

# Effect of ginger on lipid peroxidation and antioxidant status in 1,2-dimethyl hydrazine induced experimental colon carcinogenesis

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## Abstract

The prevalence of colon cancer has rapidly risen during the last decade. In this study we have evaluated the chemopreventive efficacy of ginger in 1,2-dimethyl hydrazine (DMH) induced colon carcinogenesis. Rats were given a weekly subcutaneous injection of DMH at a dose of 20mg/kg body weight for 15 weeks. Ginger (50mg/kg body weight/day) was given at the initiation and also at the post-initiation stages of carcinogenesis to DMH treated rats every day. The animals were sacrificed at the end of 30 weeks. Colon cancer incidence was 100% in DMH treated rats. The incidence of cancer as well as the number of tumours in the colon was significantly reduced on supplementing ginger to DMH treated rats. The levels of lipid peroxidation and the activities of the enzymic antioxidants such as superoxide dismutase and catalase in the colon and intestines were significantly decreased whereas the activities of glutathione and its dependent enzymes such as, glutathione peroxidase, glutathione-S-transferase and glutathione reductase and the levels of non-enzymic antioxidants such as vitamin C and vitamin E were significantly elevated in DMH treated rats as compared to control animals. Ginger supplementation to DMH treated rats inhibited colon carcinogenesis, as evidenced by the significantly decreased number and incidence of tumours. In addition ginger optimized tissue lipid peroxidation and antioxidant status in DMH treated rats.

**Keywords:** Antioxidants, 1, 2-dimethyl hydrazine, ginger, lipid peroxidation

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## Introduction

Colon cancer mortality in developed countries has been steadily rising throughout most part of this century (Bandaru et al 1999). Epidemiological evidences show that certain factors in the diet may play an important role in inducing colon carcinogenesis, whereas others may retard carcinogenesis. In animal studies, repeated treatment with the colon specific carcinogen, 1,2-dimethyl hydrazine (DMH), induces the formation of colon tumours in rodents (Pozharisski et al. 1979; Druckrey 1972). DMH is metabolized in the liver, resulting in the production of an intermediate electrophilic diazonium ion, which is known to initiate lipid peroxidation (Fiala 1977; Fiala and Sohn, 1987).

Previous studies have shown that dietary fat influences membrane structure and function, and those membranes with a high unsaturation index are more sensitive to peroxidation. Lipid peroxides may affect the regulation of DNA synthesis and cell division and this is said to be involved in a number of diseases such as inflammation of the large bowel and cancer.

Natural dietary antioxidants including phenolic or thiolic compounds could protect against damages caused by reactive oxidants (Janssen et al 1995; Ozer et al. 1993). Ginger (*Zingiber officinale* Rosc) is one of the most widely used spices in India and has been utilized frequently in traditional oriental medicine for common cold, digestive disorders and rheumatism (Surh et al. 1998). Several constituents of ginger including gingerol, shogaol and zingerone have been shown to possess many interesting pharmacological and physiological activities such as antioxidant, anti-inflammatory, analgesic, anticarcinogenic and cardiogenic effects (Surh et al. 1998; Masuda et al. 1995). In addition ginger was found to inhibit platelet aggregation *in vitro* (Lumb 1994) and tumour promotion by phorbol ester (Surh et al, 1999; Park et al, 1998; Kim et al 2005). Dietary ginger is also known to induce prostacyclin biosynthesis *in vivo* (Srivastava, 1984).

Spices are heterogeneous collections of a wide variety of volatile and non-volatile dietary additives. Spices have the capacity to affect markers associated with cancer prevention mechanisms provides

**Table 1:** Composition of the diet

	Commercial diet 84.2%	Peanut oil 15.8%	Total
Protein	17.7	-	17.7
Fat	4.2	15.8	20.0
Carbohydrate	50.5	-	50.5
Fiber	3.4	-	3.4
Mineral	6.7	-	6.7
Vitamin	1.7	-	1.7

important leads that can guide chemoprevention strategies (Kochhar, 2008). Ginger is used worldwide as a cooking spice, condiment and herbal remedy. It is also extensively consumed as a flavoring agent; it is estimated that in India, the average daily consumption is 8-10 grams of fresh ginger root (Murray, 1995). So far no study on the colon cancer inhibitory property of ginger has been reported, we deemed it important to conduct this assay and to determine the efficacy of ginger as a chemopreventive agent. We have also evaluated the role of ginger at the initiation; post-initiation stages of DMH induced colon carcinogenesis by using tissue lipid peroxidation and antioxidant status as biomarkers.

## Methods

### Chemicals

DMH was obtained from Sigma Chemical Company, St. Louis, USA. All other chemicals and reagents used were of analytical grade.

### Preparation of ginger

Fresh ginger rhizomes (*Zingiber officinale* Rosc) were directly purchased from the nearby gardens of Chidambaram town, Tamil Nadu, India and its botanical identity was confirmed by a Botanist in the Department of Botany, Faculty of Science, Annamalai University. The rhizomes were then thoroughly washed, peeled, completely dried, coarsely minced and made into a fine powder. The ginger powder was suspended in tap water and each animal received 1ml of ginger suspension at a dose of 50mg/kg body weight every day. The low dose of ginger used here is with reference to the average daily intake by human beings, learnt from a survey conducted in India (Srinivasan and Sambaiah 1991).

### Tumour induction

DMH was dissolved in 1mM EDTA just prior to use and the pH was adjusted to 6.5 with 1mM sodium bicarbonate to ensure the stability of the chemical. Animals were given a weekly subcutaneous injection of DMH in the groin at a dose of 20mg/kg body weight for 15 weeks (Nalini et al. 2004).

