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Dose-dependent effect of oregano (*Origanum vulgare* L.) on lipid peroxidation and antioxidant status in 1,2-dimethylhydrazine-induced rat colon carcinogenesis

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Abstract

Colon cancer is a major cause of morbidity and mortality in developed and developing countries. Diet and dietary constituents play a major role in the aetiology of colon cancer. We have investigated the effect of an aqueous extract of oregano (*Origanum vulgare*. L.) on lipid peroxidation and antioxidant status in 1,2-dimethylhydrazine (DMH)-induced rat colon carcinogenesis. We aimed to identify the important antioxidants present in Indian oregano using RP-HPLC. DMH (20 mg kg^{-1}) was administered subcutaneously once a week for the first four weeks and then discontinued. Oregano was supplemented every day orally at a dose of 20, 40 or 60 mg kg^{-1} to different groups of rats for 15 weeks. After this time the rats were killed and the colons were examined visually and evaluated biochemically. The levels of lipid peroxidation products, such as thiobarbituric acid reactive substances and conjugated dienes were significantly higher in the liver whereas in caecum and colon the levels were lower in DMH-treated animals as compared with control rats. The levels of the antioxidants superoxide dismutase, catalase, reduced glutathione, glutathione reductase, glutathione peroxidase and glutathione-S-transferase were decreased in DMH-treated rats, but were significantly reversed on oregano supplementation. Oregano supplementation (40 mg kg^{-1}) had a modulatory role on tissue lipid peroxidation and antioxidant profile in colon cancer-bearing rats, which suggested a possible anti-cancer property of oregano.

Introduction

Colon cancer is a major cause of morbidity and mortality in Western countries. Surgery and chemotherapy have made differences in survival only in the early stages of the disease. The prognosis of advanced disease has not improved significantly over the last 20 years. Harmful products in man's diet can promote colon cancer and hence prevention of colon cancer through the design of an optimal diet holds considerable promise. 1,2-Dimethylhydrazine (DMH), a potent and complete carcinogen, has been reliably used to induce the initiation and promotion steps of colon carcinogenesis in rodents. DMH and related compounds induce neoplasms specifically in the colon of rats even after a single dose (Ward 1974), the metabolic events of which are believed to occur in the liver with the formation of active intermediates such as azoxymethane and methylazoxymethanol, and are subsequently transported to the colon via bile or the blood (Fiala 1977). The decomposition of methylazoxymethanol results in the formation of methyldiazonium ion, which generates reactive carbonium ions capable of methylating DNA, RNA, or protein of colonic epithelial cells (Lang & Pelkonen 1999). Moreover, previous reports have pointed out the tendency of DMH to produce free radicals in blood, liver and large bowel (Weisburger 1971) in experimental models.

Lipid peroxidation is a paradox of aerobic life, affecting man's health and the quality of modern life (Davies 1995). Biological systems are lipid-rich matrices susceptible to autoxidation unless protected by either endogenous enzymatic or non-enzymatic mechanisms. Many herbs ingested with ordinary meals are known to possess some antioxidant components. Oregano (*Origanum vulgare* L.) is one such herb, which is known to consist of many effective antioxidants, such as rosmarinic acid, caffeic acid, and various flavonoids (Tada 2000). These compounds also function as substrates for peroxidase. In this study, we have

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investigated the effect of oregano on lipid peroxidation and antioxidant status in DMH-induced rat colon carcinogenesis. By using RP-HPLC we have attempted to identify the important antioxidants present in Indian oregano. The system we used was a high-resolution chromatographic technique widely used for the simultaneous identification, separation and quantification of phenolic compounds.

Materials and Methods

Materials

Compounds rosmarinic acid and caffeic acid were purchased from Laila Research Centre, Vijayawada, India. The notified purity was >95%. Methanol, acetonitrile (both HPLC grade), and phosphoric acid (analytical grade) were purchased from Qualigens (Mumbai, India). Ultrapure water generated by the Barnstead nanopure system (Model D3750, US) was used.

HPLC instrumentation

HPLC experiments were performed on a Shimadzu HPLC system equipped with Phenomenex Luna C_{18}, 5-μm (4.6×250 mm) column, LC10AT VP pumps, a SCL-10AVP system controller, SIL-10 AD VP auto-injector, and SPD-M10 AVP photodiode array detector. Class VP software was used.

