats in each group.
Group-1-Isocaloric Fiber Free Diet
Group-2-Isocaloric Fiber Free Diet + Dimethylhydrazine
Group-3-Coconut Fiber Diet
Group-4-Coconut Fiber Diet + Dimethylhydrazine
*$P \leq 0.05$.

apparent increase in FFA concentration in the proximal colon at both the 15th and 30th weeks. There was an increase in the concentration of FFA in the distal colon of Group 4 when compared with Group 3 at the 15th week, but there was no significant change at the 30th week.

The levels of TBARS and conjugated dienes (*Tables 3 and 4*) were significantly low in all of the tissues of the DMH-treated control group compared with the control group. The DMH-treated fiber group had significantly higher levels of both TBARS and conjugated dienes at the 15th and 30th weeks compared with the DMH-treated control group.

Hydroperoxide levels (*Table 5*) in the DMH-treated control group were elevated in the liver and significantly decreased in all other tissues when compared with the control group. In the DMH-treated fiber group, at the 15th week, there was a significant increase in the intestine, proximal colon, and distal colon and a significant decrease at the 30th week compared with the DMH-treated control group.

The response of the antiperoxidative enzymes superoxide dismutase and catalase in all the tissues (*Tables 6 and 7*) was significantly greater at the 15th and 30th weeks in the fiber groups compared with the control group treated with DMH. The DMH-treated control groups had significantly higher levels of glutathione content (*Table 8*) at the 15th and 30th weeks in all the tissues compared with the control group. The carcinogen-treated fiber group showed a significant decrease in the levels of glutathione in all tissues when compared with the carcinogen-treated control group.

Discussion

The results of the present study show that the preneoplastic colon mucosa of rats given DMH for 15 weeks had decreased levels of lipid peroxides. The higher levels of

Table 4 Levels of conjugated dienes in liver, intestine, proximal colon, and distal colon

	Week	Group 1	Group 2	Group 3	Group 4
Liver (mmol/ 100 g tissue)	15th	59.91 ± 3.35	45.23 ± 1.26*	48.35 ± 1.56*	68.22 ± 2.513*
	30th	31.86 ± 1.33	24.37 ± 1.206*	42.09 ± 1.76*	29.8 ± 0.865*
Intestine (mmol/100 g tissue)	15th	109.78 ± 6.8	75.89 ± 2.88*	76.84 ± 2.99*	82.72 ± 3.39
	30th	67.6 ± 2.5	25.6 ± 1.31*	58.97 ± 3.30*	73.9 ± 4.22*
Proximal colon (mmol/100 g tissue)	15th	47.66 ± 2.19	36.13 ± 1.3*	43.57 ± 1.87*	49.9 ± 2.44*
	30th	24.09 ± 1.15	18.05 ± 0.45*	45.57 ± 2.05*	28.28 ± 0.84*
Distal colon (mmol/100 g tissue)	15th	58.13 ± 1.44	48.85 ± 2.34*	57.84 ± 3.12*	81.71 ± 4.25*
	30th	54.01 ± 2.808	25.64 ± 0.89*	24.67 ± 0.863*	29.49 ± 0.943*

Note: Values are mean ±SE of six rats in each group.
Group-1-Isocaloric Fiber Free Diet
Group-2-Isocaloric Fiber Free Diet + Dimethylhydrazine
Group-3-Coconut Fiber Diet
Group-4-Coconut Fiber Diet + Dimethylhydrazine
*$P \leq 0.05$.

Table 5 Levels of hydroperoxides in liver, intestine, proximal colon, and distal colon

	Week	Group 1	Group 2	Group 3	Group 4
Liver (mmol/ 100 g tissue)	15th	9.06 ± 0.253	23 ± 0.82*	13.41 ± 0.469*	29.62 ± 1.24*
	30th	28.78 ± 1.007	51.03 ± 2.29*	20.52 ± 0.779*	38.56 ± 1.118*
Intestine (mmol/100 g tissue)	15th	31.01 ± 1.39	28.8 ± 1.09	28.95 ± 0.652*	57.2 ± 29.09*
	30th	70.5 ± 3.92	55 ± 3.025*	40.81 ± 2.12*	53.02 ± 4.45*
Proximal colon (mmol/100 g tissue)	15th	34.36 ± 1.23	30.2 ± 1.57*	18.92 ± 0.643*	44.8 ± 1.29*
	30th	64.83 ± 3.37	43.7 ± 2.09*	18.83 ± 0.64*	15.57 ± 0.872*
Distal colon (mmol/100 g tissue)	15th	32.06 ± 1.15	20.39 ± 0.782*	28.24 ± 1.35*	30.95 ± 1.609*
	30th	51.68 ± 2.75	46.5 ± 1.91	41.24 ± 2.302*	32.28 ± 2.63*

Note: Values are mean ±SE of six rats in each group.
Group-1-Isocaloric Fiber Free Diet
Group-2-Isocaloric Fiber Free Diet + Dimethylhydrazine
Group-3-Coconut Fiber Diet
Group-4-Coconut Fiber Diet + Dimethylhydrazine
*$P \leq 0.05$.

