Effect of *Cocos nucifera* and red chilli on intestinal β-glucuronidase and mucinase activity in experimental colon cancer

N Nalini[1] MSc, S Chitra[1] MSc, K Sabitha[1] MSc, P Viswanathan[2] MD and Venugopal P Menon[1] MSc PhD

1. *Department of Biochemistry, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India*
2. *Department of Pathology, Rajah Muthiah medical College,Annamalai University, India*

Effect of Cocos nucifera and red chilli on intestinal B-glucuronidase and faecal mucinase activity, was studied in rats given 1 ,2-dimethylhydrazine (DMH) . The average weight gain by the animals given coconut kernel was more than the DMH and chilli treated groups. The activity of B-glucuronidase decreased in the kernel groups, in most of the tissues studied, as compared to the DMH and chilli treated groups. A similar pattern was observed in the case of mucinase. Morphological studies showed that the number of visible malignant tumours decreased in the colon and intestine of the animals, when their diet was supplemented with coconut kernel. Histopathological studies also showed that the animals had fewer papillae, lesser infiltration into the sub-mucosa and lesser changes in the cytoplasm with decreased mitotic figures, when kernel was included in the diet. Coconut kernel, thus reduced the mutagenic and carcinogenic effect of chilli and DMH respectively.

Introduction

Dietary intervention can protect humans from a variety of diseases and has led to the formulation of a number of explanatory hypotheses, several of which involve the bacteria of the lower intestine[1-5]. These bacteria are capable of a wide variety of metabolic activities including production of toxic metabolites, transformation of bile acids, reduction and hydrolysis of drugs that may positively act as carcinogens and/or co-carcinogens.

β–glucuronidase and mucinase are two important enzymes which reflect the activity of these bacteria. Mucinase is the enzyme which hydrolyses the protective mucins and β–glucuronidase hydrolyses biliary glucuronides. If glucuronide hydrolysis is a rate-limiting step in this process, then the levels of microbial β–glucuronidase in the colon may influence the risk of colon cancer.

Among the spices, red-chilli is consumed in large quantities in different parts of India[6,7]. Coconut kernel is also an important constituent of Indian food. *In vitro* studies have shown that red chilli and its irritative phenolic compound, capsaicin, known to have an established structure of N-(4-hydroxy-3-methoxy benzyl)-8-methyl trans-6-enamide[6,7] to be a mutagenic, carcinogenic and tumour promoting agent[10,11]. Since *Cocos nucifera* forms an important constituent of Indian food, we have studied the effect of coconut kernel on 1, 2-dimethylhydrazine (DMH) induced colon carcinoma and also its effect, in the presence of red chilli.

Materials and methods

Wistar male albino rats bred in the Animal House of Rajah Muthiah Medical College, Annamalai University, weighing 120-150 g were divided into 7 groups of 10 rats each. They were all fed a commercial diet (Lipton Lever Limited) containing 20% peanut oil . Water was given *ad libitum*.

Group 1 were control rats, group 2 were rats fed fresh coconut kernel (30%), group 3 were rats administered DMH*, group 4 were rats fed red chilli powder (8mg/day/ 100g body weight in water), group 5 chilli + DMH, group 6 fresh coconut kernel + red chilli, group 7 fresh coconut kernel + chilli + DMH.

The fat intake by the animals in groups 2, 6 and 7 were adjusted, so that it was similar to the fat intake in groups 3, 4 and 5. The caloric intake of animals in groups 3, 4 and 5 were also similar to that of 2, 6 and 7. p-nitrophenyl β-D-glucuronide, mucin and 1,2-dimethylhydrazine were purchased from Sigma Chemical Co, St. Louis, MO, USA. All the other chemicals used were of analytical grade and were purchased from SD Fine Chemicals, Bombay, India.

DMH was administered as reported earlier[12]. After 15 weeks, the DMH injection was discontinued and the rats were given only commercial diet. The animals were observed daily and weighed every week. At the end of 30 weeks, fresh faecal pellets were collected and the activity of mucinase was estimated by the method of Shiau and Chang[13]. The rats were then sacrificed and the neoplasms in the intestine and colon were counted after cutting open the tissues longitudinally taking care not to disturb the tumours. Part of the tissues were sent for histopathological examination. The rest of the tissues and colon contents (bacterial contents) were transferred to ice cold containers, for measuring the activity of β-glucuronidase[14]. Protein was estimated by the method of Lowry *et al*[15].

Results obtained are expressed as mean ± SE from 6 rats in each group. The statistical significance of difference in means was analysed by Student's t-test. A one way analysis of variance (ANOVA) was also determined[16].

