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Dietary Fiber from *Musa paradisiaca* and *Artocarpus heterophyllus* on Intestinal Mucosal and Bacterial β-Glucuronidase Activity in Hexachlorocyclohexane-Treated Rats

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Dietary fiber, causes changes in the morphological and functional parameters of the small intestine (Cassidy et al. 1982; Sigleo et al. 1984). Yoshida et al. (1983) reported that dietary fiber influenced hypercholesterolemia induced by polychlorinated biphenyls. Burkitt (1975) observed that dietary fibers like cellulose and alfalfa have protective action against toxic agents. Different plant fibers may act differently to counteract the toxicity of xenobiotics.

Hexachlorocyclohexane (HCH) is one of the extensively used insecticides having long persistence and reported to be present in human tissues (Kaphalia et al. 1978; Gupta et al. 1980). No detailed work has been reported in literature on the effect of various dietary fibers on intestinal mucosal and bacterial β-glucuronidase activity in the rats fed on HCH. The objective of this study is to investigate the effect of Neutral detergent fiber (NDF) extracted from commonly used vegetables in Kerala such as plantain stem (inflorescence stalk of Musa paradisiaca, MP) and Jack tender (Artocarpus heterophyllus, AH) on intestinal mucosal and bacterial β-glucuronidase activity and digestibility of NDF in rats fed on HCH, the in vitro effect of HCH on β-glucuronidase activity in different tissues and in vitro binding of HCH by NDF.

MATERIALS AND METHODS

Neutral detergent fiber (NDF) was prepared from dry defatted material (Van Soest et al. 1973). It was then subjected to exhaustive digestion with α-amylase in 0.1M phosphate buffer (pH 7.2) containing 0.0067M NaCl till all the residual starch was completely removed. Fiber components such as acid detergent residue (ADF), hemicellulose, lignin, cellulose, cutin and silica were determined by the neutral detergent system of analysis of Van Soest et al. (1973)

Male albino rats (Sprague Dawley strain) weighing 80-100g bred and reared in our laboratory were grouped into 4 of six each and maintained on the respective diets (Table - 1).

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Table 1. Composition of diet

	0% Fiber	7% Fiber
	(.....g/100g diet....)	
CHO*	65.00	58.00
Casein	20.00	20.00
Groundnut oil	10.00	10.00
Salt mixture	4.00	4.00
Vitamine mixture	1.00	1.00
Fiber	-	7.00

* CHO - Equal parts of Glucose; Dextrin; Sucrose and Corn Starch

Group 1, control rats fed on fiber free diet (FF): Group 2, rats fed on fiber free diet + HCH (FF+HCH): Group 3, rats fed on 7% Musa paradisiaca NDF+HCH (7% MPNDF+HCH): Group 4, rats fed on 7% Artocarpus heterophyllus NDF+HCH (7% AHNDF+HCH). 7% Fiber was added at the expense of CHO. The caloric intake of all the groups was maintained unchanged by adjusting the food intake. 10g of 7% NDF diet provided the same calories as that by 9.33g FF diet. NDF was taken to contribute very little towards caloric value of the diet. Technical grade HCH (Premier Pesticides Ltd., Cochin, India, 99% pure) was administered (60 mg/kg body wt/day) in groundnut oil. The dose selected was LD_{25} as by pilot tests. The control group was given the same amount of groundnut oil. The duration of the experiment was 30 days. 24 hr feces were collected during the last 5 alternate days. Aliquots of each diet and 5 day fecal collection of each group was analysed for fiber content to determine the percentage of digestibility

i.e. $\frac{\text{intake (g/5 days)} - \text{feces (g/5 days)}}{\text{intake(g/5 days)}} \times 100$

At the end of 30 days, rats were sacrificed under ether anaesthesia; small intestine, cecum, colon and the contents of cecum and colon were immediately collected and used for the determination of β-glucuronidase (E.C.3.2.1.31) activity (Reddy and Mangat 1977). The protein content was determined by Lowry et al. (1951).

In vitro effect of HCH on tissue β-glucuronidase activity was determined by using tissue homogenates of normal Sprague Dawley strain rats (1:4 wt/vol) in 0.25 M sucrose. The homogenates were preincubated with 1 µg and 2 µg HCH for 5 minutes at assay temperature prior to the addition of substrate.

In vitro binding of HCH by NDF from MP and AH was studied by shaking 500 mg of NDF with 0.3 M NaCl for an hour and then with the solution of HCH in 0.3M NaCl for 2 hr at 37°C. In the control, NDF in 0.3M NaCl was mixed with HCH solution and centrifuged immediately. HCH present in the supernatant of both tests and control was extracted with acetonitril and analysis of HCH was done by GLC (Arnold et al. 1985). Statistical significance of differences among values were analysed by one way analysis of variance (Snedecor & Cochran 1968) followed by multiple range test (Kramer 1956).

Table 2. Composition of Neutral Detergent Fiber (NDF) from MP, AH (values are mean ± SEM from 10 estimations)

Materials	Water g/100g	NDF	ADF	Hemi cellulose	Lignin	Cellulose	Cutin and Silica
		g/100g defatted material					
1. Musa paradisiaca(MP)	95.70±2.57	43.61±1.98	35.7±1.63	7.9±0.57	9.60±1.50	25.0±1.50	1.70±0.23
2. Artocarpus heterophyllus(AH)	83.03±2.37	40.30±1.87	36.7±1.72	4.8±0.41	15.40±1.65	17.3±1.42	4.00±0.37

NDF : Neutral detergent fiber, ADF : Acid detergent fiber.