Carcinogen administration

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

Oregano aqueous extract preparation

Dry oregano leaves were purchased from Herbs & Herbs, a quality-maintaining herbal supplier based in Chennai, India. The plant was previously identified and authenticated by M. Rejdali, Agronomy and Veterinary Institute, Rabat (Eddouks et al 2002). Preparation of oregano aqueous extract was according to the traditional Moroccan method (Eddouks et al 2003). The leaves were air-dried at 40°C and ground into a fine powder. Powdered leaves (1 g) were mixed with 100 mL distilled water, boiled for 10 min and then cooled for 15 min. Thereafter, the aqueous extract was filtered using a Millipore filter (Millipore 0.2 mm, St Quentin En Yvelines, France), freeze-dried and the desired dose (mg of lyophilized aqueous extract of oregano leaves per kg body weight) was then prepared and reconstituted in 1.5 mL daily (oregano aqueous extract), just before administration. The aqueous extract was administered by intragastric intubation (20, 40 or 60 mg kg^{-1}) at approximately 10.30 h every day for 15 weeks to avoid diurnal variation.

Animals

Male Wistar albino rats (100–120 g; Central Animal House, Annamalai University, India) were used. The rats were kept in polypropylene cages (four per cage) with paddy husk for bedding and fed commercial pellet diet for one week's stabilization. The rats were randomly divided into six groups, each containing 10 rats, and maintained under controlled conditions of temperature (24±2°C) and humidity (50±10%), with 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, as detailed in Table 1. Rats were allowed free access to this modified powdered pellet diet and tap water throughout the 15-weeks experimental period.

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 Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA).

Treatment regimen

Group 1 rats served as the untreated control. Group 2 rats were given 60 mg kg^{-1} oregano aqueous extract orally, every day for 15 weeks. Groups 3–6 were injected with DMH 20 mg kg^{-1} subcutaneously once a week for the first four weeks. In addition to DMH 20 mg kg^{-1} subcutaneously once a week for the first four weeks, rats in groups 4–6 received 20, 40 or 60 mg kg^{-1} oregano aqueous extract orally every day, respectively, for a total duration of 15 weeks. Rats were monitored daily for general health, and body weight was recorded every week over the entire study period. At the end of the experimental period, food was with held overnight and the rats were killed by decapitation.

Experimental protocol (see Figure 1)

Homogenization of tissues

Immediately after rats were killed, liver, caecum, proximal and distal colonic tissues were washed with saline. The tissues were then cut into fragments and homogenized with 3 vol (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 biochemical estimations.

Biochemical estimations

Lipid peroxidation (LPO) was estimated by measuring the level of tissue LPO byproducts, such as conjugated dienes (CD) and

Table 1 Composition of the diet

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

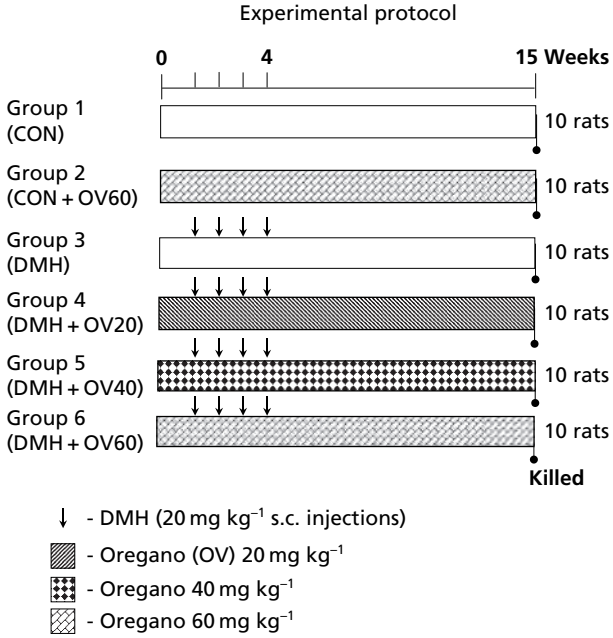

Figure 1 Experimental protocol.

thiobarbituric acid reactive substances (TBARS) by using the methods of Rao & Recknagel (1968) and Ohkawa et al (1979), respectively. The values were expressed as mmol (mg tissue)$^{-1}$.