lipid peroxides in the DMH-treated coconut fiber group (compared with the control rats not given DMH) suggest that in the fiber group the cells are active and most of the normal metabolic activity is intact. At the same time, the fiber groups had low levels of lipid peroxides compared with the control group. The decrease in the levels of lipid peroxides in the fiber group suggests that coconut fiber can counteract the increased intake of fat and thereby maintain most of the biochemical parameters at the normal level.[11]

Our studies also show that at the neoplastic stage (30th week), the DMH-treated control group had decreased levels of lipid peroxides. Similar results have been noted in other tumor model systems.[16] Here again, the DMH-treated fiber group had higher levels of the products of lipid peroxidation.

These findings suggest that coconut fiber can protect cells from loss of their oxidative capacity with the administration of the procarcinogen DMH. The low rates of lipid peroxidation in the carcinogen-treated group may be due to an increase of natural antioxidants, free radical trapping materials, and/or a significant decrease of polyunsaturated fatty acids (PUFA), especially arachidonic acid.[17] Mechanisms have been proposed to explain diminished lipid peroxidation in tumor cells on the basis of reduction in NADPH-cytochrome P-450 electron transport and a decrease in lipid soluble antioxidants often associated with reduction in the content of PUFA.[18]

There was a significant decrease in the levels of FFAs in the liver, intestine, proximal colon, and distal colon of the DMH-treated control rats. On the other hand, animals given DMH and the coconut fiber had higher levels of FFA compared with control rats given DMH, but it was close to normal. This accounts for the higher levels of lipid peroxidation (near control) in the DMH-treated fiber groups than in the DMH-treated control group. Massoti et al.[19] also observed decreased levels of FFAs. In the case of hydroperoxides and conjugated dienes, which are intermediates in the formation of different lipid peroxide products (cyclooxygenase and lipoxygenase pathway), the levels decreased in the different tissues, except in the liver, where it was increased in the DMH-treated control group.

The liver is an important organ in which a number of

Table 6 Activity of superoxide dismutase in liver, intestine, proximal colon, and distal colon

	Week	Group 1	Group 2	Group 3	Group 4
Liver	15th	8.8 ± 0.316	5.18 ± 0.16*	7.1 ± 0.338*	7.92 ± 0.1402*
	30th	7.6 ± 0.208	2.59 ± 0.069*	7.02 ± 0.3444	6.515 ± 0.38*
Intestine	15th	4.25 ± 0.153	3.16 ± 0.131*	4.445 ± 0.12	4.446 ± 0.245*
	30th	4.17 ± 0.162	2.32 ± 0.12*	3.233 ± 0.199	2.823 ± 0.146
Proximal colon	15th	4.1 ± 0.125	3.6 ± 0.1212*	4.5 ± 0.261*	6.59 ± 0.401*
	30th	4.12 ± 0.196	2.162 ± 0.12*	3.2165 ± 0.18*	3.61 ± 0.184*
Distal colon	15th	3.64 ± 0.164	2.8 ± 0.106*	4.56 ± 0.26*	5.2 ± 0.312*
	30th	3.74 ± 0.12	1.67 ± 0.052*	3.17 ± 0.18	2.56 ± 0.13*

Note: Values are mean ±SE of six rats in each group. Expressed in units/mg protein. (A unit is an enzyme concentration required to inhibit optical density at 560 nm of chromogen production by 50% in 1 minute.
Group-1-Isocaloric Fiber Free Diet
Group-2-Isocaloric Fiber Free Diet + Dimethylhydrazine
Group-3-Coconut Fiber Diet
Group-4-Coconut Fiber Diet + Dimethylhydrazine
*$P \leq 0.05$.

Table 7 Activity of catalase in liver, intestine, proximal colon, and distal colon

	Week	Group 1	Group 2	Group 3	Group 4
Liver	15th	1.21 ± 0.038	1.22 ± 0.045	1.3 ± 0.032	2.91 ± 0.052*
	30th	1.2 ± 0.039	0.78 ± 0.021*	1.09 ± 0.029	1.2 ± 0.034*
Intestine	15th	1.55 ± 0.043	1.26 ± 0.06[b]	2.51 ± 0.094	1.77 ± 0.063*
	30th	1.79 ± 0.052	1.4 ± 0.04*	2.4 ± 0.06	2.33 ± 0078*
Proximal colon	15th	2.09 ± 0.201	2.22 ± 0.07*	2.55 ± 0.13*	2.6 ± 0.13*
	30th	1.89 ± 0.05	1.7 ± 0.07	1.8 ± 0.10	1.7 ± 0.09
Distal colon	15th	2.91 ± 0.045	2.16 ± 0.03*	2.68 ± 0.14	2.25 ± 0.11*
	30th	2.23 ± 0.10	1.3 ± 0.13*	2.5 ± 0.13	3.0 ± 0.16*

