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Dose–response efficacy of caraway (*Carum carvi* L.) on tissue lipid peroxidation and antioxidant profile in rat colon carcinogenesis

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Abstract

Colon cancer is a leading cause of cancer death and its prevention is of great interest throughout the world. This study was conducted to examine the efficacy of different doses of dietary caraway (*Carum carvi* L.) on tissue lipid peroxidation (LPO) and antioxidant profile in rat colon carcinogenesis. Wistar male rats were divided into 6 groups and were fed a modified pellet diet for the whole of 30 weeks. To induce colon cancer, rats were given a weekly subcutaneous injection of 1,2-dimethylhydrazine (DMH) at a dose of 20 mg kg^{-1} (based on body weight) for the first 15 weeks. Caraway was supplemented every day orally at doses of 30, 60 and 90 mg kg^{-1} for different groups of rats for the total period of 30 weeks. All rats were sacrificed at the end of 30 weeks, the colons were examined visually for masses and were subsequently evaluated histologically. The results showed diminished levels of intestinal, colonic and caecal LPO products, such as conjugated dienes (CD), lipid hydroperoxides (LOOH) and thiobarbituric acid reactive substances (TBARS) and also the antioxidants superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and glutathione reductase (GR) in DMH treated rats, which were significantly reversed ($P < 0.05$) on caraway supplementation. Moreover, enhanced activity of intestinal, colonic and caecal glutathione peroxidase (GPx), glutathione S-transferase (GST) and colonic ascorbic acid and α-tocopherol levels were observed in carcinogen-treated rats, which were significantly ($P < 0.05$) reduced on caraway supplementation. Thus, our study showed that caraway supplementation at a dose of 60 mg kg^{-1} had a modulatory role on tissue LPO, antioxidant profile and prevented DMH-induced histopathological lesions in colon cancer rats.

Introduction

Cancer can be defined as a genetic disease, resulting from multiple events associated with initiation, promotion and metastatic growth. Cancer results from the loss of control of cellular homoeostasis (Jemal et al 2004). Colorectal cancer is a leading cause of cancer death and its prevention is of great interest throughout the world (Potter et al 1993). Induction of colon cancer by 1,2-dimethylhydrazine (DMH), a complete procarcinogen, in rats is the best experimental model of colon cancer, since it parallels human colon cancer with respect to its histological and proliferation characteristics (Weisburger et al 1997). Most carcinogens operate by a common cellular mechanism. However, the relative importance of the individual steps that lead to malignant transformation depends on the type of carcinogen, the target tissue and individual genetic susceptibility (Cerutti 1994).

Diet, especially saturated fat intake, has been regarded as the most important nutritional influence on the development of colon cancer (Pickering et al 1995). Lipid peroxidation (LPO) is a free-radical-initiated chain oxidation of polyunsaturated fatty acids (PUFAs) and it has been investigated in wide range of cell types, including malignant cells (Dreher & Junod 1996). Substantial experimental evidence so far strongly implicates free radicals in both the initiation and promotion stages of carcinogenesis (Oberley & Oberley 1986). DMH can be metabolized to a methyl free radical and generate hydroxy radicals or hydrogen peroxide that may contribute to the initiation of LPO and its products, which in turn can modify DNA, causing mutation and carcinogenesis (Kawanishi & Yamamoto 1991).

Mammalian cells possess elaborate defence mechanisms for free radical detoxification. Under physiological conditions oxy radicals are part of normal regulatory circuits

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and the cellular redox state is directly correlated by antioxidants (Cerutti 1994). Antioxidants are believed to induce their own effects on cell signalling in pre-cancerous cells to decrease tumour promotion, a critical stage in carcinogenesis (Gopalakrishna & Jaken 2000). Recently, interest has been focused on the possible role of enzymic and non-enzymic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione S-transferase (GST) and glutathione (GSH), vitamin C (ascorbic acid) and vitamin E (α-tocopherol) on carcinogenesis (Evans et al 1997).

Cancer chemoprevention can be defined as the prevention, inhibition or reversal of carcinogenesis by administration of one or more chemical entities either as an individual drug or as a dietary supplement (Kelloff et al 1994). A wide range of compounds has been tested for possible chemopreventive activity against DMH-induced rat colon carcinogenesis (Mori et al 2004). Previous studies from our laboratory have shown that some plant products and spices, such as coconut cake (Nalini et al 2004), cumin, black pepper (Nalini et al 1998), fenugreek (Devasena & Menon 2002) and ginger (Manju & Nalini 2005), effectively modulate colon carcinogenesis.

Herbal-based dietary supplements contain a large array of phytochemicals (flavonoids) that may mediate physiological functions related to cancer suppression. Medicinal herbs and their active principles have attracted the focus of attention as potential chemopreventive agents (Johnson 1997). Spices are dried herbs that have been effectively used in the indigenous systems of medicine in India and also in other countries (Nadkarni & Nadkarni 1976). Apart from their traditional use, beneficial physiological effects have been brought to the fore by extensive animal studies during the past three decades (Srinivasan 2006).

Caraway (*Carum carvi* L. Umbelliferae) is one of the spices globally distributed with a long history as a medicinal plant since ancient times. The primary active constituent in caraway is volatile oil (4–6% on average), which itself is made up of carvone (50–60%) and limonene (40%). Caraway has been widely used as an anti-dyspeptic, antispasmodic, antibacterial, antioxidant, anti-ulcerogenic and anti-proliferative agent (Eddouks et al 2004). Recently, caraway was identified as a novel natural product with cancer chemopreventive potential (Zheng et al 1992) and hence a trial was made in our laboratory to delineate the possible protective effects of caraway on DMH-induced colon tumorigenesis using the rat as an experimental model.