Superoxide dismutase (SOD, EC1.15.1.1) was assayed using the method of Kakkar et al (1984) based on 50% inhibition of the formation of NADH phenazine methosulfate-nitro blue 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 min^{-1} (mg protein)$^{-1}$. The activity of catalase (CAT, EC 1.11.16) was determined by the method of Sinha (1972). The activity of CAT was expressed as μmol H_2O_2 utilized min^{-1} (mg protein)$^{-1}$. Reduced glutathione (GSH) was determined by the method of Ellman (1959). The values were expressed as mmol (mg tissue)$^{-1}$. Glutathione reductase (GR, EC1.6.4.2) activity was assayed using the method of Carlberg & Mannervik (1985) and the values were expressed as μmol NADPH oxidized min^{-1} (mg protein)$^{-1}$. Glutathione peroxidase (GPx, EC 1.11.1.9) activity was assayed by the method of Rotruck et al (1973), the values were expressed as μmol GSH utilized min^{-1} (mg protein)$^{-1}$. The activity of glutathione S-transferase (GST, EC.2.5.1.18) was estimated by the method of Habig et al (1974) and the values were expressed as μmol 1-chloro –2,4-dinitro benzene (CDNB)–GSH conjugate formed min^{-1} (mg protein)$^{-1}$. The protein content was determined by the method of Lowry et al (1951) using bovine serum albumin as the standard at 660 nm.

HPLC analytical method

We used a reverse-phase HPLC column and a mixture of 0.1% phosphoric acid and acetonitrile as the mobile phase at a flow rate of 1 mL min^{-1}. Detection was with a PDA detector and the column temperature was maintained at 30°C. The detection wavelength was set at 330 nm. The injection volume was 20 μL. The total run time was 35 min. The method applied to the oregano extract and its reference compounds **1** and **2** (rosmarinic acid and caffeic acid, respectively) ran successfully.

Statistical analysis

Data were analysed by one-way analysis of variance and the 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 oregano on the incidence of colon tumours and the number of tumours/polyps per tumour-bearing rat

The effect of oregano on colonic tumour incidence, number of tumours/polyps per tumour-bearing rat is summarized in Table 2. In rats treated with DMH (group 3) the tumour incidence in the colon was 60%. Supplementing DMH-treated rats with oregano at different doses (20, 40 or 60 mg kg^{-1} given to groups 4,5 and 6, respectively) reduced tumour incidence (40, 20 and 30%, respectively). The total number of tumours in DMH-treated rats was 11, which was markedly reduced to three after 40 mg kg^{-1} oregano supplementation (group 5).

HPLC method validation

The specificity of the method was ascertained by analysing the standards and the sample. The peaks for compounds **1**

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

Group	No. of rats	No. of tumour-bearing rats	Tumour incidence (%)	Total no. of tumours/polyps	No. of tumours/polyps per tumour-bearing rat
DMH	10	6	60	11	1.86[a]
DMH + oregano 20 mg kg^{-1}	10	4	40	7	1.75[b]
DMH + oregano 40 mg kg^{-1}	10	2	20	3	1.50[c]
DMH + oregano 60 mg kg^{-1}	10	3	30	5	1.66[d]

Tumour incidence was expressed as (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 $P < 0.05$ (analysis of variance followed by DMRT).

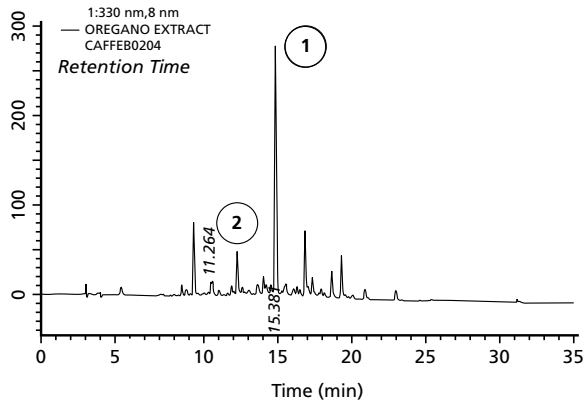

Figure 2 HPLC chromatogram of oregano. Peaks are (**1**) rosmarinic acid and (**2**) caffeic acid. For conditions see Materials and Methods.