### Experimental animals

Male Wistar rats 100-120g bodyweight were obtained from the Central Animal House, Department of Experimental Medicine, Annamalai University, Tamil Nadu, India and maintained at  $27 \pm 2^\circ\text{C}$  with 12h-light/12h-dark cycles. Commercial pellet diet (Table 1) containing 4.2% fat (Hindustan Lever Ltd., Mumbai, India) was powdered and mixed with 15.8% peanut oil making a total of 20% fat was fed to rats throughout the experimental period of 32 weeks (including 2 weeks of acclimatization) to all the rats. Water was given *ad libitum*. Rats were randomized into 5 groups of ten animals each.

Rats in group 1 received no treatment and served as the untreated control. Group 2 animals received ginger by intragastric intubation daily at a dose of [50mg/kg body weight] everyday. Rats in groups 3 to 5 received DMH [20mg/kg body weight] injection once a week subcutaneously for the first 15 weeks. Group 4 rats received ginger as in group 2 starting one week before DMH injections and continued till

one week after the final exposure [DMH + ginger (initiation)]. Group 5 rats received ginger as in group 2 starting one week after the cessation of DMH injections and continued till the end [DMH + ginger (post-initiation)]. The experimental protocol is clearly represented in figure 1.

The experiment was terminated at the end of 30 weeks and all the animals were killed by cervical dislocation after an overnight fast. The colon was split open longitudinally and gross tumours were counted. Colonic and intestinal tissues were then processed and used for various biochemical estimations. Tissue samples were immediately transferred to ice-cold containers weighed and homogenized using the appropriate buffer in a tissue homogeniser.

Lipid peroxidation was estimated by measuring the levels of thiobarbituric acid reactive substances (TBARS) in tissues by the method of Ohkawa et al (1979). The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was estimated at 532 nm. The values are expressed as nmoles/100 g tissue. The level of conjugated dienes was assessed using the method of Rao and Recknagel et al (1968). This method is based on the arrangement of the double bonds in polyunsaturated fatty acids (PUFA) to form conjugated dienes with an absorbance maximum at 233 nm. The values are expressed as mmoles/100 g tissue. The lipid hydroperoxide contents were measured using the method of Jiang et al (1992). Hydroperoxides are detected by their ability to oxidize ferrous iron leading to the formation of a chromophore with an absorbance maximum at 560nm. The values are expressed as mmoles/100 g tissue. Reduced glutathione (GSH) content was determined via the method of Ellman (1959). GSH determination is based on the development of a yellow color when 5, 5' dithio (2-nitro benzoic acid) (DTNB) is added to compounds containing sulfhydryl groups. The values are expressed as mg/g tissue. Glutathione peroxidase (GPx, EC.1.11.1.9) activity was assayed via the method of Rotruck et al (1968) with a modification. A known amount of enzyme preparation was incubated with  $\text{H}_2\text{O}_2$  in the presence of GSH for a specified time period. The amount of  $\text{H}_2\text{O}_2$  utilized was determined using the method of Ellman (1959). The values are expressed as  $\mu\text{moles}$  of GSH utilized/min/mg protein. The activity of glutathione-S-transferase (GST, EC. 2.5.1.18) was estimated via the method of Habig et al (1974) by following the increase in absorbance at 340 nm using 1-chloro-2, 4-dinitrobenzene (CDNB) as the substrate. The values are expressed as  $\mu\text{moles}$  of CDNB-GSH conjugate formed/min/mg protein. Glutathione reductase activity was assayed using the method of Carlberg and Mannervik (1985) by measuring GSH formed by NADPH. The values are expressed as  $\mu\text{moles}$  of NADPH oxidised/min/mg protein.

Superoxide dismutase (SOD, EC.I.15.1.1) was assayed using the method of Kakkar et al (1984) based on the 50% inhibition of the formation of NADH-phenazine methosulfate-nitroblue tetrazolium formazan at 520 nm. One unit of the enzyme is taken as the amount of enzyme required for 50% inhibition of NBT reduction/min/mg protein. The activity of catalase (CAT, EC.1.11.16) was determined via the method of Sinha (1972). Dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), with the formation of perchromic acid as an unstable CAT intermediate. The chromic acetate formed was measured at 590 nm. Catalase was allowed to split  $\text{H}_2\text{O}_2$  for different periods of time. The reaction was stopped at different time intervals via the addition of a dichromate-acetic acid mixture, and the remaining  $\text{H}_2\text{O}_2$  was determined by heating the reaction mixture and measuring chromic acetate colorimetrically. The values are expressed as  $\mu\text{moles}$  of  $\text{H}_2\text{O}_2$  utilised/min/mg protein. Vitamin C (ascorbic acid) content was estimated by the method of Roe and

Table 4: Effect of ginger on thiobarbituric acid reactive substances, conjugated dienes and lipid hydroperoxides in control and experimental rats