The aim of this study was to evaluate the effect of caraway in a dose-dependent manner on DMH-induced tissue LPO and antioxidant profile in rat colon carcinogenesis.

Materials and Methods

Animals

The experiments were carried out using male albino Wistar rats, 100–120 g, obtained from the Central Animal House, Annamalai University, India. The rats were kept in polypropylene cages (4 per cage) with paddy husk for bedding and fed commercial pellet diet (Table 1) for one week's stabilization. Thereafter, the rats were randomly divided into six groups, each containing 12 rats and maintained under controlled conditions of temperature ($24 \pm 2^{\circ}$C) and humidity ($50 \pm 10\%$) and a 12-h light–dark cycle. Commercial pellet diet containing 4.2% fat (Hindustan Lever Ltd, Mumbai, India) was powdered and mixed with 15.8% peanut oil, making a total of 20% fat in the diet (Table 1). Rats were allowed free access to this modified powdered pellet diet and tap water throughout the experimental period of 30 weeks. The rats were cared for in compliance with the principles and guidelines of the Ethical Committee for Animal Care of Annamalai University in accordance with the Indian National Law on Animal Care and Use (Reg no.160/2004/CPCSEA).

Table 1 Composition of the diet

	Commercial diet 84.2%	Peanut oil 15.8%	Total 100%
Protein	17.7	—	17.7
Fat	4.2	15.8	20.0
Carbohydrate	50.5	—	50.5
Fibre	3.4	—	3.4
Minerals	6.7	—	6.7
Vitamins	1.7	—	1.7

Carcinogen administration

DMH (Sigma Chemical Co., St Louis, MO, USA) was dissolved in 1 mM EDTA, adjusted to pH 6.5 with 1 mM NaOH and administered subcutaneously in the right thigh of rats at a dose of 20 mg kg^{-1} body weight once a week for the first 15 weeks.

Supplementation of caraway seed suspension

Dry caraway seeds were purchased from a local herbal dealer and subsequently authenticated by a botanist from our University (Chidambaram, Tamilnadu, India). The composition of caraway was determined to have 333 calories: 9.9% water, 19.8 g protein, 14.6 g fat, 49.9 g carbohydrates, 12.6 g fibre and 5.9 g ash (Duke & Ayensu 1985). The seeds were further dried and ground into a fine powder. The powdered seeds were suspended in 1% carboxymethylcellulose (CMC) at room temperature to prepare a crude suspension of desired concentration just before use. Caraway seed suspension (cc) was fed by intragastric intubation (30, 60 and 90 mg kg^{-1}) at about 10:30 h every day for the whole experimental period of 30 weeks to avoid diurnal variation. The low dose of caraway used here was based on the average daily intake by man (1.5–6 g) (Blumenthal et al 1998).

Treatment regimen

The experimental design is shown in Figure 1. A total of 72 rats were randomly assorted to 6 experimental groups

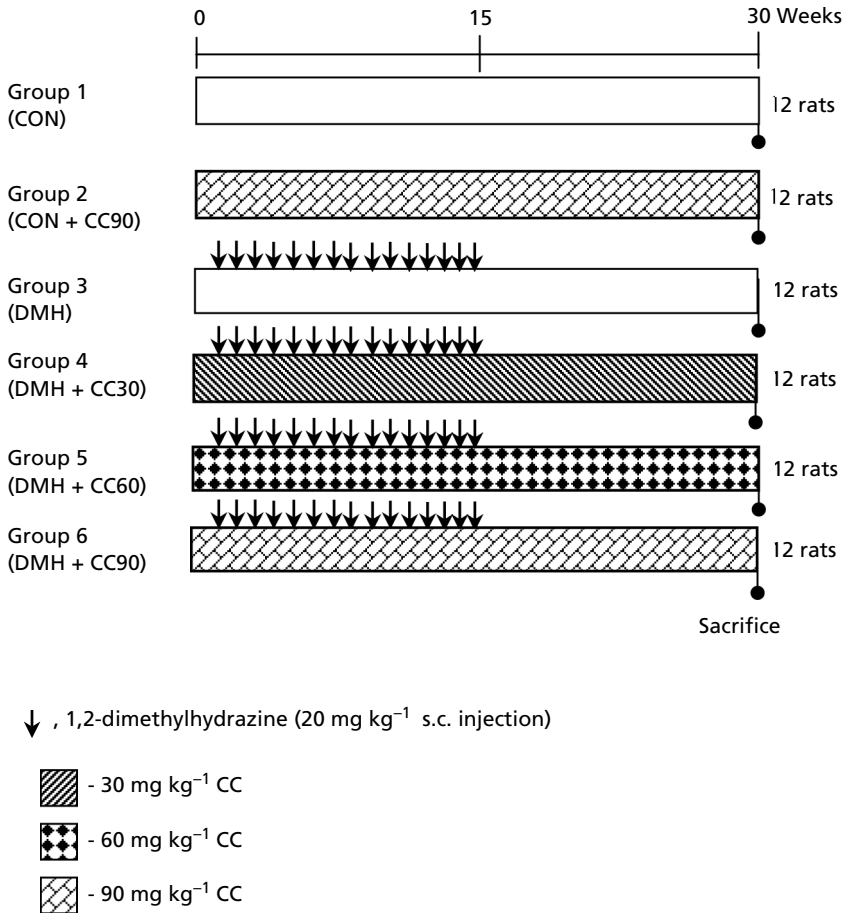

Figure 1 Experimental protocol.