(rosmarinic acid 15.381) and **2** (caffeic acid 11.264) in the sample were confirmed by comparing the retention times of the peaks with that of standards (Figure 2). The results proved that the oregano (Indian oregano) extract contained rosmarinic acid and caffeic acid as the main antioxidants.

Effect of oregano and DMH on the lipid peroxidation products of control and experimental rats

Table 3 summarizes the data on the effect of oregano supplementation on the LPO byproducts such as TBARS and conjugated dienes in liver, caecal, proximal and distal colonic tissues of control and experimental rats. TBARS and conjugated diene levels were lower in DMH-treated rats (group 3) in caecal, proximal and distal colonic tissues as compared with the untreated control rats (group 1), whereas in the liver, the TBARS and conjugated diene levels were significantly elevated in the DMH-treated rats as compared with the untreated control rats. However, on oregano supplementation (groups 4–6) tissue LPO byproducts in the caecum, proximal and distal colon were elevated significantly ($P<0.05$), the significance being marked in rats on 40 mg kg^{-1} oregano supplementation (group 5). Liver TBARS and conjugated diene levels were also optimized on oregano supplementation to DMH-treated rats.

Effect of oregano and DMH on the activity of SOD and CAT of control and experimental rats

Table 4 depicts the activity of the antioxidant enzymes SOD and CAT in the liver, proximal, distal and caecal tissues of control and in all experimental rats. Our results indicated that the activity of tissue SOD and CAT in the DMH-administered rats (group 3) was significantly lowered as compared with the control (group 1). However, treatment with oregano at a daily dose of 20, 40 or 60 mg kg^{-1} significantly ($P<0.05$) increased the activity of these enzymes, a more pronounced effect being observed in the 40 mg kg^{-1} supplemented group (group 5).

Effect of oregano and DMH on tissue GSH and GSH-dependent enzymes

The levels of GSH and GSH-dependent enzymes in the liver, proximal, distal colonic tissues and caecum are shown in Tables 5 and 6. On DMH treatment (group 3), GSH and GR activity in the liver, 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 the DMH + oregano-treated rat tissues, and a moderate increase was observed in the 40 mg kg^{-1} (group 5) as compared with rats treated with DMH alone (group 3). GST and GPx activity significantly declined on treatment with DMH ($P<0.05$), whereas on oregano supplementation the GST and GPx activity significantly ($P<0.05$) elevated. Among the oregano-supplemented groups, the rats that received 40 mg kg^{-1} (group 5) showed a statistically significant improvement in GST and GPx activity.

Table 3 Effect of oregano on the levels of tissue LPO byproducts of control and experimental rats

Parameters	Control	Control + oregano 60 mg kg^{-1}	DMH	DMH + oregano 20 mg kg^{-1}	DMH + oregano 40 mg kg^{-1}	DMH + oregano 60 mg kg^{-1}
Thiobarbituric acid reactive substances (TBARS) (mmol (mg tissue)$^{-1}$)						
Liver	0.35 ± 0.04^{a}	0.36 ± 0.04^{b}	0.70 ± 0.06^{c}	0.68 ± 0.04^{c}	0.44 ± 0.06^{a}	0.62 ± 0.05^{d}
Proximal colon	0.66 ± 0.03^{a}	0.69 ± 0.06^{a}	0.31 ± 0.05^{b}	0.37 ± 0.05^{b}	0.56 ± 0.02^{a}	0.45 ± 0.03^{c}
Distal colon	0.71 ± 0.06^{a}	0.73 ± 0.03^{a}	0.37 ± 0.04^{b}	0.48 ± 0.04^{c}	0.62 ± 0.04^{a}	0.49 ± 0.06^{c}
Caecum	0.69 ± 0.04^{a}	0.72 ± 0.05^{a}	0.35 ± 0.06^{b}	0.39 ± 0.04^{b}	0.61 ± 0.03^{a}	0.49 ± 0.05^{c}
Conjugated dienes (mmol (mg tissue)$^{-1}$)						
Liver	36.20 ± 4.13^{a}	36.65 ± 2.72^{a}	55.92 ± 4.72^{c}	50.59 ± 4.01^{bc}	37.44 ± 2.86^{a}	48.62 ± 3.71^{b}
Proximal colon	46.09 ± 5.46^{a}	50.59 ± 4.01^{a}	32.76 ± 2.50^{b}	37.44 ± 2.86^{c}	45.57 ± 3.48^{a}	43.67 ± 3.33^{d}
Distal colon	55.06 ± 4.20^{a}	55.92 ± 4.27^{a}	41.02 ± 3.13^{b}	42.54 ± 3.24^{b}	51.78 ± 3.95^{ac}	47.33 ± 3.61^{c}
Caecum	52.86 ± 4.03^{a}	53.25 ± 4.06^{a}	36.44 ± 2.85^{b}	44.95 ± 3.43^{c}	49.45 ± 3.77^{ad}	46.43 ± 3.54^{cd}