	Thiobarbituric acid reactive substances (nmoles/100g tissue)			Conjugated dienes (mmoles/100g tissue)			Lipid hydroperoxides (mmoles/100g tissue)		
	Proximal colon	Distal colon	Intestine	Proximal colon	Distal colon	Intestine	Proximal colon	Distal colon	Intestine
Control	3.86 ± 0.31 <sup>a</sup>	4.26 ± 0.33 <sup>a</sup>	4.24 ± 0.33 <sup>a</sup>	48.55 ± 3.82 <sup>a</sup>	48.45 ± 3.83 <sup>a</sup>	57.97 ± 4.52 <sup>a</sup>	74.74 ± 5.82 <sup>a</sup>	72.34 ± 5.61 <sup>a</sup>	78.12 ± 6.10 <sup>a</sup>
Ginger	2.87 ± 0.21 <sup>b</sup>	3.48 ± 0.32 <sup>b</sup>	3.29 ± 0.31 <sup>b</sup>	39.80 ± 3.12 <sup>b</sup>	35.82 ± 2.84 <sup>b</sup>	48.75 ± 3.85 <sup>b</sup>	62.33 ± 4.93 <sup>b</sup>	61.68 ± 4.81 <sup>b</sup>	63.73 ± 5.02 <sup>b</sup>
DMH	1.18 ± 0.13 <sup>c</sup>	1.35 ± 0.12 <sup>c</sup>	1.41 ± 0.14 <sup>c</sup>	26.73 ± 2.11 <sup>c</sup>	23.76 ± 1.91 <sup>c</sup>	28.71 ± 2.32 <sup>c</sup>	48.54 ± 3.82 <sup>c</sup>	47.52 ± 3.74 <sup>c</sup>	53.61 ± 4.21 <sup>c</sup>
DMH + ginger (initiation)	2.77 ± 0.22 <sup>b</sup>	2.47 ± 0.21 <sup>d</sup>	2.82 ± 0.22 <sup>d</sup>	37.62 ± 2.91 <sup>bd</sup>	34.65 ± 2.71 <sup>b</sup>	47.52 ± 3.71 <sup>b</sup>	71.52 ± 5.61 <sup>a</sup>	71.93 ± 5.63 <sup>a</sup>	71.92 ± 5.73 <sup>a</sup>
DMH + ginger (post-initiation)	2.47 ± 0.22 <sup>d</sup>	2.07 ± 0.21 <sup>e</sup>	2.34 ± 0.23 <sup>e</sup>	35.64 ± 2.81 <sup>d</sup>	31.68 ± 2.42 <sup>b</sup>	42.57 ± 3.33 <sup>d</sup>	69.44 ± 5.54 <sup>ab</sup>	69.30 ± 5.41 <sup>ab</sup>	68.93 ± 5.42 <sup>b</sup>

Values are mean ± S.D of ten rats in each group. Values not sharing a common superscript (a-e) differ significantly at p<0.05 (DMRT).

Kuether (1943), in which dehydro ascorbic acid is coupled with 2, 4 dinitro phenyl hydrazine (DNPH) and then treated with sulfuric acid, forming an orange-red colored compound, the content of which was measured at 520 nm. The values are expressed as μmoles/mg tissue. Vitamin E (α-tocopherol) content was estimated using the methods of Barker and Frank (1980). The method involves the α-tocopherol-mediated reduction of ferric ions to ferrous ions, and the formation of a red colored complex with 2, 2'-dipyridyl. The absorbance of the chromophore was measured at 520 nm. The values are expressed as μmoles/mg tissue. The protein content was determined via the method of Lowry et al (1951). Proteins react with Folin-Ciocalteu reagent to give a coloured complex. The colour so formed was due to the reaction of alkaline copper with protein and the reduction of phosphomolybdate by the tyrosine and tryptophan present in the protein. The intensity of the colour depends on the amount of these aromatic amino acids present.

**Statistics**

The data presented here are mean ± SD of 10 rats in each group. The results were analysed using one-way analysis of variance [ANOVA] and the group means were compared by Duncan's Multiple Range Test [DMRT] using SPSS version 12 for windows (Chicago, IL, USA). The findings were considered statistically significant if p<0.05.

**Results**

The average growth rate of the animals in the control and experimental groups are shown in Table III. It was observed that the weight gained by rats in the control group > ginger group > DMH + ginger (initiation) group > DMH + ginger (post-initiation) group > DMH group. The effect of ginger on incidence, percentage, multiplicity, and number of tumours per tumour bearing rat and size of colonic tumours in DMH-treated rats are summarized in Table II.

Table 2: Incidence of colonic neoplasms

Group	Number of rats examined	Number of rats with tumour	Incidence of Tumour (%)	Tumour size
Control	10	0	0	-
Ginger	10	0	0	-
DMH	10	10	100	2cm
DMH+ginger (initiation)	10	0	0	-
DMH+ginger (post-initiation)	10	1	10	0.5cm

There were no tumours in the control rats (group I), ginger-treated control rats (group II) and DMH + ginger rats (initiation, group IV). In rats treated with DMH injections (group III) the tumour incidence in the colon was 100% and the average size of the tumour was approximately 2cm. Ginger supplementation to DMH treated rats (initiation, group IV) markedly suppressed tumour formation. The

size of the tumour was reduced to 0.5 cm and the tumour incidence was 10% in group V rats (post-initiation).

Table 3: Average growth rate of control and experimental animals

Group	Initial body weight	Final body weight
Control	119 ± 12	329 ± 33
Ginger	124 ± 12	311 ± 31
DMH	129 ± 13	264 ± 26*
DMH+ginger (initiation)	129 ± 13	299 ± 30
DMH+ginger (post-initiation)	129 ± 13	289 ± 29

Values are mean ± S.D of ten rats in each group.\* Significantly different from other groups by Students 't' Test at p < 0.001.

Table IV shows the tissue levels of TBARS, lipid hydroperoxides and conjugated dienes in control and experimental animals. The levels of TBARS, hydroperoxides, and conjugated dienes were significantly decreased in the proximal colon, distal colon and intestines of DMH treated rats (group III) as compared to the control group rats (group I). Ginger supplementation to rats at both the initiation and post-initiation stages (group IV and V) of DMH treatment significantly restored the levels of TBARS, lipid hydroperoxides and conjugated dienes of the colon and intestines to near those of control rats. Moreover the levels of TBARS, lipid hydroperoxides and conjugated dienes were significantly reduced in the colon and intestines of control rats treated with ginger (group II) as compared to untreated control rats (group I).

