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Murraya koenigii and *Brassica juncea* – Alterations on lipid profile in 1-2 dimethyl hydrazine induced colon carcinogenesis

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Summary

The influence of the two commonly used spices *Murraya koenigii* (curry leaf) leaf and *Brassica juncea* (mustard) seeds on the levels of lipids, fecal bile acids and neutral sterols was studied in rats administered 1,2-dimethyl hydrazine (1,2 DMH). The levels of cholesterol and phospholipids decreased in the experimental groups when compared with the control. The cholesterol phospholipid ratio showed an elevated level in the DMH treated control compared with the spices group. Bile acids and neutral sterols showed a sharp increase in the spices treated groups in liver and feces when compared with the control. Morphological and histological studies revealed that the mean number of neoplasms in the colon and intestine were significantly low in the spices fed groups.

Introduction

There is no cuisine without the addition of one or more spices. Every spice has medicinal, preservative or antiseptic value. However, in regard to *Murraya koenigii* leaf and *Brassica juncea* seeds, which are used together, very little information is available about its biochemical effects. The carcinogenic [1] and anti-carcinogenic [2] property of certain spices have been documented.

Large bowl cancer is the most commonly diagnosed malignancy all over. It is suggested that colon cancer is environmental in origin and is thus preventable [3, 4]. Among this dietary factors play a major role. Both synthetic and natural hydrazines exist in the environment as industrial and food contaminants [5]. Therefore, investigations were carried out into the role of these two spices in rats, inducing colon cancer by 1,2-dimethyl hydrazine.

Materials and methods

Chemicals

1,2 Dimethyl hydrazine (1,2 DMH) was purchased from Sigma Chemicals Co. (St. Louis, MO, USA).

Plant products

The spices *Murraya koenigii* leaf (curry leaf) and *Brassica juncea* seeds (mustard) were purchased locally. The curry leaves and mustard seeds were ground well and mixed in the powdered Hisdustan Lever "Gold mohur" pellet diet. The dose of these spices was fixed strictly according to the Ayurveda [6] literature.

Rat tumour experiments

Four groups of 10 male Sprague-Dawley strain rats, 8 weeks old, weighing 80–100 g, were used for the experiments. Group I, the negative control, received only pellet diet whereas Group II, the positive control, received a 1,2 DMH injection and the pellet diet. Group III and Group IV received curry leaf and mustard respectively, in addition to the 1,2 dimethyl hydrazine

Table 1. Concentration of serum and tissue cholesterol

	Serum (mg/dl)	Liver (mg/100 g tissue)	Intestine	Colon
Group 1	110.82±2.3	421.8±8.8	438.18±9.2	478.2±10.0
Group 2	178.42±3.7	507.4±10.6	499.7 ±10.5	627.5±13.2
Group 3	113.6 ±2.3[a]	430.9±8.8[a]	432.9 ±9.1[a]	471.8±9.9[a]
Group 4	115.7 ±2.4[a]	412.5±8.6[a]	440.6 ±9.2[a]	468.6±9.8[a]

Group 2 has been compared with group 3 and group 4.
Values are mean ± SE of 6 rats
$a_p < 0.01$

Table 2. Concentration of phospholipids (g/100 g tissue)

	Intestine	Colon	Liver
Group 1	0.57±0.012	0.65±0.013	2.0±0.04
Group 2	0.58±0.012	0.67±0.014	0.618±0.013
Group 3	0.50±0.011[a]	0.50±0.010[a]	0.481±0.01[a]
Group 4	0.49±0.010[a]	0.52±0.011[a]	0.512±0.011[a]

Group 2 has been compared with group 2 and group 3.
Values are mean ± SE of 6 rats
$a_p < 0.01$

injection. The animals received these spices only during the period of carcinogen administration, i.e., during the first 15 weeks. Then until the 30th week they were given normal pellet diet. At the end of this period they were sacrificed by decapitation after an over-night fast. The neoplasms were counted by cutting the colon longitudinally. The tissue lipids were extracted [7] and used for various estimations. Total cholesterol [8] and phospholipids [9] were estimated.

Stool samples were homogenised with equal weight of water and lyophilized to a fine powder. Sterols and bile acids (liver, feces) were extracted [10] and estimated by Snell [11] and Snell (1961).

Statistical analysis

Student's t-test [12] was used to analyse the significance of the results.

Results

The concentration of cholesterol in the serum, liver, intestine and colon showed a significant decrease in all the groups compared with the control (DMH group) (Table 1).

The concentration of phospholipids decreased in liver, intestine and colon in both the experimental groups when compared with the DMH control group (Table 2).