The values were means ± s.d. from 10 rats in each group. The values with different superscripts are significantly different from each other, $P<0.05$. Mean values were significantly different from the DMH-treated groups (analysis of variance followed by DMRT).

Table 4 Effect of oregano on the tissue SOD and CAT activity of control and experimental rats

Parameters	Control	Control + oregano 60 mg kg^{-1}	DMH	DMH + oregano 20 mg kg^{-1}	DMH + oregano 40 mg kg^{-1}	DMH + oregano 60 mg kg^{-1}
Superoxide dismutase (SOD) (enzyme required for 50% inhibition of NBT reduction)						
Liver	3.49 ± 0.26^{a}	3.29 ± 0.25^{ad}	2.45 ± 0.18^{b}	2.83 ± 0.20^{c}	3.19 ± 0.24^{ad}	2.77 ± 0.21^{c}
Proximal colon	4.21 ± 0.33^{a}	3.69 ± 0.28^{b}	2.56 ± 0.20^{c}	2.79 ± 0.21^{c}	3.90 ± 0.30^{a}	3.01 ± 0.24^{d}
Distal colon	3.77 ± 0.28^{a}	3.67 ± 0.28^{ac}	2.46 ± 0.19^{b}	2.58 ± 0.19^{b}	3.47 ± 0.24^{c}	2.80 ± 0.21^{d}
Caecum	4.19 ± 0.31^{a}	4.10 ± 0.31^{a}	2.73 ± 0.21^{b}	2.85 ± 0.21^{b}	3.48 ± 0.26^{a}	3.12 ± 0.22^{c}
Catalase (CAT) (μmol H_2O_2 utilized min^{-1} (mg protein)$^{-1}$)						
Liver	33.43 ± 2.55^{a}	32.12 ± 2.46^{a}	22.29 ± 1.70^{b}	23.26 ± 1.77^{b}	29.15 ± 2.14^{ac}	26.32 ± 1.85^{c}
Proximal colon	38.20 ± 2.91^{a}	41.13 ± 3.14^{a}	23.75 ± 1.81^{b}	26.71 ± 2.04^{b}	36.46 ± 2.40^{a}	29.41 ± 2.24^{c}
Distal colon	41.13 ± 3.14^{a}	40.31 ± 3.37^{a}	33.43 ± 2.55^{b}	36.65 ± 2.72^{c}	38.20 ± 2.91^{ac}	35.47 ± 2.91^{bc}
Caecum	39.86 ± 3.04^{a}	41.13 ± 3.14^{a}	25.71 ± 1.99^{b}	31.46 ± 2.40^{c}	36.65 ± 2.72^{a}	29.72 ± 2.26^{c}

The values were means ± s.d. from 10 rats in each group. The values with different superscripts are significantly different from each other, $P < 0.05$. Mean values were significantly different from the DMH-treated groups (analysis of variance followed by DMRT).

Table 5 Effect of oregano on tissue glutathione of control and experimental rats

Parameters	Control	Control + oregano 60 mg kg^{-1}	DMH	DMH + oregano 20 mg kg^{-1}	DMH + oregano 40 mg kg^{-1}	DMH + oregano 60 mg kg^{-1}
Glutathione (GSH) (mmol (mg tissue)$^{-1}$)						
Liver	20.68 ± 1.58^{a}	21.32 ± 1.62^{a}	14.70 ± 0.81^{b}	15.78 ± 0.93^{bc}	18.31 ± 1.25^{a}	16.60 ± 0.96^{c}
Proximal colon	21.84 ± 1.68^{a}	20.38 ± 1.72^{a}	12.65 ± 0.98^{b}	16.50 ± 1.02^{c}	18.52 ± 1.26^{ac}	14.18 ± 1.07^{d}
Distal colon	20.96 ± 1.69^{a}	20.63 ± 1.60^{a}	14.63 ± 0.88^{b}	16.32 ± 1.01^{b}	18.88 ± 1.37^{ac}	17.93 ± 1.06^{c}
Caecum	18.85 ± 1.06^{a}	17.44 ± 1.57^{a}	11.09 ± 0.85^{b}	14.87 ± 0.97^{c}	16.42 ± 1.25^{ac}	12.57 ± 1.00^{bd}

The values are means ± s.d. from 10 rats in each group. The values with different superscripts are significantly different from each other, $P < 0.05$. Mean values were significantly different from the DMH-treated groups (analysis of variance followed by DMRT).