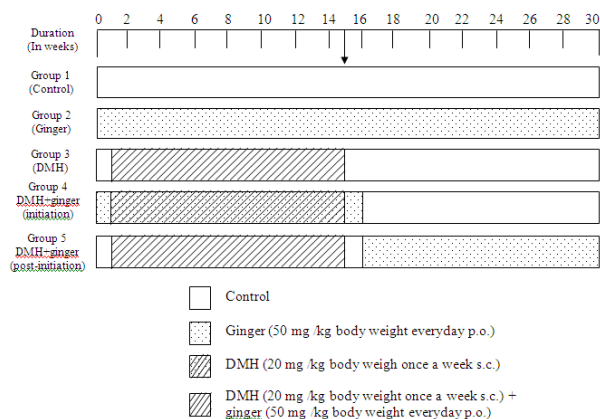


Figure 1: Experimental protocol

Table V and VI represents the levels of tissue glutathione and its dependent enzymes (GSH, GPx, GST and GR) in control and experimental animals. The levels of glutathione and its dependent enzymes in the proximal colon, distal colon and intestines, were significantly elevated on DMH treatment (group III) as compared to the control rats (group I), whereas the tissue levels of GSH, GPx,

**Table 5:** Effect of ginger on reduced glutathione and glutathione peroxidase in control and experimental rats

Groups	Reduced glutathione (mg/g tissue)			Glutathione peroxidase (units/mg protein)		
	Proximal colon	Distal Colon	Intestine	Proximal colon	Distal colon	Intestine
Control	1.10 ± 0.08 <sup>a</sup>	1.12 ± 0.08 <sup>a</sup>	1.19 ± 0.09 <sup>a</sup>	4.70 ± 0.03 <sup>a</sup>	5.34 ± 0.04 <sup>a</sup>	6.09 ± 0.04 <sup>a</sup>
Ginger	1.45 ± 0.11 <sup>b</sup>	1.35 ± 0.12 <sup>a</sup>	1.47 ± 0.31 <sup>b</sup>	7.36 ± 0.25 <sup>b</sup>	6.56 ± 0.15 <sup>b</sup>	8.55 ± 0.26 <sup>b</sup>
DMH	3.33 ± 0.31 <sup>c</sup>	3.26 ± 0.22 <sup>b</sup>	3.50 ± 0.31 <sup>c</sup>	11.28 ± 0.82 <sup>a</sup>	10.94 ± 0.83 <sup>c</sup>	11.28 ± 0.84 <sup>c</sup>
DMH + ginger (initiation)	2.54 ± 0.31 <sup>d</sup>	2.66 ± 0.24 <sup>c</sup>	2.67 ± 0.11 <sup>d</sup>	7.93 ± 0.63 <sup>bd</sup>	8.01 ± 0.61 <sup>d</sup>	8.41 ± 0.64 <sup>d</sup>
DMH + ginger (post-initiation)	2.61 ± 0.21 <sup>d</sup>	2.73 ± 0.23 <sup>c</sup>	2.81 ± 0.20 <sup>d</sup>	8.22 ± 0.61 <sup>d</sup>	8.48 ± 0.64 <sup>d</sup>	8.91 ± 0.72 <sup>bd</sup>

Values are mean ± S.D of ten rats in each group. Values not sharing a common superscript (a-d) differ significantly at p<0.05 (DMRT). \* μ moles of GSH utilized/min/mg protein.

GST and GR were significantly decreased in the tissues of rats supplemented ginger (initiation and post-initiation, group IV and V) as compared to the untreated DMH administered rats (group III). The levels of glutathione and glutathione-dependent enzymes were elevated in the colon and intestine of control rats treated with ginger (group II) as compared to untreated control rats (group I).

Table VIII represents the levels of tissue non-enzymic antioxidants (vitamin C and vitamin E) in control and experimental animals. The levels of vitamin C and vitamin E in the proximal colon, distal colon and intestines, were significantly elevated on DMH treatment (group III) as compared to the control rats (group I), whereas the levels of vitamin C and vitamin E were significantly decreased in the tissues

**Table 6:** Effect of ginger on glutathione-S-transferase and glutathione reductase in control and experimental rats.

Groups	Glutathione-S-transferase (units <sup>a</sup> /mg protein)			Glutathione reductase (units <sup>b</sup> /mg protein)		
	Proximal colon	Distal colon	Intestine	Proximal colon	Distal colon	Intestine
Control	4.74 ± 0.72 <sup>a</sup>	5.38 ± 0.41 <sup>a</sup>	6.14 ± 0.43 <sup>a</sup>	14.9 ± 1.14 <sup>a</sup>	15.4 ± 1.21 <sup>a</sup>	15.7 ± 1.24 <sup>a</sup>
Ginger	6.20 ± 0.82 <sup>b</sup>	6.26 ± 0.52 <sup>b</sup>	7.32 ± 0.52 <sup>b</sup>	15.8 ± 1.21 <sup>a</sup>	16.1 ± 1.23 <sup>a</sup>	16.5 ± 1.22 <sup>a</sup>
DMH	11.38 ± 0.94 <sup>c</sup>	11.04 ± 0.84 <sup>c</sup>	11.80 ± 0.90 <sup>c</sup>	34.90 ± 2.84 <sup>c</sup>	35.35 ± 2.74 <sup>c</sup>	36.53 ± 2.82 <sup>c</sup>
DMH + ginger (initiation)	6.53 ± 0.54 <sup>b</sup>	6.03 ± 0.43 <sup>b</sup>	6.44 ± 0.54 <sup>a</sup>	19.40 ± 1.52 <sup>ad</sup>	19.5 ± 1.51 <sup>ad</sup>	19.8 ± 1.51 <sup>ad</sup>
DMH + ginger (post-initiation)	6.63 ± 0.52 <sup>b</sup>	6.41 ± 0.50 <sup>b</sup>	6.85 ± 0.51 <sup>a</sup>	21.70 ± 1.73 <sup>d</sup>	21.4 ± 1.62 <sup>d</sup>	21.3 ± 1.64 <sup>d</sup>

Values are mean ± S.D of ten rats in each group. Values not sharing a common superscript (a-d) differ significantly at p<0.05 (DMRT). <sup>a</sup> - μmoles of CDNB-GSH conjugate formed / minute / mg protein. <sup>b</sup> - μmoles of NADPH oxidised / mg protein

Table VII represents the levels of tissue enzymic antioxidants (SOD and CAT) in control and experimental animals. The levels of enzymic antioxidants (SOD and CAT) in the proximal colon, distal colon and intestines, were significantly decreased on DMH treatment (group III) as compared to the control rats (group I), whereas the tissue levels of SOD and CAT were significantly increased in the tissues of rats supplemented ginger (initiation and post-initiation, group IV and V) as compared to the untreated DMH administered rats (group III). The activities of enzymic antioxidants (SOD and CAT) were significantly elevated in the colon and intestine of control rats treated with ginger (group II) as compared to untreated control rats (group I).

of rats supplemented with ginger (initiation and post-initiation, group IV and V) as compared to the untreated DMH administered rats (group III). The levels of vitamin C and vitamin E were enhanced in the colon and intestine of control rats treated with ginger (group II) as compared to untreated control rats (group I).