(12 rats/group). All the rats received a modified powdered pellet diet (20% high fat). Group 1 rats served as untreated control, group 2 rats were given 90 mg kg^{-1} caraway suspended in 1% CMC orally, every day for 30 weeks and groups 3–6 were injected subcutaneously with DMH 20 mg kg^{-1} once a week for the first 15 weeks. In addition, rats in groups 4–6 received 30, 60 and 90 mg kg^{-1} of caraway seed powder in 1% CMC orally every day, respectively, for the total duration of 30 weeks. Rats were monitored daily for general health, and body weight was recorded every week during the entire period of the study. At the end of the experimental period, food was withheld overnight and the rats were sacrificed by decapitation.

Homogenization of tissues

Immediately after sacrifice, intestinal, caecal, proximal and distal colonic tissues were washed with ice-cold saline. The tissues were then cut into fragments and homogenized with 3 volumes (w/v) of the appropriate buffer using a Potter-Elvehjam homogenizer with a Teflon pestle and centrifuged at 12 000 *g* for 20 min at 4°C. The supernatant was used for the biochemical estimations.

Histopathological analysis

For histopathological examination, colons were fixed in 10% neutral buffered formalin, embedded in paraffin and stained with hematoxylin and eosin (H&E).

Biochemical assays

Lipid peroxidation was estimated by measuring the level of tissue LPO byproducts, such as conjugated dienes (CD), lipid hydroperoxides (LOOH) and thiobarbituric acid reactive substances (TBARS) by using the methods of Ohkawa et al (1979), Rao & Recknagel (1968) and Jiang et al (1992), respectively. The values are expressed as mmol (mg tissue)$^{-1}$.

SOD (EC.1.15.1.1) was assayed using the method of Kakkar et al (1984) based on 50% inhibition of the formation of NADH phenazine methosulfate-nitroblue tetrazolium (NBT) formazan at 520 nm. One unit of the enzyme was taken as the amount of enzyme required for 50% inhibition of NBT reduction per minute per mg protein. The activity of CAT (EC.1.11.16) was determined by the method of Sinha (1972). The values of CAT activity are expressed as μmol of H_2O_2 utilized per minute per mg protein.

Reduced glutathione (GSH) was determined by the method of Ellman (1959). The values are expressed as mmol (mg tissue)$^{-1}$. GR (EC.1.6.4.2) activity was assayed using the method of Carlberg & Mannervik (1985) and the values are expressed as μmol of NADPH oxidized per minute per mg protein. GPx (EC.1.11.1.9) activity was assayed by the method of Rotruck et al (1973); the values are expressed as μmol of GSH utilized per minute per mg protein. The activity of GST (EC. 2.5.1.18) was estimated by the method of Habig et al (1974) and the values are expressed as μmol of 1-chloro-2,4-dinitrobenzene (CDNB)-GSH conjugate formed minute per mg protein.

Vitamin C (ascorbic acid) content was estimated by the method of Roe & Kuether (1943) and vitamin E (α-tocopherol) content by the method of Baker et al (1980). The content of vitamins C and E are expressed as mg (mg tissue)$^{-1}$. The protein content was determined by the method of Lowry et al (1951) using bovine serum albumin as the standard at 660 nm.

Statistical analysis

Data were analysed by one-way analysis of variance and a significant difference among treatment groups were evaluated by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant at $P < 0.05$. All statistical analyses were made using SPSS 11.0 software package (SPSS, Tokyo, Japan).

Results

Effect of caraway on the incidence of colon tumours and the number of tumours per tumour-bearing rat

The effect of caraway on colonic tumour incidence, number of tumours per tumour-bearing rat and size of the tumour is summarized in Table 2. In rats treated with DMH (group 3) the tumour incidence in the colon was 83.33% and the average tumour size was approximately 13.8 mm^2. On supplementing DMH-treated rats with caraway at different doses of 30, 60 and 90 mg kg^{-1} (groups 4, 5 and 6) reduced tumour size (10.1, 1.3 and 5.2 mm^2) and tumour incidence (66.6, 24.99 and 49.98%) was noticed. The total number of tumours in DMH-treated rats was 16, which was markedly reduced to 3 after 60 mg kg^{-1} (group 5) caraway supplementation.

Histopathological analysis

Table 3 and Figure 2 represent the histopathological changes in the colon of control and experimental rats observed microscopically. Tissue sections of control rats (group 1) displayed normal crypts and colonic architecture with no signs of apparent abnormality (Figure 2A). On histological examination of rats supplemented with caraway alone (group 2), the colonic mucosal epithelium appeared normal. The mucosa of rats treated with DMH alone (group 3) showed distorted crypt architecture with densely packed lymphoid aggregates, vascular granulation and congestion, and moreover the tumours were macroscopically sessile with well-defined margins (Figure 2B, C; Table 3). In all caraway-supplemented rats, colonic histopathological observations showed reduced DMH-induced neoplasms, with a more pronounced effect being observed only in the DMH + CC60-treated rats (group 5) (Figure 2D; Table 3).