Table 6 Effect of oregano on tissue glutathione-dependent antioxidants of control and experimental rats

Parameters	Control	Control + oregano 60 mg kg^{-1}	DMH	DMH + oregano 20 mg kg^{-1}	DMH + oregano 40 mg kg^{-1}	DMH + oregano 60 mg kg^{-1}
Glutathione peroxidase (GPx) (μmol of GSH utilized min^{-1} (mg protein)$^{-1}$)						
Liver	7.20 ± 0.54^{a}	6.89 ± 0.52^{a}	3.39 ± 0.25^{b}	3.58 ± 0.27^{b}	5.27 ± 0.32^{c}	4.71 ± 0.28^{d}
Proximal colon	7.21 ± 0.52^{a}	7.40 ± 0.56^{a}	4.92 ± 0.24^{b}	5.18 ± 0.24^{b}	6.94 ± 0.34^{a}	6.46 ± 0.26^{c}
Distal colon	6.54 ± 0.51^{a}	6.39 ± 0.55^{a}	4.35 ± 0.17^{b}	4.71 ± 0.21^{b}	5.96 ± 0.33^{ac}	5.20 ± 0.27^{c}
Caecum	6.75 ± 1.51^{a}	6.71 ± 0.54^{a}	4.99 ± 0.22^{b}	5.82 ± 0.24^{c}	6.35 ± 0.33^{a}	5.58 ± 0.25^{c}
Glutathione-S-transferase (GST) (μmol CDNB-GSH conjugate formed min^{-1} (mg protein)$^{-1}$)						
Liver	3.47 ± 0.26^{a}	3.09 ± 0.23^{a}	1.95 ± 0.14^{b}	2.10 ± 0.16^{b}	3.20 ± 0.24^{a}	2.50 ± 0.19^{c}
Proximal colon	3.19 ± 0.24^{a}	2.96 ± 0.22^{a}	1.57 ± 0.11^{b}	2.33 ± 0.13^{c}	2.87 ± 0.21^{ac}	1.99 ± 0.15^{d}
Distal colon	3.16 ± 0.24^{a}	2.97 ± 0.21^{a}	1.68 ± 0.12^{b}	2.41 ± 0.13^{c}	2.92 ± 0.22^{a}	2.66 ± 0.15^{c}
Caecum	3.32 ± 0.25^{a}	3.09 ± 0.23^{a}	1.77 ± 0.13^{b}	2.00 ± 0.15^{bc}	3.08 ± 0.22^{a}	2.12 ± 0.16^{c}
Glutathione reductase (GR) (μmol NADPH oxidized min^{-1} (mg protein)$^{-1}$)						
Liver	12.65 ± 0.98^{a}	13.50 ± 1.02^{a}	8.89 ± 0.52^{b}	10.35 ± 0.63^{c}	11.70 ± 0.81^{ac}	9.55 ± 0.69^{bc}
Proximal colon	13.44 ± 1.02^{a}	13.85 ± 1.06^{a}	9.68 ± 0.51^{b}	10.87 ± 0.52^{c}	12.97 ± 1.00^{a}	10.40 ± 0.56^{c}
Distal colon	14.23 ± 1.10^{a}	14.06 ± 1.08^{a}	9.15 ± 0.69^{b}	10.51 ± 0.80^{b}	13.32 ± 1.01^{a}	11.39 ± 0.85^{c}
Caecum	12.60 ± 0.96^{a}	13.13 ± 1.06^{a}	8.35 ± 0.63^{b}	9.15 ± 0.69^{b}	11.63 ± 0.88^{a}	10.51 ± 0.80^{bc}

The values are means ± s.d. from 10 rats in each group. The values with different superscripts are significantly different from each other, $P < 0.05$. Mean values are significantly different from the DMH-treated groups (analysis of variance followed by DMRT).