The ratio of cholesterol to phospholipids was higher in the DMH group when compared with all the experimental groups (Table 3). Fecal sterols and fecal bile acids excretion studies showed a sharp increase in the spices fed groups compared with the DMH control. A similar enhancement of bile acids and total sterols in

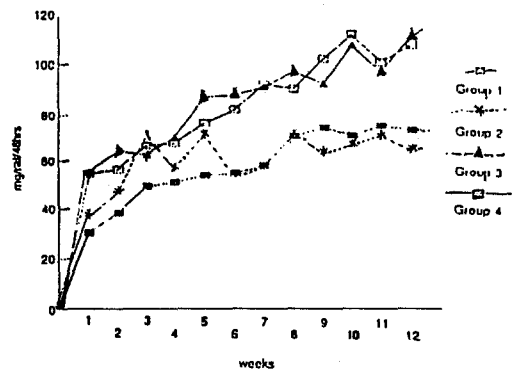

Figure 1. Level of fecal neutral sterol (mg/rat/48 hrs)

the liver was also observed in the experimental groups (Figs. 1–4). Malignant tumours were observed in the DMH group and the average number of malignant neoplasms observed is given in Figure 5. Fecal dry weight of the rat per day is given in Figure 6.

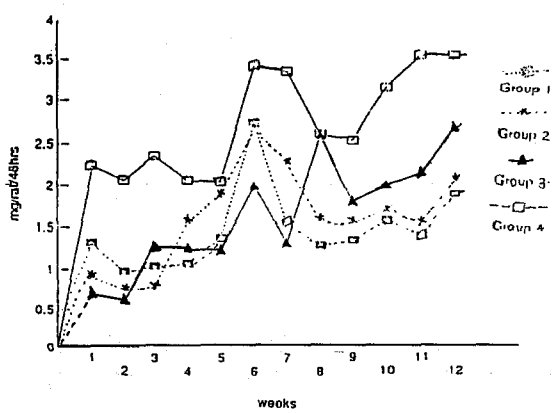

Figure 2. Level of fecal bile acids (mg/rat/48 hrs)

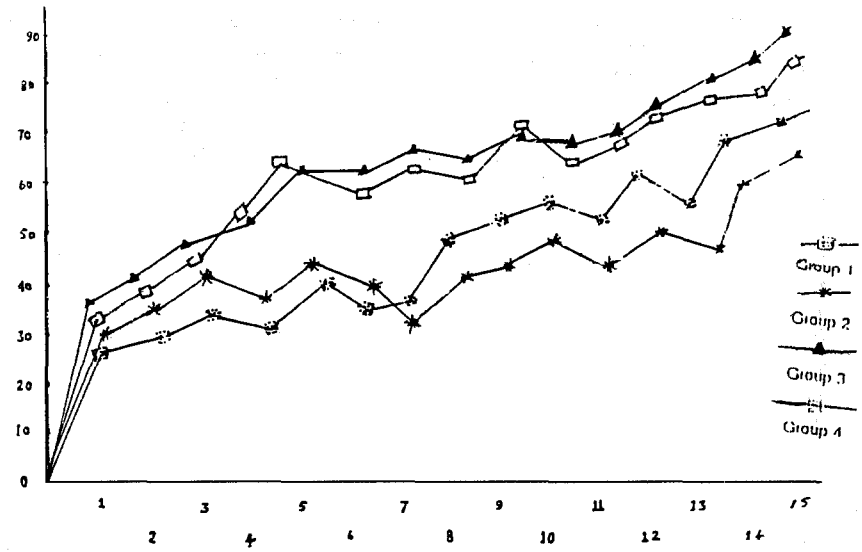

Figure 3. Level of liver neutral sterol (mg/rat/48 hrs)

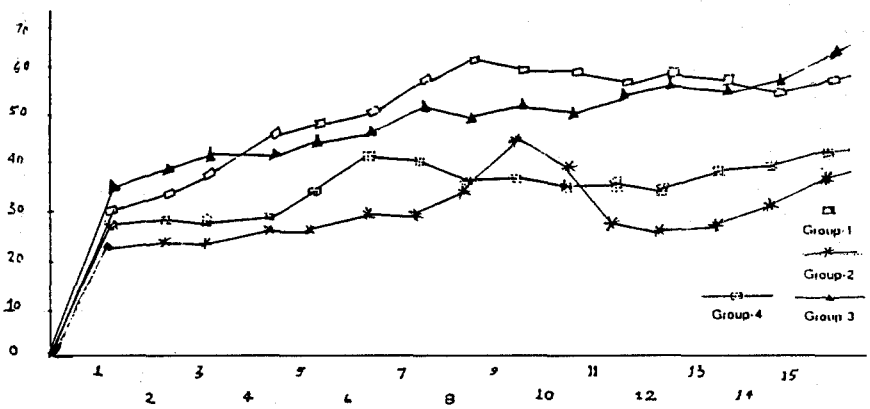

Figure 4. Level of liver bile acids (mg/rat/48 hrs)