Note: Values are mean ±SE of six rats in each group. Values $\times 10^{-1}$ units/mg protein. (A unit is the velocity constant/sec.
Group-1-Isocaloric Fiber Free Diet
Group-2-Isocaloric Fiber Free Diet + Dimethylhydrazine
Group-3-Coconut Fiber Diet
Group-4-Coconut Fiber Diet + Dimethylhydrazine
*$P \leq 0.05$.

metabolic reactions occur. Changes in other tissues to a certain extent also are reflected in the metabolic activity of the liver. Administration of DMH results in decreased levels of TBARS and no change in FFAs, but the levels of hydroperoxides increase (in the fiber group). This increase in hydroperoxides may result from the metabolic conversion of fatty acid to products in the eicosanoid pathway, which under normal conditions may be beneficial, but under altered conditions may be deleterious.

Associated with the decreased levels of FFAs and lipid peroxides in the DMH-treated fiber group compared with the DMH-treated control group, the fiber groups had higher activity of superoxide dismutase and catalase. The enzymes superoxide dismutase and catalase play important roles in scavenging toxic intermediates of reactive oxygen species. The activity of these enzymes in the DMH-treated group (Group 2) was found to be lower compared with the control group.

Intracellular antioxidant enzyme concentrations have been found to be indicators of a cell's susceptibility to oxidative damage in vitro.[9] Higher levels of the antioxidant enzymes have been correlated with decreased susceptibility to chemically induced cancer and cell damage in vivo.[20,21] The increased activity of these enzymes is probably due to (1) the removal of any inhibitor, if present, in the intestine and colon by dietary fiber, (2) the removal of any toxic intermediate by dietary fiber, which may be synthesized by the intestinal colon microflora (because of a high-fat diet), which can poison the enzyme; and/or (3) activation of these enzymes by the components of dietary fiber. In addition to this, the increased intake of dietary fiber can induce indirect diet restriction. This may be responsible for the molecular expression and increased synthesis of these antiperoxidative enzymes, which will result in decreased peroxidation. Several investigators have proposed that diet restriction modulates the aging process through free radical reactions,[22,23] and it has been reported that catalase activity was higher in liver tissue from rodents given energy-restricted diets than in tissue from rodents fed ad libitum.[24,25] This diet-restriction–mediated increase in activity of these enzymes was achieved at the level of transcription.[26,27]

The DMH-treated control diet group showed higher levels of glutathione in all of the tissues compared with the control group. The level of glutathione decreased in the

Table 8 Levels of glutathione in liver, intestine, proximal colon, and distal colon

	Week	Group 1	Group 2	Group 3	Group 4
Liver (mmol/ 100 g tissue)	15th	353.7 ± 18.39	353 ± 19.5	289 ± 11.27*	224.12 ± 10.26*
	30th	299.3 ± 13.16	397.5 ± 14.7*	360.1 ± 13.29*	321 ± 16.89*
Intestine (mmol/100 g tissue)	15th	208.9 ± 12.74	266.69 ± 12.6*	259 ± 13.74*	151.15 ± 7.859*
	30th	174.2 ± 8.88	349.1 ± 13.7*	153.75 ± 6.91*	199 ± 10.40*
Proximal colon (mmol/100 g tissue)	15th	296.16 ± 13.32	305 ± 14.8	198.7 ± 10.81*	187.5 ± 6.87*
	30th	279 ± 9.5	391.9 ± 13.8*	134.8 ± 7.27*	146.1 ± 7.59*
Distal colon (mmol/100 g tissue)	15th	261.4 ± 13.5	307.1 ± 14.2*	153.83 ± 9.7*	129.83 ± 12.56*
	30th	186.5 ± 9.87	315.7 ± 11.2*	143.7 ± 10.07*	122 ± 12.43*

Note: Values are mean ±SE of six rats in each group.
Group-1-Isocaloric Fiber Free Diet
Group-2-Isocaloric Fiber Free Diet + Dimethylhydrazine
Group-3-Coconut Fiber Diet
Group-4-Coconut Fiber Diet + Dimethylhydrazine
*$P \leq 0.05$.

fiber group. It has been reported recently that the DMH treatment results in an increase in the reduced glutathione concentration.[28] The decreased level of glutathione (a reductant) suggests that the microsomal lipid peroxidation system follows a controlled normal pathway. The levels of reduced glutathione increase in the DMH-treated rats suggest an increased concentration of the reductant. This increase in the concentration of the reductant should normally result in increased lipid peroxidation. The observed decrease in concentration of lipid peroxidation in spite of the increased levels of reduced glutathione suggests that the peroxides that formed should have been channeled through the eicosanoid pathway and these may manifest their toxic effects in colon carcinoma. Supplementation of the fiber protects the colon by preventing the formation of unwanted free radicals that may damage the mucosal lining of the lower gastrointestinal tract. The observed "no change" with the control agrees with this view. Thus the dietary fiber from coconut, controls the level of free radicals, preventing any lipid peroxide associated damage to occur in the colon.

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