## Discussion

The results clearly indicate that administration of procarcinogen DMH in the presence of ginger brings about profound alterations in tissue lipid peroxidation and antioxidant status of the rat colon and intestines.

**Table 7:** Effect of ginger on superoxide dismutase and catalase in control and experimental rats

Groups	Superoxide dismutase (Units*/mg protein/minute)			Catalase (μmoles of H <sub>2</sub> O <sub>2</sub> utilized/mg protein/min)		
	Proximal colon	Distal colon	Intestine	Proximal colon	Distal colon	Intestine
Control	4.79 ± 0.32 <sup>a</sup>	4.39 ± 0.31 <sup>a</sup>	4.23 ± 0.31 <sup>a</sup>	37.55 ± 2.92 <sup>a</sup>	37.97 ± 2.94 <sup>a</sup>	39.93 ± 3.11 <sup>a</sup>
Ginger	6.08 ± 0.41 <sup>b</sup>	6.30 ± 0.43 <sup>b</sup>	5.46 ± 0.44 <sup>b</sup>	47.16 ± 3.72 <sup>b</sup>	47.25 ± 3.71 <sup>b</sup>	49.55 ± 3.93 <sup>b</sup>
DMH	2.50 ± 0.22 <sup>c</sup>	2.74 ± 0.22 <sup>c</sup>	2.87 ± 0.22 <sup>c</sup>	21.87 ± 1.70 <sup>c</sup>	23.88 ± 1.84 <sup>c</sup>	24.75 ± 1.91 <sup>c</sup>
DMH + ginger (initiation)	4.05 ± 0.33 <sup>d</sup>	4.00 ± 0.31 <sup>ad</sup>	4.07 ± 0.33 <sup>a</sup>	35.38 ± 2.72 <sup>ad</sup>	37.95 ± 2.94 <sup>ad</sup>	34.99 ± 2.72 <sup>d</sup>
DMH + ginger (post-initiation)	3.09 ± 0.32 <sup>e</sup>	3.86 ± 0.33 <sup>d</sup>	3.94 ± 0.31 <sup>a</sup>	31.95 ± 2.51 <sup>d</sup>	34.99 ± 2.82 <sup>d</sup>	33.16 ± 2.43 <sup>d</sup>

Values are mean ± S.D of ten rats in each group. Values not sharing a common superscript (a-e) differ significantly at p<0.05 (DMRT). \*enzyme required for 50% inhibition of NBT (nitroblue tetrazolium) reduction/ min/mg protein.

DMH, a potent colon specific carcinogen used in the present study is metabolized in the liver to azoxymethane (a known colon carcinogen) and ultimately to methyl diazonium ion and carbonium ion, which are active carcinogenic electrophiles that manifest their action in the colon.

In this context Szatrowski and Van Driel et al have shown that human cancer lines produce large amounts of hydrogen peroxide (Szatrowski, Nathan, 1991; Van Driel et al. 1997).

**Table 8:** Effect of ginger on vitamin C and vitamin E in control and experimental rats.

Groups	Vitamin C ( $\mu$ moles/mg tissue)			Vitamin E ( $\mu$ moles/mg tissue)		
	Proximal colon	Distal colon	Intestine	Proximal colon	Distal colon	Intestine
Control	1.28 $\pm$ 0.11 <sup>a</sup>	1.32 $\pm$ 0.14 <sup>a</sup>	1.35 $\pm$ 0.14 <sup>a</sup>	0.65 $\pm$ 0.05 <sup>a</sup>	0.68 $\pm$ 0.05 <sup>a</sup>	0.72 $\pm$ 0.05 <sup>a</sup>
Ginger	1.40 $\pm$ 0.12 <sup>a</sup>	1.44 $\pm$ 0.11 <sup>a</sup>	1.50 $\pm$ 0.11 <sup>a</sup>	0.71 $\pm$ 0.04 <sup>a</sup>	0.72 $\pm$ 0.05 <sup>a</sup>	0.77 $\pm$ 0.06 <sup>a</sup>
DMH	2.39 $\pm$ 0.21 <sup>b</sup>	2.41 $\pm$ 0.22 <sup>b</sup>	2.42 $\pm$ 0.21 <sup>b</sup>	1.81 $\pm$ 0.14 <sup>b</sup>	1.79 $\pm$ 0.12 <sup>b</sup>	1.84 $\pm$ 0.31 <sup>b</sup>
DMH + ginger (initiation)	1.68 $\pm$ 0.23 <sup>a</sup>	1.76 $\pm$ 0.22 <sup>a</sup>	1.70 $\pm$ 0.32 <sup>a</sup>	0.88 $\pm$ 0.06 <sup>c</sup>	0.85 $\pm$ 0.06 <sup>c</sup>	0.89 $\pm$ 0.07 <sup>c</sup>
DMH + ginger (post-initiation)	1.71 $\pm$ 0.23 <sup>a</sup>	1.92 $\pm$ 0.21 <sup>a</sup>	1.93 $\pm$ 0.11 <sup>a</sup>	1.10 $\pm$ 0.08 <sup>d</sup>	1.12 $\pm$ 0.08 <sup>d</sup>	1.18 $\pm$ 0.09 <sup>d</sup>

Values are mean  $\pm$  S.D of ten rats in each group. Values not sharing a common superscript (a-d) differ significantly at  $p < 0.05$  (DMR)