Results

Table 1 shows the incidence of colon and intestinal tumours in all the 7 groups. The values expressed are the sum of about 30 surviving rats from different experiments. The incidence and

Correspondence address: Dr Venugopal P Menon, Professor and Head, Department of Biochemistry, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India
Tel: +91-4144-22282 Fax: +91-4144-23080

number of tumours decreased when coconut kernel was supplemented in the diet.

The macroscopy and light microscopic observations (histopathological) of the colon of rats in different groups are given in Table 2. The table shows that when kernel was supplemented in the diet the animals showed fewer papillae, lesser infiltration into the submucosa and less changes in the cytoplasm with decreased mitotic figures.

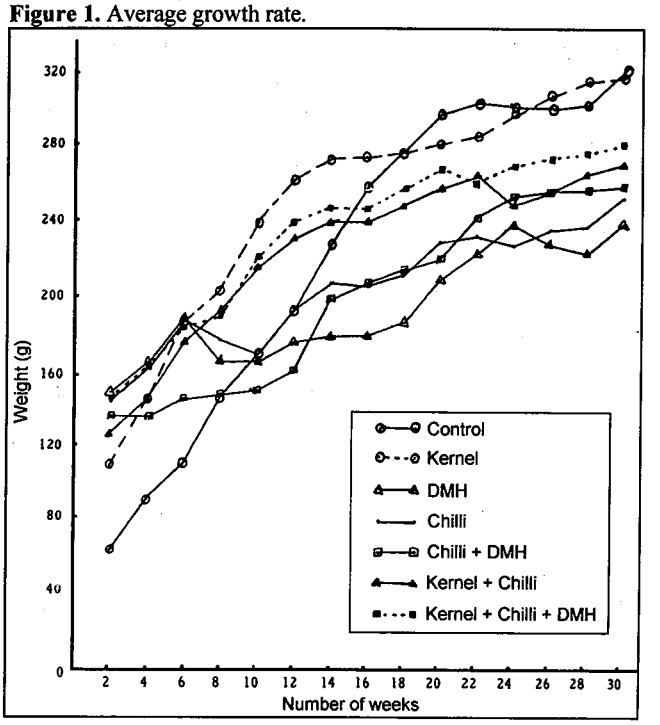
Table 1. Incidence of colon and intestinal tumours.

Rat group	Rats with tumours/ total rats	Incidence of colon tumours (%)	Tumours in colon/ tumour-bearing rat	Tumours in intestine/ tumour-bearing rat
Group 1 Control	Nil	Nil	Nil	Nil
Group 2 Kernel	Nil	Nil	Nil	Nil
Group 3 DMH	27/30	90.0	26	12
Group 4 Chilli	25/30	83.3	17	8
Group 5 Chilli + DMH	28/30	93.3	34	15
Group 6 Kernel + chilli	6/30	20.0	2	Nil
Group 7 Kernel + chilli + DMH	22/30	73.3	12 3	

Figure 1 gives the average growth rate of the animals in the various groups. It was observed that the weight gained by the control group > kernel group > kernel + chilli + DMH > kernel + chilli > chilli + DMH > chilli > DMH, even though the average food intake by the animals of the various groups were more or less similar. The energy intake was the same in all the groups. Figure 2 shows the intestine and colon of rat.

β-glucuronidase activity showed a significant increase in the DMH, chilli and chilli + DMH groups when compared with the control rats (Table 2).

Figure 1. Average growth rate.



In the chilli treated animals the β-glucuronidase levels increased significantly in the colon, intestines and liver, and also in the colon contents (bacterial) when compared with kernel + chilli. Similarly, when the chilli + DMH group was compared with the kernel + chilli + DMH group, the β-glucuronidase level

Table 2. Histopathological changes in the colon.