Table 3. Cholesterol-phospholipid ratio

	Liver	Intestine	Colon
Group 1	0.210	0.768	0.735
Group 2	0.821	0.861	0.936
Group 3	0.895	0.195	0.943
Group 4	0.805	0.236	0.911

Discussion

An elevated level of fecal dry weight was observed in the spices fed group in agreement with many reports [1]. This effect is attributed to one of the possible mechanisms by which curry leaf and mustard can inhibit colon tumourogenesis. Both of these may have a higher water-holding capacity, and/or may be dilutable and absorbe any carcinogens or promoters contained within the intestinal lumen.

In this context we have observed a decrease in the levels of cholesterol in different tissues in the spices fed group along with DMH. This may be due to decreased cholesterogenesis in these tissues [13]. The total bile acids and neutral sterol output was found to be higher in feces and liver in the curry leaf and mustard fed rats given DMH than the control. It has been shown

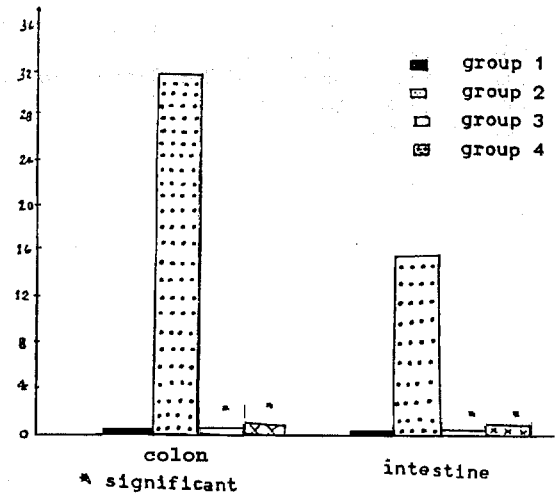

Figure 5. Number of tumors observed

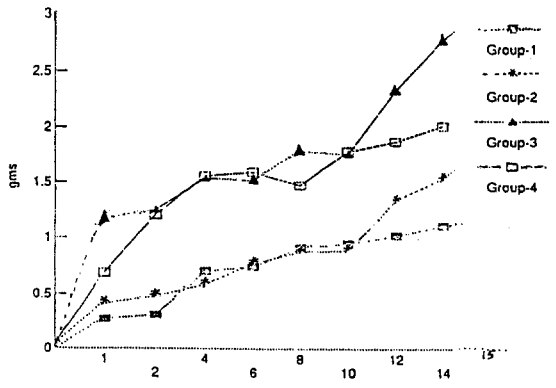

Figure 6. Fecal dry weight (g/day/rat)

that increasing the excretion of bile acids sequestrants leads to decreased plasma cholesterol concentration [14]. The carcinogen treated spices groups had a very low incidence of colon cancer.

The excretion of total sterols and bile acids was found to be higher than that of the carcinogen treated control group. This suggests that both these spices help to prevent the proliferative damage in the colonic epithelium induced by the colon specific carcinogen 1,2-dimethyl hydrazine. So it can be assumed that both these spices may reduce the absorption of bile acids in the ileum and thereby enterohepatic circulation of bile acids may be affected. The higher level of bile acids in the feces may be due to the absorption of less water-soluble bile acids by these spices. Thus the enterohepatic pool is initially reduced and may be renewed by increased synthesis of bile acids from cholesterol thereby reducing the body cholesterol. Srinivasan et al. [16] suggested that spices like tumeric, red pepper, ginger and mustard can stimulate the coversion of cholesterol to bile acids.

In the animals given the spices, the level of phospholipids in the various tissues shows a decline when compared with the control. The decreased level of phospholipids in the experimental groups may be due to the higher degradation of phospholipids. These changes in the cholesterol and phospholipids reflected their ratios. The lower ratio of cholesterol to phospholipid in the experimental groups is closely associated with membrane stability. Changes in the cholesterol concentration will greatly affect the membrane fluidity and thereby can bring about abnormal changes in the membrane property and function [15].

From this study we can see that feeding the spices curry leaf and mustard reduced the incidence of colon tumor in rats induced by 1,2-dimethyl hydrazine. Thus, the inclusion of these spices in a daily diet plays a significant role in the protection of the colon against chemical carcinogenesis.

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