Decreased lipid peroxidation observed in the colon and intestinal tissues of DMH treated rats is based on the estimation of thiobarbituric acid reactive substances (TBARS) such as lipid hydroperoxides (LOOH) and conjugated dienes (CD) formed during the chain reaction of lipid peroxidation, in addition to malondialdehyde (MDA). Previous studies have shown reduced rates of lipid peroxidation in the tumour tissue of various types of cancer (Tanaka et al. 1998; Schmelz et al. 2000) and indicate an apparent inverse relationship between lipid peroxidation and rates of cell proliferation and/or extent of differentiation. Cancer cells are known to acquire certain characteristics that benefit proliferation (Nakagami et al. 1990) and they tend to proliferate faster when lipid peroxidation is low. Therefore, the decreased colon and intestinal lipid peroxidation observed in DMH treated rats could be due to increased cell proliferation. Thus, malignant tissues are less susceptible and more resistant to free radicals attack and hence lipid peroxidation is less intense (Masotti et al. 1998). In addition to this the decreased levels of lipid peroxidation in DMH treated rats may also be due to increased resistance and/or decreased susceptibility of the target organs to free radical attack.

Ginger supplementation to DMH treated rats resulted in optimal TBARS, LOOH and CD levels in our study. In this context, ginger is known to possess antioxidant properties, the reactive phenolic group helping to scavenge reactive oxygen species that initiate lipid peroxidation (Burton et al. 1983; Cao et al. 1993; Krishnakantha and Lokesh et al. 1993; Reddy AA, Lokesh et al. 1992). Ginger is also known to induce apoptosis in cancer tissues, thus preventing cell proliferation. Since proliferation and LPO are inversely related and since ginger is known as an antiproliferative agent luteolin could contribute to the observed increase in the colon and intestine lipid peroxidation. This could increase the susceptibility and decrease the resistance of tumor cells to free radical attack leading to decreased cell proliferation. Hence, we suggest that ginger prevents DMH-induced colon cancer through its antiproliferative effects.

SOD and CAT are two important enzymes that act against toxic oxygen derived free radicals such as superoxide ( $O_2^-$ ) and hydroxyl ions ( $^*OH$ ) in the biological system. They are involved in the direct elimination of reactive oxygen metabolites, which probably is one of the most effective defense of the living body against diseases (Nagao et al. 1986; Ray et al. 2000). SOD and CAT are reported to be more sensitive to oxidative damage induced by the carcinogen treatment (Nordman et al. 1992; Taniguchi et al. 1999). Thus decreased activities of SOD and CAT observed in the DMH treated rats in our study, may suggest their increased utilization to scavenge the dangerous increase in reactive oxygen species in the cancer tissues. In

Administration of ginger to group 4 (initiation) and group 5 rats (post-initiation) enhanced the activities of SOD and CAT in the colon and intestinal tissues of DMH-treated rats. Moreover ginger due to its ability to scavenge free radicals and toxic carcinogenic electrophiles may spare the antioxidant enzymes, which may be the cause for the enhanced SOD and CAT levels in the colon and intestinal tissues. These results prove the excellent chemopreventive efficacy of ginger, against DMH induced colon carcinogenesis.

GSH is a major non-protein thiol, which plays a significant role in protecting cells against cytotoxic and carcinogenic chemicals by scavenging reactive oxygen species (Michiels et al. 1994). GPx, GST and GR are predominantly involved in the detoxification of xenobiotics (Arrick, Nathan, 1984) carcinogens, free radicals and peroxides, by conjugating the toxic substances with GSH, ultimately protecting cells and organs against carcinogen-induced toxicity (Hayes, Pulford 1995). Particularly GST increases the capacity of the tumour cells to withstand the burden of toxins and procarcinogens. It is a well known phenomena that GSH, GPx, GST and GR are expressed in greater amounts by the neoplastic cells, conferring a selective growth advantage to those cells (Nijhoff et al. 1993). Our results also correlate with the above findings as we had observed elevated levels of GSH, GPx, GST and GR in the colonic and intestinal cells of rats treated with DMH, which may be due to the increased rates of cell proliferation (Obrador et al. 1977). Manoj et al have also observed decreased lipid peroxidation associated with enhanced GSH and GSH dependent enzymes in the colon and intestines (Manoj et al 1999). Thus, in addition the elevation of GSH, GPx, GST and GR levels in the colon and intestinal tissues may be used as markers of cell proliferation.

Vitamin E is a fat-soluble vitamin, which functions as a free radical quencher and prevents lipid peroxidation of polyunsaturated fatty acids while vitamin C is a water-soluble antioxidant, which scavenges reactive oxygen metabolites generated during the metabolism of carcinogen, thus protecting genetic material from getting transformed. The enhanced levels of vitamin C and vitamin E observed in our study on DMH treatment may be due to the increased levels of GSH in tissues and also due to the active rate of cell proliferation (Broquist 1991). In this context overexpression of antioxidants have been already documented in many malignant tumours (Skrzydowska et al. 2001; Iscan et al. 2002). Moreover decreased levels of GSH and its dependent enzymes observed in the colonic and intestinal tissues during carcinogenesis, might in turn lead to increased utilization of vitamin C and vitamin E to scavenge the reactive oxygen species. This may be one of the reasons for the observed decrease in the levels of these vitamins in DMH treated rats.

Ginger is known to be a good natural antioxidant and has been found to efficiently scavenge superoxide and hydroxyl radicals produced by the cancer cells (Manju and Nalini, 2005). Ginger is also anticarcinogenic, non-toxic and non-mutagenic even at high concentrations (Manju and Nalini, 2005). Moreover ginger and its chemical compounds are able to influence cell proliferation and programmed cell death (Mirand et al. 1999; Ko et al. 2000). In our study ginger is shown not only to suppress colon cancer incidence but also to reduce the size of colonic neoplasms. Here, ginger dramatically inhibits colon carcinogenesis by blocking the metabolic activation of carcinogens and also by suppressing carcinogenesis both at the initiation and post-initiation stages. Since ginger and its active principles, are known to exhibit antiproliferative activity against various types of cancers we could postulate that ginger regulates cell proliferation. In this regard already we have reported the antioxidant and colon cancer inhibitory role of ginger in 1, 2-dimethyl hydrazine colon carcinogenesis (Manju and Nalini, 2005).