	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Macroscopy						
1. Size	-	2 cm	1 cm	2 cm	< 0.5 cm	1 cm
2. Margin	-	Well defined	Defined	Ill defined	Ill defined	Ill defined
3. Nature	-	Pedunculated	Pedunculated	Pedunculated	Sessile	Sessile
Microscopy						
1. Transitional zone with foci of dysplasia	-	Not present	Focal areas of dysplasia	Present	Occasional dysplastic gland	Present
2. Inflammatory cell infiltrate into the mucosa	-	Mixed population	Mixed population	Mixed population	Mixed population	Mixed population
3. Lymphoid aggregates in the submucosa	-	Not observed	Not observed	Occasional lymphoid aggregate	Not observed	Not observed
4. Papillary pattern	-	Large number of papillae	No papillae	Few papillae	No papillae	Significant number of papillae
5. Mucin secretion	-	Few glands dilated, filled with mucin	-	Glands dilated and filled with mucin	-	Some glands filled with secretion
6. Infiltration in the submucosa	-	Observed	-	Several areas of infiltration	-	Occasional focus of infiltration seen
7. Cell morphology						
i) Nuclear pleomorphism	-	Marked	Less severe	Marked	Less severe	Marked
ii) Nucleoli	-	Prominent	Less prominent	Prominent	Less prominent	Prominent
iii) Cytoplasm	-	Scanty	Moderate	Scanty	Moderate	Scanty
iv) Mitotic figures	-	Numerous	Present	Numerous	Not present	Present
Others						
1. Vascular granulation	Increased vascular granulation	Not seen	Not seen	Vascular granulation (not very prominent)	Not seen	Vascular granulation present

Table 3. β-glucuronidase activity (mg of p-nitrophenol liberated/ hr/ g protein).

	Group1	Group2	Group3	Group4	Group5	Group6	Group7	F-ratio
Distal colon	55.84±6.916	40.317±5.862 a	108.74±9.275 a	44.76±4.350 b	83.40±14.500	42.15±3.101 a	61.60±2.184 NS	68.26*
Proximal colon	47.25±3.783	30.00±2.484 a	47.36±3.730 NS	50.97±5.449 NS	56.91±7.269 b	47.95±2.020 NS	59.50±3.301 a	29.78*
Distal intestine	53.66±6.245	42.01±3.357 b	54.26±9.501 NS	85.767±12.810 a	80.88±8.648 a	47.75±3.416 NS	61.525±2.729 b	28.55*
Proximal intestine	53.96±7.636	39.00±2.755 a	51.01±7.00 NS	79.53±12.591 a	65.34±3.959 a	48.85±3.261 NS	56.183±2.874 NS	20.69*
Liver	99.95±8.286	85.60±2.137 a	145.12±12.981 a	96.08±12.595 NS	131.63±23.790 b	91.00±2.156 NS	119.43±4.525 a	19.22*
Colon contents	102.13±8.238	71.70±4.093 a	112.18±13.732 NS	149.00±24.390 a	173.98±15.330 a	121.40±8.400 b	160.20±9.640 a	6.29*

Values are mean ± SE from 6 rats in each group. Group 1 has been compared with groups 2-7.
a: $p < 0.01$ b: $p < 0.05$ NS - Not significant *ANOVA - Significant at 1% level.

Table 4. Mucinase activity (n moles of glucose liberated/min/mg protein)

	Group1	Group2	Group3	Group4	Group5	Group6	Group7	F-ratio
Colon contents	1.997±0.106	1.097±0.191 a	3.065±0.420 a	2.693±0.328 a	4.518±0.663 a	1.990±0.293 NS	3.380±0.509 a	44.61*
Faecal contents	1.921±0.261	3.480±0.569	5.580±0.625 a	4.300±0.652 a	7.360±0.916 a	3.500±0.443 a	4.870±0.834 a	43.72*

Values are mean ± SE from 6 rats in each group. Group 1 has been compared with groups 2-7.
a: p0.01; b: $p<0.05$; NS - Not significant; *ANOVA - Significant at 1% level

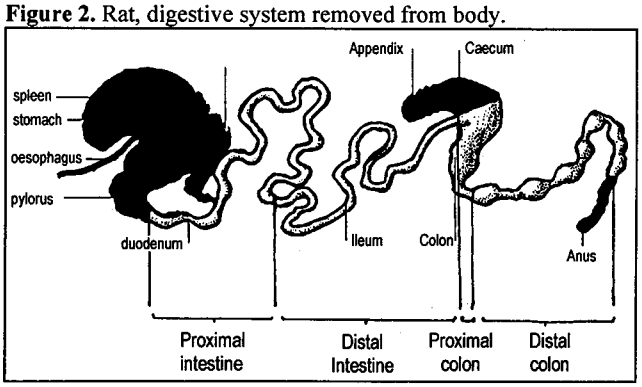
fell significantly in the latter group in the distal colon, intestines, liver and also in the colon contents (bacterial).

A similar pattern was noted in the case of mucinase (Table 3). The chilli group showed a significant increase when compared with the kernel group, and kernel + chilli group.

The kernel + chilli + DMH group showed a significant fall in the activity of mucinase both in the colon contents as well as in the faecal contents when compared with chilli + DMH group.