Changes in the levels of tissue LPO byproducts of control and experimental rats

Table 4 summarizes the data on the effect of caraway supplementation on the LPO byproducts such as CD, LOOH and TBARS in intestinal, caecal, proximal and distal colonic tissues from control and experimental rats. The CD, LOOH and TBARS levels were lower in DMH-treated rats (group 3) as compared with the untreated control rats (group 1). However, in all caraway-supplemented rat (groups 4–6) tissue LPO byproducts were elevated significantly ($P < 0.05$), the significance ($P < 0.01$) being marked on 60 mg kg^{-1} caraway supplementation (group 5).

Changes in the levels of antioxidant enzymes SOD and CAT

Table 5 depicts the activity of the antioxidant enzymes SOD and CAT in intestinal, proximal, distal and caecal tissues in all experimental rats. Our results indicate that the activity of tissue SOD and CAT in DMH-administered rats (group 3) was significantly lowered compared to

Table 2 Incidence of colon tumours and the number of tumours per tumour-bearing rat

Group	No. of rats	No. of tumour-bearing rats	Tumour incidence (%)	Total No. of tumours	No. of tumours per tumour-bearing rat	Tumour size (mm^2)
DMH	12	10	83.33	16	1.60^a	13.8 ± 1.1^a
DMH + CC30	12	8	66.66	11	1.37^b	10.1 ± 0.9^a
DMH + CC60	12	3	24.99	3	1.00^c	1.3 ± 0.1^b
DMH + CC90	12	6	49.98	7	1.16^d	5.2 ± 0.4^c

Tumour incidence is expressed as means ± s.d. (No. of tumour-bearing rats/total number of rats in each group) × 100. Values not sharing common superscript letter (a–d) differ significantly from each other at $P < 0.01$ (analysis of variance followed by DMRT).

Table 3 Histopathological changes in the colon of DMH-treated and caraway-supplemented rats

S. No.	Microscopy	DMH	DMH + CC60
1	Nature	Sessile	Pedunculated
2	Margin	Well defined	Ill defined
3	Microscopic observations		
	Focal atypia	Observed within the superficial mucosa	Not observed
	Mucosal crypt architecture		
	Crypt branching	Irregular crypt	Regular crypt
	Crypt enlargement-transitional mucosa	Observed in large numbers	Not observed
	Crypt epithelium		
	Pleomorphism	Marked	Less severe
	Pseudo stratification	Observed	Not observed
	Mitotic figures	Numerous	Not present
	Lymphoid aggregates	Densely packed lymphoid aggregates in sub mucosa	Scattered aggregates
	Vascular granulation and congestion	Observed in the lamina propria	Not observed

the control (group 1). However, treatment with caraway at a daily dose of 30, 60 and 90 mg kg^{-1} significantly ($P < 0.05$) increased the activity of these enzymes, a more pronounced effect being observed in the 60 mg kg^{-1} supplemented group (group 5).

Changes in GSH- and GSH-dependent enzyme activity

Table 6 represents GSH- and GSH-dependent enzyme activity in all experimental groups. On DMH treatment (group 3), the GSH and GR activity in rat intestinal, proximal, distal and caecal tissues declined as compared with the control (group 1). There was a gradual increase in the GSH and GR levels in all DMH + CC-fed rat tissues, and a moderate increase ($P < 0.01$) was observed in the 60 mg kg^{-1} (group 5) caraway-supplemented rats as compared with rats treated with DMH alone (group 3). GST and GPx activity was significantly elevated on treatment with DMH ($P < 0.05$) whereas this activity significantly ($P < 0.05$) declined on caraway supplementation (group 4–6). Among the caraway-supplemented groups, the rats that received 60 mg kg^{-1} (group 5) showed a statistically significant ($P < 0.01$) decline.

Changes in colonic ascorbic acid and α-tocopherol concentration

The concentration of proximal and distal colonic tissue ascorbic acid and α-tocopherol in control and experimental rats is shown in Figure 3. DMH administration elevated these concentrations (group 3) significantly as compared with control. Feeding caraway to DMH-treated rats significantly decreased ($P < 0.05$) ascorbic acid and α-tocopherol levels, the concentrations being markedly decreased ($P < 0.01$) in the 60 mg kg^{-1} supplemented rats (group 5) as compared with those treated with DMH alone (group 3).

Discussion

These data reveal the modulatory role of caraway in a dose-dependent manner (30, 60 and 90 mg kg^{-1} body weight) on tissue lipid peroxidation and antioxidant profile in DMH-induced rat colon cancer. Caraway at a dose of 60 mg kg^{-1} was able to exert a more pronounced effect, as shown histologically by a significant reduction in the extent and severity of lesions in the tissue, and also biochemically by the modulation of intestinal and colonic LPO and antioxidant status.

Free radical attack on biomembranes lead to oxidative destruction of PUFAs by a process called LPO (Chiu et al 1989). Studies carried out both in man and experimental animals show that LPO has a very important role in the initiation and promotion stages of cancer (Capel & Thornley 1983). DMH, a potent colon specific procarcinogen, poses an acute oxidative stress to both the liver and colon of experimental animals and methyl hydrazines, which are intermediates of DMH metabolism, have been reported to produce free radicals following liver microsomal metabolism (Pence 1991).

In our study, the levels of LPO byproducts, such as CD, LOOH and TBARS, were measured in the intestinal and colonic tissues of long-term DMH-treated rats. This may be due to inhibition of NADPH cytochrome P450 electron transport system and an increase in lipid soluble antioxidants. Such a high degree of resistance to peroxidation in the tumour cells has been attributed to marked decrease in PUFAs (Das 1991). These results are also very similar to those obtained by Cheeseman et al (1984). In addition, LPO has been suggested to be involved in the control of cell division (Diplock et al 1994). Dreher & Junod (1996) also showed that low levels of oxygen free radicals stimulate cell proliferation, whereas high levels induce cytotoxicity and cell death in later stages of carcinogenesis and this in turn may confer the cancer cells with an ability to overwhelm oxidative stress.