## Conclusions

Thus our present data strongly suggest that administration of ginger during the initiation as well as the post-initiation stages of carcinogenesis significantly inhibited colon cancer incidence. In addition our results reveals that tissue lipid peroxidation, glutathione, glutathione-dependent enzymes, vitamin C and vitamin E levels are optimized and the activities of enzymic antioxidants such as SOD and CAT in the colonic and intestinal tissues are elevated on supplementing ginger to DMH treated rats. Thus our findings support the view that ginger is an effective chemopreventive agent. However molecular mechanistic pathways of chemoprevention remains to be elucidated.

## References

- Arrick B, Nathan C (1984) Glutathione metabolism as determinant of the therapeutic efficacy: a review. *Cancer Res* 33:4224-4232
- Baker H, Frank O, DeAngelis B et al. (1980) Plasma  $\alpha$ -tocopherol in man at various times after ingesting free or acetylated  $\alpha$ -tocopherol. *Nutr Rep Int* 21:531-536
- Bandaru SR, Toshiko K, Ronald AL et al (1999) Chemopreventive efficacy of sulindac sulfone against colon cancer depends on the time of administration during carcinogenic process. *Cancer Res* 59:3387-3391
- Broquist HP (1991) Buthionine sulfoximine an experimental tool to induce glutathione deficiency: elucidation and ascorbate in their role as antioxidants. *Nutr Rev* 50:110-111
- Burton GW, Cheesman, KN, Ingold KV, Seater TF (1983) Lipid antioxidants and products of lipid peroxidation as potential tumor protective agents. *Biochem Soc Trans* 11: 261-262
- Cao ZF, Chen ZG, Guo P et al (1993) Scavenging effect of ginger on superoxide anion and hydroxyl radical. *Chung – kuo Chung Yao Tsa Chih* 18:750-764
- Carlberg I, Mannervik B (1985) Glutathione reductase, In: Meister A, Methods in enzymology, New York Academic Press 484
- Druckrey E (1972) Organ-specific carcinogenesis in the digestive tract. In: Topics in Chemical Carcinogenesis, Nakahara W, Takayama S, Sugimura T, Odashima S.E, Baltimore Maryland: University Park Press, 73-101
- Ellman GL (1959) Tissue sulphhydryl groups. *Arch Biochem Biophys* 82:72-77
- Fiala ES, Sohn OS, Hamilton SR (1987) Effects of chronic dietary ethanol on the *in vivo* and *in vitro* metabolism of methylazoxymethanol and methylazoxymethanol induced DNA methylation in the rat colon and liver. *Cancer Res* 47:5939-5943
- Fiala ES (1977) Investigations into the metabolism and mode of action of the colon carcinogen 1,2-dimethyl hydrazine and azoxymethane. *Cancer* 40:2436-2445
- Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione-S-transferases, the first enzymatic step in mercapturic acid formation. *J Biol Chem* 249:7130-7139
- Hayes JD, Pulford DJ (1995) The glutathione-S-transferase supergene family: regulation of GST and contribution of the isoenzymes to cancer chemoprevention and drug resistance. *Crit Rev Biochem Mol Biol* 31:445-600
- Iscan M, Coban T, Cok I et al (20024) The organochlorine pesticide residues and antioxidant enzyme activities in human breast tumours: is there any association?. *Breast Cancer Res Treat* 72:173
- Janssen JWW, Heintz NH, Mossman BT (1995) Induction of C-fos and N-jun proto-oncogene expression of asbestos is ameliorated by N-acetyl-cysteine in mesothelial cells. *Cancer Res* 55: 2085-2089
- Jiang ZY, Hunt JV, Wolf SP (1992) Detection of lipid hydroperoxides using Fox method. *Anal Biochem* 202:384-389
- Kakkar PS, Das B, Viswanathan PN. (1984) A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys* 21: 130-132
- Kim SO, Kundu JK, Shin YK et al (2005) Gingerol inhibits COX-2 expression by blocking the activation of p38 MAP kinase and NF- $\kappa$ B in phorbol ester-stimulated mouse skin. *Oncogene* 25:2558-2567
- Ko WG, Kang TH, Kim NY et al (2000) Lavandulyflavonoids: A new class *in vitro* apoptogenic agents from (*Sophora flavescens*). *Toxicol in vitro* 14:429-433
- Kochhar KP (2008) Dietary spices in health and diseases: 1. *Indian J Physiol Pharmacol.*: 52(2): 106-122
- Krishnakantha TP, Lokesh BR (1993) Scavenging of superoxide anions by spice principles. *Indian J Biochem Biophys* 30:133-134
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. *J Biol Chem* 193:265-275
- Lumb AB (1994) Effect of dried ginger on human platelet function. *Thromb Haemost* 71:110-111
- Manju V, Nalini N (2005) Chemopreventive efficacy of ginger, a naturally occurring anticarcinogen during the initiation, post-initiation stages of 1, 2 dimethyl hydrazine-induced colon cancer. *Clin chim acta* 358: 60-67
- Manoj GP, Thampi BSH, Menon VP et al (1999) Influence of dietary fibre from coconut kernel (*Cocos nucifera*) on 1,2-dimethyl hydrazine induced lipid peroxidation in rats. *J Nutr Biochem* 10:555-560
- Masotti L, Casali E, Galconi, T (1998) Lipid peroxidation in tumor cells. *Free Rad Med* 4: 377-386
- Masuda T, Jitoe A, Mabry TJ (1995) Isolation and structure determination of cassumunarin A, B, C: new anti-inflammatory antioxidants from a tropical ginger, *Zingible cassumunar*. *J Am Oil Chem Soc* 72: 1053-1057
- Michiels C, Raes M, Toussaint O et al (1994) Importance of Se-glutathione peroxidase, catalase and Cu/Zn superoxide dismutase for cell survival against oxidative stress. *Free Radic Biol Med* 17:235-248
- Miranda CL, Stevens JF, Helmrich A et al (1999) Antiproliferative and cytotoxic effects of prenylated flavonoids from hops (*Humulus lupulus*) in human cancer cell lines. *Food Chem Toxicol* 37:271-285
- Murray MT (1995) The healing power of herbs: the enlightened persons guide to the wonders of medicinal plants. Rocklin, CA: Prima Pub 410
- Nagao M, Wakabayashi K, Suwa Y et al (1986) Alteration of mutagenic potential by peroxidase, catalase, and superoxide dismutase. In: Hartman APE, Kada T, Hollaender A, Eds.