Discussion

The results have revealed the protective role of oregano (20, 40 and 60 mg kg^{-1}) on lipid peroxidation and antioxidant status in DMH-induced rat colon cancer. Oregano at a dose of 40 mg kg^{-1} was able to exert a more pronounced effect, as shown by the incidence of tumours/polyps and also biochemically by a significant reduction in the extent and severity of colon carcinogenesis. The lower dose of oregano (20 mg kg^{-1}) was not found to be effective, possibly because the concentration might not have been enough to counteract the damaging effects of DMH (a potent carcinogen). The high dose (60 mg kg^{-1}) was effective but not as effective as 40 mg kg^{-1}. This might have been because at the higher concentration oregano might have resulted in the production of byproducts interfering with the antioxidant activity of oregano, thus decreasing its effect. Rukkumani et al (2004) reported that ferulic acid, a natural antioxidant, was less effective in higher doses and they proposed the same mechanism. Therefore from our study we concluded that the optimal dose, 40 mg kg^{-1}, was potent/beneficial against DMH-induced colon carcinogenesis.

Alterations in the oxidant and antioxidant profile are known to occur in cancer (Saroja et al 1999). Oxidative stress is due to damage brought about by free radical attack on cellular molecules such as lipids and DNA (Van Rossen 2000). Recent evidence has indicated that the generation of reactive oxygen species may be involved in various carcinogenic processes (Fang et al 2002). Free radical-mediated mechanisms represent a significant pathway for metabolic activation of carcinogens (Fiala 1977). DMH is a carcinogen that undergoes metabolic activation in the liver to produce active electrophilic carbonium ion, which in turn through several processes, is known to elicit oxidative stress.

In this study, the levels of LPO byproducts, such as TBARS and conjugated dienes, were measured in the liver, caecal and colonic tissues of short-term DMH-treated rats. Levels of lipid peroxidation products, conjugated dienes and malondialdehyde determined in the liver were significantly higher, whereas the levels of lipid peroxidation products were significantly lowered in the colon and caecum in DMH only treated rats as compared with control rats. Previous studies have shown reduced levels of lipid peroxidation in the tumour tissue of various types of cancer (Cheesman et al 1986; Tanaka 1997; Tanaka et al 1998; Manoj et al 1999). Cancer cells acquire particular characteristics that benefit their proliferation (Schmelz et al 2000), and they tend to proliferate faster when the lipid peroxidation level is low. Therefore, the decreased lipid peroxidation observed in the colon and caecum of DMH-treated rats could be due to increased cell proliferation. Thus, malignant tissues are less susceptible and more resistant to free radical attack, and hence lipid peroxidation is less intense (Nakagami et al 1990). Our results correlated with Masotti et al (1998), who suggested that malignant cells were better protected than their normal counterparts against free radical mediated damage. In addition to this, the decreased levels of lipid peroxidation in DMH-treated rats may also have been due to increased resistance and/or decreased susceptibility of the target organs to free radical attack.

Oregano administration to DMH-treated rats restored the lipid peroxidation levels to near those of the control rats, which may have been due to the antioxidant properties of the active principles in oregano such as carvacrol, thymol etc. (Koparal & Zeytinoglul 2003). Oregano may have facilitated increased susceptibility and decreased resistance of tumor cells to free radical attack, leading to decreased cell proliferation.

SOD and CAT are two important enzymic antioxidants that act against toxic oxygen free radicals such as superoxide ($O_2^{-\bullet}$) and hydroxyl ($^{\bullet}OH$) ions in biological systems. They are involved in the direct elimination of reactive oxygen metabolites, which is probably one of the most effective defenses of the living body against disease (Burton et al 1983). Impaired efficiency of these enzymes leads to disease, including cancer (Rajesh Kumar & Kuttan 2003). Slaga (1995) has shown that carcinogen administration usually decreases the levels of SOD and CAT. Our results also show a similar trend in the SOD and CAT activity of the liver, colon and caecal tissues of DMH-treated rats. The decreased activity of SOD and CAT may have been due to the dangerous increase in the levels of reactive oxygen species, and thus enhanced oxidative stress and proliferation of colonocytes in colorectal carcinoma (Burton et al 1983). Janssen et al (1999) showed that low levels of SOD and CAT activity in precancerous tissues promoted growth of cancer and its infiltration into the surrounding tissues, which was important for invasion and metastasis.