We observed that caraway supplementation increased the levels of CD, LOOH and TBARS in intestinal and colonic tissues of DMH-treated rats. Caraway supplementation to DMH-treated rats restored the LPO levels to near those of the control rats, which may be due to the antiproliferative activity of caraway (Eddouks et al 2004). Since proliferation and LPO are inversely related (Schmelz et al 2000), and since caraway is a known antiproliferative agent, it could contribute to the observed increase in LPO in the colon and intestine. Elevated LPO may in turn increase the susceptibility and decrease

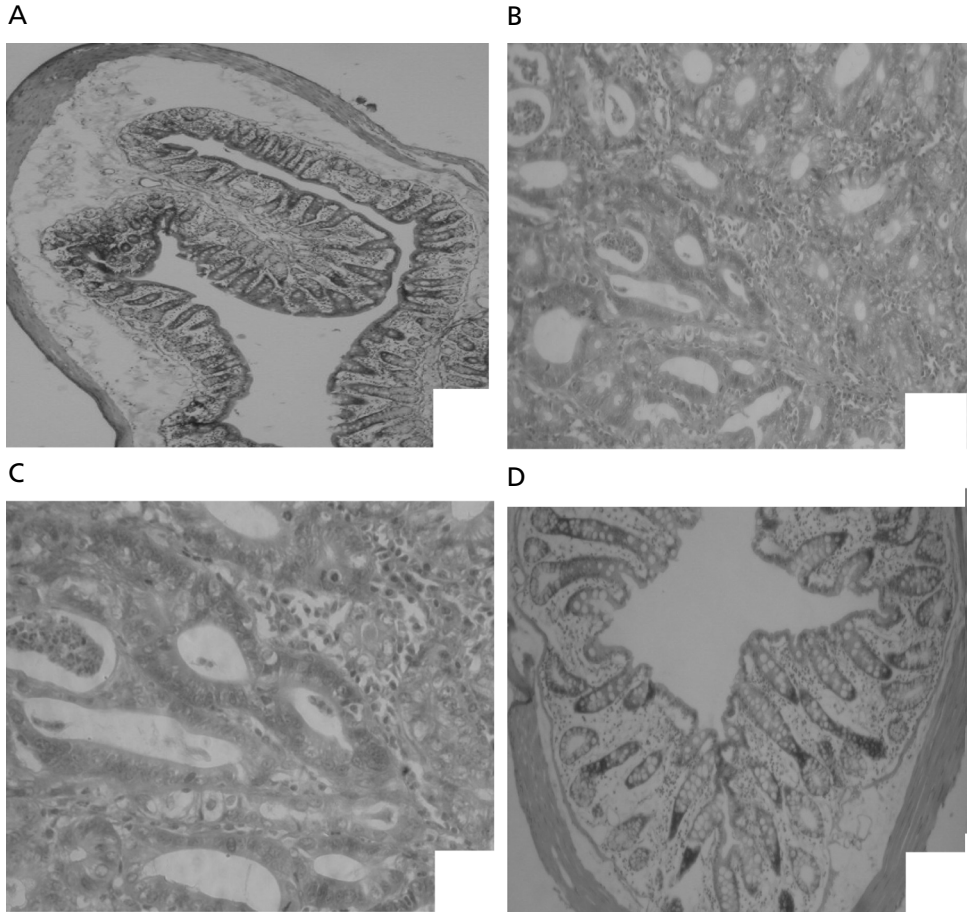

Figure 2 Histopathological changes in colon of rats. A. Colon of control rat showing normal mucosal and submucosal layers (H&E 20×). B. DMH-treated rat colon showing carcinomatous glands (H&E 20×). C. Colon of DMH-treated rat showing adenocarcinoma with papillary pattern and dysplastic zone (H&E 40×). D. Colon of DMH + caraway (60 mg kg^{-1})-treated rat showing lymphoid aggregates in submucosa and scattered inflammatory cell infiltration (H&E 20×).

Table 4 Effect of caraway on the levels of lipid peroxidation byproducts in control and experimental rats