The F-value showed that there was a significant difference between and the within the groups at 1% level in all the parameters studied.

Figure 2. Rat, digestive system removed from body.



Discussion

Treatment with red chilli, DMH and coconut kernel brings about profound alterations in the activity of both β-glucuronidase and mucinase. Chilli treated rats and those given DMH showed increased incidence of tumours both in the colon and intestine. When coconut kernel was included in the diet, the incidence of tumours decreased, the size of the tumours (visible) were significantly reduced and were more or less diffused.

Histopathological studies showed a great degree of variation in the different groups. In the case of kernel treated control animals there was vascularisation in the colon, but the colon was otherwise normal. The chilli group showed the size of the tumour to be about 1 cm, pedunculated with defined margin. It showed areas of dysplasia which were less severe, the nucleoli was less prominent, moderate cytoplasm and with mitotic figures. In the DMH treated group, the size of the tumour was around 2 cm, pedunculated, with well-defined margin, with large number of papillae and an invasive adenocarcinoma, which showed marked pleomorphism. The nucleoli was also very prominent, with scanty cytoplasm and numerous mitotic figures. In the chilli + DMH group, the size of the tumour was more than 2 cm. There was a transitional zone with areas of marked dysplasia and infiltrating adenocarcinoma. The nucleoli was also prominent. In the kernel + chilli group, the size of the tumour was less than 0.5 cm, with ill defined margin, sessile with occasional dysplasia. Nuclear pleomorphism was less severe, nucleoli was less prominent with moderate cytoplasm. In kernel + chilli + DMH group, the size of the tumours was 1 cm, sessile with ill defined margin, had a significant number of papillae with few glands filled with mucin and showed occasional infiltration into the submucosa. Nuclear pleomorphism was marked with prominent nucleoli scanty, cytoplasm and vascular granulation. The vascular granulation observed in the kernel group may be a protective mechanism, by which the animal tries to resist the invasion of the tumour into the deeper layer.

Glucuronide formation is a major detoxification mechanism in mammals[17]. Many exogenous compounds that are excreted in the bile as glucuronide conjugates are deconjugated by bacterial β-glucuronidase and modified further by intestinal bacteria in the large bowel[17,18]. The activity of this microflora is affected by diet, ie, they can alter the biological activity, toxicity , excretion and reabsorption of many of the exogenous and endogenous compounds which are considered as carcinogens and/or co-carcinogen metabolites[19]. Studies have also shown, β-glucuronidase to be a key enzyme in the activation of DMH metabolites to carcinogens[20]. These substances can trigger the formation of neoplastic changes in the colon and intestine[21]. The composition of coconut kernel is approximately: fibre 3.1%, protein 3.6%, fat 38.1%, digestible carbohydrates 9.1% and the rest moisture. The inclusion of coconut kernel in our study

significantly decreased the activity of this enzyme in the presence of chilli or DMH, or both, emphasising the protective role of the kernel.

Mucins are glycoproteins consisting of a large number of carbohydrate side chains attached to a protein core. They serve as a source of energy for the intestinal bacteria and are consequently degraded by them[21-23]. Supplying the microflora with fermentable dietary fibre (ie, coconut kernel) may permit them to use these substrates preferentially. Thus, the treatment with coconut kernel showed a decrease in the activity of mucinase, while chilli and DMH treatment showed an increase.

Thus our studies show that inclusion of coconut kernel in the diet, results in alteration in the intestinal and colon tissue as well as in the colon microflora. Biochemically the activity of β-glucuronidase and mucinase is decreased, while histopathologically the degree of invasion by the tumour is controlled in the colon. Macroscopically also the number of tumours as well as the tumour size is reduced.

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椰子和紅辣椒對實驗性結腸癌大鼠腸β—葡糖苷酸酶和黏多糖酶活性的影響

摘要

作者以大白鼠爲對象，給予 1,2—二甲基肼（DMH），並觀察椰子與紅辣椒對腸β—葡糖苷酸酶和糞便黏多糖酶活性的影響。椰子組大鼠的平均體重較 DMH 組和紅辣椒組大。椰子組大鼠的β—葡糖苷酸酶活性較 DMH 組和紅辣椒組低。黏多糖酶活性與前者相似。當膳食補充椰子時，形態學的研究顯示大鼠小腸和大腸的可見性腫瘤減少。組織學的研究顯示，大鼠乳頭較少，透入黏膜下較少和細胞漿變化較少，有絲分裂下降。作者得出結論，椰子可分別減少紅辣椒和 DMH 的誘變和致癌作用。

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