- Antimutagenesis and anticarcinogenesis mechanism. New York: Plenum Press, 73-80
- Nakagami K, Uchida T, Okwada S et al (1990) Increased choline kinase activity in 1,2 dimethylhydrazine induced rat colon cancer. *Jpn J Cancer Res* 90:1212-1217
- Nalini N, Manju V, Menon VP (2004) Effect of coconut cake on the bacterial enzyme activity in 1,2-dimethyl hydrazine induced colon cancer. *Clin Chim Acta* 342:203-210
- Nijhoff WA, Groen GM, Peters WHM (1993) Induction of rat hepatic and intestinal glutathione-S-transferase and GSH by dietary naturally occurring anticarcinogens. *Int J Onc* 3: 1131-1139
- Nordman R, Ribere C, Rouach H (1992) Implication of free radical mechanisms in ethanol induced cellular injury. *Free Radical Med* 12:219-240
- Obrador E, Navarro J, Mompou J et al (1977) Glutathione and the rate of cell proliferation determine tumour cell sensitivity to tumour necrosis factor in vivo. *Biochem J* 325:183-189
- Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95:351-358
- Ozer NK, Palozza P, Boscoboinik D et al (1993)  $\alpha$ -tocopherol inhibits low density lipoprotein induced proliferation and protein kinase C activity in vascular smooth muscle cells. *FEBS Lett* 322: 307-310
- Park KK, Chu KS, Lee JM et al (1998) Inhibitory effects of gingerol, a major pungent principle of ginger, on phorbol ester-induced inflammation, epidermal ornithine decarboxylase activity and skin tumour promotion in IC mice. *Cancer Lett* 129: 139-144
- Pozharisski KM, Likhachev AJ, Klimashevski VF et al (1979) Experimental intestinal cancer research with special reference to human pathology. *Adv Cancer Res* 30:165-237
- Rao KS, Recknagel RO (1968) Early onset of lipid peroxidation in rat liver after carbon tetrachloride administration. *Exp Mol Pathol* 9:271-278
- Ray G, Batra S, Shukla NK et al (2000) Lipid peroxidation, free radical production and antioxidant status in breast cancer. *Breast Cancer Res Treat* 59:163-170
- Reddy AA, Lokesh BR (1992) Studies on spice principles as antioxidants in the inhibition of lipid peroxidation on rat liver microsomes. *Mol Cell Biochem* 111:117-124
- Roe JM, Kuether, CA (1943) Detection of ascorbic acid in whole blood, and urine through 2,4-DNPH derivative of dehydroascorbic acid. *J Biol Chem* 147:399-407
- Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973) Selenium: biochemical role as a component of glutathione peroxidase. *Science* 179:588-590
- Schmelz EM, Sullards MC, Dillachay DL et al (2000) Colonic cell proliferation and aberrant crypt foci formation are inhibited by dairy glycerosphingolipid in 1,2-dimethylhydrazine-treated CFI mice. *J Nutr* 130: 522-527
- Sinha KA (1972) Colorimetric assay of catalase. *Anal Biochem* 47: 389-394
- Skrzydowska E, Stankiewicz A, Sulkowska M et al (2001) Antioxidant status and lipid peroxidation in colorectal cancer. *J Toxicol Environ Health* 64:213-222
- Srinivasan K, Sambaiah K (1991) Effect of spices on cholesterol 7 $\alpha$ -hydroxylase activities and on serum and hepatic cholesterol levels in the rat. *Int J Vit Nutr Res* 61:364-369
- Srivastava KC (1984) Effects of aqueous extracts of onion, garlic acid and ginger on platelet aggregation and metabolism of arachidonic acid in the blood vascular system: in vitro study. *Prostaglandins Leukotrienes Med* 13:227-235
- Surh YJ, Loe E, Lee JM (1998) Chemopreventive properties of some pungent ingredients present in red pepper and ginger. *Mutat Res* 402:259-267
- Surh YJ, Park KK, Chun KS et al (1999) Anti-tumour promoting activities of selected pungent phenolic substances present in ginger. *J Environ Pathol Toxicol Oncol* 18: 131-139
- Szatrowski TP, Nathan CF (1991) Production of large amounts of hydrogen peroxide by human tumour cells. *Cancer Res* 51:794-798
- Tanaka K, Kawabata K, Kakumoto M et al (1998) Citrus auranthol exerts dose dependent chemopreventive activity in rat large bowel tumourigenesis: The inhibition correlates with suppression of cell proliferation and lipid peroxidation and with induction of Phase II drug metabolizing enzymes. *Cancer Res* 58:2550-2556
- Taniguchi M, Yasutake A, Takedomi K et al (1999) Effect of N-Nitrosodimethylamine (NDMA) on the oxidative status of rat liver. *Arch Toxicol* 73:141-146
- Van Driel BEM, Lyon H, Hoogenraad DCJ et al (1997) Expression of Cu-Zn and Mn-superoxide dismutase in human colorectal neoplasms. *Free Radical Biol Med* 23:435-444