Tissues	CON	CON + CC90	DMH	DMH + CC30	DMH + CC60	DMH + CC90
Thiobarbituric acid reactive substances (TBARS) (mmol (mg tissue)$^{-1}$)						
Intestine	0.60 ± 0.06^{a}	0.63 ± 0.06^{a}	0.33 ± 0.03^{b}	0.36 ± 0.03^{b}	0.56 ± 0.05^{b}*	0.44 ± 0.03^{c}*
Proximal colon	0.68 ± 0.06^{a}	0.71 ± 0.06^{a}	0.36 ± 0.03^{b}	0.38 ± 0.03^{bc}	0.62 ± 0.05^{d}*	0.41 ± 0.03^{c}
Distal colon	0.70 ± 0.06^{a}	0.72 ± 0.06^{a}	0.38 ± 0.03^{b}	0.42 ± 0.03^{b}	0.63 ± 0.05^{c}*	0.58 ± 0.04^{c}*
Caecum	0.65 ± 0.06^{a}	0.69 ± 0.06^{a}	0.35 ± 0.03^{b}	0.37 ± 0.03^{bc}	0.59 ± 0.05^{d}*	0.41 ± 0.03^{c}*
Conjugated dienes (CD) (mmol (mg tissue)$^{-1}$)						
Intestine	48.81 ± 4.82^{a}	52.04 ± 5.12^{a}	29.38 ± 2.92^{b}	31.44 ± 2.92^{b}	44.73 ± 4.42^{c}*	31.31 ± 3.33^{d}*
Proximal colon	50.89 ± 4.95^{ab}	53.23 ± 5.00^{a}	35.26 ± 3.39^{c}	37.80 ± 3.71^{c}	48.80 ± 4.63^{b}*	43.05 ± 3.78^{d}*
Distal colon	53.89 ± 5.21^{ab}	57.71 ± 5.06^{a}	38.77 ± 3.82^{c}	41.34 ± 3.42^{c}	50.85 ± 4.91^{bd}*	47.39 ± 4.20^{d}*
Caecum	51.04 ± 4.88^{ab}	54.04 ± 4.98^{a}	33.71 ± 3.23^{c}	35.99 ± 3.56^{c}	48.32 ± 4.10^{b}*	41.95 ± 3.23^{d}*
Lipid hydroperoxides (LOOH) (mmol (mg tissue)$^{-1}$)						
Intestine	53.79 ± 5.31^{a}	55.26 ± 5.44^{a}	30.39 ± 3.03^{b}	33.46 ± 3.13^{b}	49.69 ± 4.91^{c}*	44.36 ± 3.96^{d}*
Proximal colon	56.09 ± 5.46^{a}	59.36 ± 5.57^{a}	38.37 ± 3.69^{b}	40.74 ± 4.00^{b}	51.67 ± 4.90^{c}*	47.04 ± 4.13^{d}*
Distal colon	58.06 ± 5.62^{a}	61.75 ± 5.41^{a}	40.79 ± 4.02^{b}	42.36 ± 3.51^{b}	52.88 ± 5.11^{c}*	44.28 ± 3.93^{b}
Caecum	54.15 ± 5.18^{a}	57.04 ± 5.25^{a}	36.77 ± 3.52^{c}	39.99 ± 3.96^{c}	50.37 ± 4.28^{b}*	43.99 ± 3.38^{d}*

Values are expressed as means ± s.d. for 10 rats. Values not sharing common superscript (a–d) differ significantly from each other at $P < 0.05$; *$P < 0.01$ vs DMH (analysis of variance followed by DMRT).

Table 5 Effect of caraway on the activity of SOD and CAT in control and experimental rats

Tissue	CON	CON + CC90	DMH	DMH + CC30	DMH + CC60	DMH + CC90
Superoxide dismutase (SOD) (50% NBT reduced/min/mg protein)						
Intestine	3.13 ± 0.30^{a}	3.04 ± 0.26^{a}	1.47 ± 0.13^{b}	1.53 ± 0.10^{b}	$2.79 \pm 0.16^{c*}$	1.68 ± 0.10^{b}
Proximal colon	3.52 ± 0.34^{a}	3.46 ± 0.32^{a}	1.57 ± 0.13^{b}	1.68 ± 0.14^{b}	$3.14 \pm 0.22^{c*}$	1.79 ± 0.10^{b}
Distal colon	2.85 ± 0.24^{a}	2.76 ± 0.19^{a}	1.47 ± 0.12^{b}	1.51 ± 0.10^{b}	$2.45 \pm 0.18^{c*}$	1.68 ± 0.10^{b}
Caecum	3.80 ± 0.37^{a}	3.62 ± 0.34^{a}	1.75 ± 0.15^{b}	1.89 ± 0.16^{b}	$3.24 \pm 0.23^{c*}$	2.02 ± 0.11^{b}
Catalase (CAT) (μmol H_2O_2 utilized/min/mg protein)						
Intestine	39.01 ± 3.80^{a}	41.15 ± 3.88^{a}	24.41 ± 2.32^{b}	26.26 ± 2.37^{b}	$31.35 \pm 2.71^{c*}$	$29.43 \pm 2.76^{c*}$
Proximal colon	35.84 ± 3.49^{a}	37.81 ± 2.30^{a}	21.93 ± 1.95^{b}	22.25 ± 1.29^{b}	$28.29 \pm 2.52^{c*}$	23.23 ± 1.34^{b}
Distal colon	29.27 ± 2.72^{a}	30.88 ± 2.73^{a}	18.08 ± 1.78^{b}	20.24 ± 1.80^{c}	$25.27 \pm 2.25^{d*}$	$22.25 \pm 1.98^{c*}$
Caecum	37.31 ± 3.46^{a}	39.08 ± 3.46^{a}	22.35 ± 2.20^{b}	24.25 ± 2.16^{b}	$30.30 \pm 2.70^{c*}$	$27.27 \pm 2.43^{d*}$

Values are expressed as means ± s.d. for 10 rats. Values not sharing common superscript (a–d) differ significantly from each other at $P < 0.05$; *$P < 0.01$ vs DMH (analysis of variance followed by DMRT).