Table 3. Mucosal and Bacterial β-glucuronidase activity in HCH administered rats fed vegetable neutral detergent fiber (Values are mean ± SEM of 6 rats in each group)

	FF	FF + HCH	7% MPNDF+HCH	7% AHNDF+HCH
		A. Mucosal activity/mg protein		
Small intestine	75.7±3.03	93.9±6.57[b]	70.9±2.13[b]	72.4±2.53[b]
Colon	72.9±2.89	91.9±6.43[b]	59.3±1.78[b]	71.6±2.51[b]
Cecum	78.4±2.74	98.0±6.86[b]	71.0±2.13[b]	80.0±3.20[b]
		B. Bacterial activity/mg protein		
Cecum	1077±37.69	1232±86.24[b]	1040±31.20[b]	1080±43.20[b]
Colon	1037±31.11	1260±88.20[b]	1046±36.61[b]	1056±42.24[b]

A μg phenolphthaline liberated/hr at 37°C B μg phenolphthaline liberated/4 hr at 37°C
$P < 0.01$ = b

Table 3a. Intergroup comparison of mucosal and bacterial β-glucuronidase activity in HCH administered rats fed vegetable neutral detergent fiber

Parameter	F value	Comparison	Computed value	R' 5%	P 1%	Significance (P<)
A. Mucosal β-glucuronidase activity						
Small intestine	78.714	M1 Vs M2	44.58	8.10	10.93	0.01
		M3 Vs M2	56.34	8.50	11.43	0.01
		M4 Vs M2	52.66	8.31	11.19	0.01
Colon	85.261	M1 Vs M2	48.01	8.58	11.56	0.01
		M3 Vs M2	66.14	8.78	11.80	0.01
		M4 Vs M2	44.09	8.37	11.28	0.01
Cecum	136.276	M1 Vs M2	46.54	7.85	10.59	0.01
		M3 Vs M2	79.85	8.24	11.07	0.01
		M4 Vs M2	49.72	8.06	10.84	0.01
B. Bacterial β-glucuronidase activity						
Colon	44.759	M1 Vs M2	546.24	110.56	148.67	0.01
		M3 Vs M2	524.19	108.15	145.58	0.01
		M4 Vs M2	499.70	105.41	142.15	0.01
Cecum	27.887	M1 Vs M2	379.67	108.02	145.40	0.01
		M3 Vs M2	470.30	110.42	148.49	0.01
		M4 Vs M2	372.32	105.28	141.97	0.01

R' P Significance range factor; M1 - FF; M2 - FF+HCH; M3- 7% MPNDF+HCH; M4 - 7% AHNDF+HCH

RESULTS AND DISCUSSION

NDF from MP contains 7.9% hemicellulose, 9.6% lignin, 25% cellulose and 1.7% cutin and silica. NDF from AH had hemicellulose 4.8%, lignin 15.4% cellulose 17.3% cutin and silica 4% (Table - 2).

Compared to rats fed on FF diet, bacterial β-glucuronidase activity (Table - 3) in the contents of cecum and colon and in the mucosa of small intestine and colon of HCH administered FF diet group as increased. Animals fed on AHNDF and MPNDF at 7% level along with HCH showed a lower β-glucuronidase activity in the contents of cecum and colon as well as in the mucosa of small intestine and colon and the decrease was most significant in MPNDF fed group. The activity of β-glucuronidase in the mucosa of small intestine, colon, cecum and colon contents of MPNDF and HCH fed group showed significantly lower activity than that of FF control group. Digestibility of NDF from MP and AH was found to be 8% and 13% respectively.

In vitro study showed that the β-glucuronidase activity in liver, small intestine, colon and cecum was accelerated significantly by HCH at very low concentration of 1 µg and the increase was concentration dependent (Table - 4).

Table 4. In vitro effect of HCH on β-glucuronidase activity

Concentration of HCH	% Activation			
	Liver	Small Intestine	Colon	Cecum
Control	100	100	100	100
1 µg	118	112	107	115
2 µg	124	120	116	127

In vitro binding of HCH by NDF from Musa paradisiaca and Artocarpus heterophyllus showed that maximum binding was observed in the case of NDF from MP (69±0.40) and minimum in the case of AH (63±0.32).

Antonis and Bersohn (1962) stated that the fiber content of food determines transit time through the gastrointestinal tract and influences the weight, consistency and bacterial content of the feces and that a high intake of fiber increased bile acid excretion. Results of this investigation indicate that feeding of fiber from Musa paradisiaca and Artocarpus heterophyllus at 7% level along with HCH has an effect on small and large intestinal and cecal, mucosal and bacterial β-glucuronidase activity. Administration of HCH on FF diet fed rats stimulated the intestinal and bacterial β-glucuronidase activity. It is supported by in vitro study that the activity of β-glucuronidase in liver,small intestine, colon and cecum was increased significantly by HCH at a very low concentration. Glucuronide formation is an important mechanism involved in detoxification in mammals. Since bacterial activity of intestinal microflora is

enhanced by administration of HCH by FF group which in turn increases the absorption of HCH and its endogenous metabolites and these may increase the toxicity of HCH in FF diet fed rats. Fiber from MP and AH had decreased the biological activity of intestinal microflora thereby decreasing the absorption and reabsorption of HCH and their metabolites. Since the intestinal microflora are changed by fiber, these changes might alter the biological activity, toxicity, excretion and absorption of exogenous HCH and its metabolites. In vitro study demonstrated that the binding of HCH may be one of the reasons that result in the excretion of considerable quantities of HCH from the body and make less HCH available for intestinal absorption. The work is in progress to determine the residual HCH in tissues and in excreta and also the effect of NDF on detoxification systems.

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