In our study, we observed enhanced activity of SOD and CAT in the liver, colonic and caecal tissues on oregano supplementation, which may have been due to the ability of oregano to scavenge free radicals and toxic carcinogenic metabolites, thus sparing the endogenous antioxidants. This activity of oregano extract may have been due to the presence of effective antioxidants, such as rosmarinic acid, caffeic acid and various flavonoids (Wollenweber 1998).

GSH, a tripeptide, plays a vital role in the detoxification of many environmental carcinogens and free radicals. It also maintains the integrity of the gastrointestinal tract and liver (Meister 1974). It is generally accepted that reduced GSH and GSH-dependent enzymes have an important role in the protection of mammalian cells against oxidative and alkylating agents. GR is a glutathione-regenerating enzyme that permits the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) by the oxidation of NADH to NAD^+. In this study, GSH and GR levels of the colonic mucosa were reduced in rats treated with DMH as compared with control, which was consistent with previous results (Kamaleewari & Nalini 2006). The depleted levels of GSH on DMH administration suggested that the tripeptide may have been involved in the detoxification and possibly repair mechanisms in the colonic mucosa. The restoration of GSH and GR levels on oregano supplementation reflect a favourable balance between potentially harmful oxidants and natural antioxidants, which may have been due to the chemopreventive effect of oregano (Garcia & Sanz 2001).

GST constitutes a multi-gene super family of xenobiotic metabolizing enzymes, which bind various ligands and catalyses the nucleophilic addition of glutathione to diverse electrophilic substrates and carcinogens (Hayes & Pulford 1995). GPx plays a crucial protective role by removing hydrogen

peroxide and lipid (organic) hydroperoxides. The reduction in GPx activity would cause the formation of potentially carcinogenic toxic compounds. The decline in the colonic and liver GST and GPx enzyme activity in tumours, which was also observed in our study, reflected increased detoxification capacity, and may have been an adaptive mechanism by which tumour cells gained a selective growth advantage over their surrounding normal cells (Slater 1984). In our study on DMH treatment, GST and GPx levels were found to be reduced in the colonic, hepatic and caecal tissues, which were shown to be enhanced on oregano supplementation.

This activity of oregano was thought to be due to its phenolic constituents such as rosmarinic acid, caffeic acid and certain volatile compounds, namely linalool, alcohol, phenol (Deferera et al 2000; Garcia & Sanz 2001), terpenes (Mockute et al 2001) and some flavonoids (Harpaz et al 2003). Although the effect of these compounds in colon cancer has not been demonstrated, their potential antibacterial and antioxidant activity has been proved (Wollenweber 1998; Stashenko et al 2002; Burt & Reinders 2003). In this context flavonoids are considered as active compounds in many medicinal plants and natural products with positive effect on human health (Wollenweber 1998).

Meanwhile, we developed a simple method for the identification of antioxidants present in Indian oregano based on reversed-phase HPLC separation combined with UV detection.

HPLC columns differing in stationary phase and pore sizes were initially screened for their potential to resolve the compounds **1** and **2** (rosmarinic acid and caffeic acid, respectively). Special attention was paid to separation efficiency, peak shape and separation time. The best results were obtained with a Luna C_{18} column 5-μm particle size, 250 mm length and 4.6 mm i.d. (Phenomenex), using phosphoric acid (0.1%) and acetonitrile as the mobile phase, with a 1 mL min^{-1} flow rate, enabling the baseline separation of two compounds within 35 min. All the separations were performed at 30°C. Absorption measurement at 330 nm was selected and the compounds rosmarinic acid and caffeic acid were effectively detected. The study confirmed that the stable retention times of the identified compounds in the sample were comparable with retention times of standard compounds with ± 0.2 to ± 0.3 min variation (Figure 2).

Conclusion

Oregano, a medicinal herb, has been shown to possess a profound antioxidant and anticarcinogenic effect on DMH-induced tumour formation. This was probably by altering the tissue LPO and antioxidant status in colon cancer bearing rats. The findings represent for the first time an experimental confirmation of the anticarcinogenic efficacy of this herb. The precise mechanism and site of oregano's activity are still to be determined.

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