Table 6 Effect of caraway on the activity of GSH- and GSH-dependent enzymes in control and experimental rats

Tissue	CON	CON + CC90	DMH	DMH + CC30	DMH + CC60	DMH + CC90
Glutathione (GSH) (mmol (mg tissue)$^{-1}$)						
Intestine	20.09 ± 1.95^{a}	21.04 ± 1.98^{a}	12.94 ± 1.23^{b}	13.36 ± 1.21^{b}	$17.52 \pm 1.51^{c*}$	14.01 ± 1.31^{b}
Proximal colon	18.61 ± 1.81^{a}	19.36 ± 1.18^{a}	10.16 ± 0.90^{b}	11.17 ± 0.64^{b}	$16.20 \pm 1.44^{c*}$	$13.18 \pm 0.76^{d*}$
Distal colon	16.20 ± 1.50^{a}	17.20 ± 1.52^{a}	9.98 ± 0.98^{b}	10.35 ± 0.92^{b}	$13.21 \pm 1.18^{c*}$	$11.76 \pm 1.05^{d*}$
Caecum	19.00 ± 1.76^{a}	20.00 ± 1.77^{a}	11.93 ± 1.17^{b}	12.18 ± 1.08^{b}	$14.78 \pm 1.32^{c*}$	12.57 ± 1.12^{b}
Glutathione reductase (GR) (μmol NADPH oxidized/min/mg protein)						
Intestine	14.57 ± 1.43^{a}	14.76 ± 1.28^{a}	7.19 ± 0.66^{b}	7.60 ± 0.44^{b}	$13.09 \pm 0.75^{c*}$	$8.69 \pm 0.50^{d*}$
Proximal colon	15.70 ± 1.53^{a}	15.90 ± 1.51^{a}	7.50 ± 0.65^{b}	7.96 ± 0.69^{bc}	$14.05 \pm 1.01^{d*}$	8.56 ± 0.49^{c}
Distal colon	16.49 ± 1.43^{a}	16.60 ± 1.14^{a}	11.27 ± 0.95^{b}	11.61 ± 0.80^{bc}	$14.80 \pm 1.11^{d*}$	12.42 ± 0.72^{c}
Caecum	15.10 ± 1.31^{a}	15.48 ± 1.07^{a}	9.79 ± 0.82^{b}	10.14 ± 0.69^{b}	$13.40 \pm 1.00^{c*}$	$11.08 \pm 0.64^{d*}$
Glutathione peroxidase (GPx) (μmol GSH utilized/min/mg protein)						
Intestine	7.23 ± 0.62^{a}	7.01 ± 0.51^{a}	3.80 ± 0.35^{b}	3.93 ± 0.35^{b}	$6.05 \pm 0.59^{c*}$	4.13 ± 0.38^{b}
Proximal colon	6.84 ± 0.66^{a}	6.67 ± 0.40^{a}	2.97 ± 0.26^{b}	3.08 ± 0.17^{b}	$5.98 \pm 0.53^{c*}$	3.12 ± 0.18^{b}
Distal colon	5.62 ± 0.52^{a}	5.59 ± 0.49^{a}	2.01 ± 0.19^{b}	2.19 ± 0.19^{b}	$5.01 \pm 0.44^{c*}$	$4.56 \pm 0.40^{d*}$
Caecum	6.96 ± 0.64^{a}	7.03 ± 0.62^{a}	3.16 ± 0.31^{b}	3.31 ± 0.29^{b}	$6.05 \pm 0.54^{c*}$	3.43 ± 0.30^{b}
Glutathione-S-transferase (GST) (μmol CDNB-GSH conjugate formed/min/mg protein)						
Intestine	3.68 ± 0.35^{a}	3.51 ± 0.33^{a}	1.73 ± 0.16^{b}	1.79 ± 0.16^{b}	$3.01 \pm 0.26^{c*}$	1.83 ± 0.17^{b}
Proximal colon	3.27 ± 0.31^{a}	3.13 ± 0.19^{a}	1.58 ± 0.14^{b}	1.63 ± 0.09^{b}	$2.94 \pm 0.26^{c*}$	$2.47 \pm 0.14^{d*}$
Distal colon	3.08 ± 0.28^{a}	2.94 ± 0.26^{a}	1.49 ± 0.14^{b}	1.52 ± 0.13^{b}	$2.98 \pm 0.26^{c*}$	$1.59 \pm 0.14^{d*}$
Caecum	3.14 ± 0.31^{a}	3.34 ± 0.29^{a}	1.53 ± 0.14^{b}	1.55 ± 0.13^{b}	$3.03 \pm 0.26^{c*}$	1.62 ± 0.14^{b}

Values are expressed as means ± s.d. for 10 rats. Values not sharing common superscript (a–d) differ significantly from each other at $P < 0.05$; *$P < 0.01$ vs DMH (analysis of variance followed by DMRT).

the resistance of tumour cells to free radical attack, leading to decreased cell proliferation.

There is convincing evidence that the cellular pro-oxidant state can be modified or suppressed by enzymes of the cellular antioxidant defence and low-molecular-weight scavenger molecules (Wills 1985). Experimental studies suggest that affects the antioxidant mechanisms in the colonic mucosa presumably because the composition of the cell membrane reflects the fatty acid composition of the diet (Prohaska & Ganther 1976).

Enzymic antioxidants such as SOD and CAT provide the first line of defense. Impaired efficiency of these enzymes leads to diseases, including cancer (Rajeshkumar & Kuttan 2003). Slaga (1995) has shown that carcinogen administration usually decreases the levels of SOD and CAT. Our results also show a similar pattern. Satomi et al (1995) reported an association between SOD and CAT activity and the degree of malignancy in colorectal cancer patients. In addition, a majority of the tumours have low concentrations of these enzymes, relatively high amounts of vitamin E, low PUFA content and lipid peroxides, which may render them more susceptible and sensitive to free radical attack (Das 1991). Janssen et al (1999) showed that low levels of SOD and CAT activity in the cancerous tissue promotes the growth of cancer and its infiltration into the surrounding tissues, which is important for invasion and metastasis.

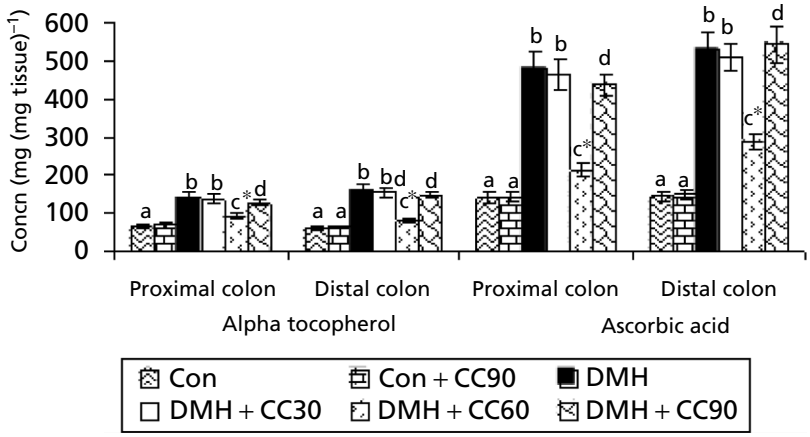

Figure 3 Changes in proximal and distal colonic α-tocopherol and ascorbic acid levels in rats. Column heights are the means ± s.d. of the values of 10 rats. Values not sharing common superscripts (a–d) differ significantly from each other at $P < 0.05$; *$P < 0.01$ vs DMH (analysis of variance followed by DMRT).

Enhanced activity of SOD and CAT in the intestinal, colonic and caecal tissues of caraway-supplemented rats may due to the ability of caraway to scavenge free radicals and toxic carcinogenic electrophiles (Satyanarayana et al 2004). In addition, the naturally occurring monoterpene *d*-limonene present in caraway has been found to inhibit various stages of tumorigenesis in a number of animal models and is now being evaluated as a chemopreventive agent in man (Uedo et al 1999). Moreover, Uedo et al (1999) have shown that *d*-limonene possesses significant chemopreventive and chemotherapeutic properties.

GSH, a principal non-protein thiol in mammalian cells, plays a crucial role in the detoxification of cellular free radicals, and maintains the integrity of the gastrointestinal tract, as well as the liver, when challenged by toxic agents. GR is a flavoprotein that permits the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) by the oxidation of NADH to NAD^+. A change in GSH status in cancer cells is associated with tumour growth in-vivo (Meister 1974). In this study the GSH and GR levels of the colonic mucosa were lower in rats treated with DMH as compared with control, which is consistent with our previous results (Manju et al 2005). The decrease in these levels as a result of DMH injections suggests that this tripeptide may be involved in the detoxification and possibly repair mechanisms in the colonic mucosa. On caraway supplementation, replenishment of GSH and GR levels reflects a favourable balance between potentially harmful oxidants and protective antioxidants, which may be due to its chemopreventive activity (Zheng et al 1992).

GST constitutes a gene superfamily of xenobiotic metabolizing enzymes, which binds various ligands and catalyses the nucleophilic addition of glutathione to diverse electrophilic substrates and carcinogens (Hayes & Pulford 1995). GPx catalyses the reduction of hydroperoxides by reduced GSH to their corresponding alcohols. Tsuchida et al (1989), using an enzyme-linked immunosorbent assay, found that the GST content in colon cancer mucosa was 6-fold higher than the level found in normal mucosa. Over-expression of GPx has been reported in a wide variety of tumours, including colon cancer (Lu et al 1997). The increase in the colonic and intestinal GST and GPx enzyme activity in tumours reflects increased detoxification capacity, which may be an adaptive mechanism by which tumour cells gain a selective growth advantage over their surrounding normal cells. There results are consistent with findings by Slater et al (1984).

Antioxidant nutrients such as vitamins C and E play an essential role in the prevention of colon carcinogenesis. Our data suggest that the levels of vitamins E and C were increased in DMH-treated rats. This suggests that the high content of vitamin E in colon tissues may contribute to the low levels of LPO observed in the DMH-induced tumour cells (Cheeseman et al 1984). Thus, both PUFA deficiency and relatively high content of the antioxidants may be responsible for the low rate of LPO in tumours. Moreover, the results of our study show that the activity of tissue GST and GPx, and levels of vitamins C and E, were gradually decreased on caraway supplementation to DMH-treated rats, which may be due to the antioxidant property of caraway (Satyanarayana et al 2004).

Caraway contains relatively high amounts of both flavonoids and monoterpenes (de Carvalho & Fonseca 2006). Flavonoids are known to suppress carcinogenesis in various animal models (Uedo et al 1999). Moreover, one of the mechanisms by which flavonoids, such as carvone and limonene, present in caraway may exert their putative anticancer effects may be through interaction with the P450 1A1 system, preventing the activation of procarcinogen (Eddouks et al 2004). In this context, Zheng et al (1992) reported that carvone and its related compound, by virtue of their antioxidant properties, have an ability to inhibit female A/J mice forestomach tumour and pulmonary adenoma formation.

Conclusion

The data reported show that caraway has an inhibitory effect on DMH-induced tumour formation, possibly by modulating histopathological changes, tissue LPO and antioxidant profile in colon cancer rats. Thus, caraway warrants more attention as a potential colon cancer chemopreventive agent.

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