The Chemopreventive Effect of *Nigella Sativa* on 1,2-dimethylhydrazine-induced Colon Tumor

Dadkhah A[a,*], Fatemi F[b], Malayeri M[c], Jahanbani A[d], Batebi F[e] and Ghorbanpour Z[e]

[a]Department of Medicine, Faculty of Medicine, Qom Branch, Islamic Azad University, Qom, Iran
[b]Nuclear Fuel Cycle research school, Nuclear Science and Technology Research Institute, Tehran, I.R. Iran
[c]Department of pathobiology, Faculty of veterinary medicine, Garmsar Branch, Islamic Azad University, Garmsar-Iran
[d]Young Researchers Club, Garmsar Branch, Islamic Azad University, Garmsar, Iran
[e]Department of Biochemistry, Faculty of Sciences, Payame-e-Noor University, Tehran, Iran

ABSTRACT

In this study, the chemopreventive efficacy of *Nigella sativa* seed powder as an important chemopreventive herb on the formation of colon tumor has been examined through measuring the levels of hepatic oxidative stress and metabolizing enzymes in 1,2-dimethylhydrazine (DMH)-induced colon cancer in rats. Male Wistar rats were divided into 6 groups as untreated control, Sham, DMH (30mg/kg b.w) received carcinogen via subcutaneously (s.c) injection once a week for 18 weeks and treatment groups received diet containing *Nigella sativa* (2 and 4%) until the end of whole experimental period of 6 months. It is indicated that *Nigella sativa* seed powder could prevent colon tumor formation by modulating the activities of hepatic detoxification enzymes, GST and CYP450 whereas antioxidant statues are not affected. The data was confirmed by histopathological findings It is obvious that one of the mechanisms of chemoprevention of colon tumorigenesis by *Nigella sativa* seed powder may be the enhancement of carcinogen detoxification but not antioxidant system in the liver.

Keywords: *Nigella sativa*, Colon tumor, DMH, Antioxidant parameters, Xenobiotic metabolizing enzymes.

INTRODUCTION

Nigella sativa L. (Ranunculaceae) (black seed, black caraway or black cumin) called in persian language Siyah Daneh, is an annual flowering plant, native to south and southwest Asia. It appears that the early Egyptians already used these seeds as a flavouring agent.[1] The seeds are naturally distributed in different parts of Iran. Also, it is extensively cultivated in various regions of Iran.[2,3] The black seeds of *N. sativa* have been widely used in Iranian traditional medicine as a natural remedy for a long time having galactagogue, carminative, laxative and antiparasitic properties.[3,4] The seeds are reputed to have many medicinal purposes[5,6] including anti-bacterial, anti-fungal, anti-viral, antihelminthic, anti-inflammatory, immunomodulatory and anticancer properties.[7–9] *N. sativa* seeds contain 35%–40% fixed oils, 0.5%–1% essential oil, proteins (23%), amino acids, sugars, mucilage, alkaloids, organic acids, tannin, resin, glycosides, metarbin, saponins, melanthigenin, lipase, phytosterols, vitamins and minerals. Thymoquinone is the major constituents of its essential oils leadsing to the most theraspeutic effects[10].

Recently, chemoprevention through medicinal plants offers a novel approach to control the incidence of cancer. Considerable attention has been directed on identifying phytochemicals by examining the various dietary components which has the ability to interfere with carcinogenic or mutagenic processes. Chemopreventive agents are known to exert their anticarcinogenic action by modulating the oxidative/antioxidant status in the tissues.[11–13]

Colorectal cancer (CRC) with age-adjusted rate of 6–7.9 per 100,000 person per year is the fourth most common cancer in Iran[14] and one of the leading causes of worldwide morbidity and mortality due to cancer.[15] Epidemiological studies have suggested

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Address for correspondence
Dadkhah A, Ph.D.
Professor of Biochemistry
Faculty of Medicine,
Qom Branch, Islamic Azad University,
P.O. Box 37185/364, Qom, Iran.
E-mail:
Dadkhah_bio@yahoo.com

www.ijper.org

that specific components of the Western diet, including dietary fat and red meat, are risk factors in colorectal cancer pathogenesis whereas other dietary components, including fruit, vegetables, and dietary fiber, are protective factors.[16] 1,2-Dimethylhydrazine (DMH), a toxic environmental pollutant, is a well established procarcinogen with selectivity for colon. DMH undergoes metabolism in the liver, resulting in the production of electrophilic diazoniumion, which are known to elicit oxidative stress[17,18] finally leaded to colon tumors.

There are scientific reports indicating the chemopreventive potential of *Nigella sativa* seeds,[19–23] however, the present study has focused on the mechanism or the way of the chemopreventive effect of *Nigella sativa* seed powder -cultivated in Iran- in colon carcinogenesis induced by DMH *in vivo* system regarding parameters related to oxidative stress and xenobiotic metabolizing enzymes in liver and plasma of rats accompany with histopathological examinations.

MATERIALS AND METHODS

Plant preparation

Fresh *Nigella sativa* seeds cultured in Iran were purchased from market. The seeds were powdered ready to use within the diet in 2 and 4%, respectively.

Induction of colon tumor in rats

Young male Wistar rats (100±20 g) were purchased from Pasteur Institute of Iran and maintained at 25±2°C with a 12-h light/12-h dark cycle. Animal studies were approved by the Medical Ethics Committee of Tarbiat Modares University. This Ethics Committee was based on the World Medical Association Declaration of Helsinki (adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964).

DMH was dissolved in 1 mM EDTA just before use and the pH was adjusted to 6.5 with 1 mM NaOH to ensure the stability of the chemical. The rats were randomly assigned to 6 groups (8 rats/group) (Table 1). The rats in group 1 received 0.5ml of EDTA- the vehicle of DMH- subcutaneously (s.c) once a week for 18 weeks and considered as control group. The rats in groups 2 & 3 received only pellet diet containing 2 and 4% of *Nigella sativa* respectively for 6 months, and served as sham groups. Rats in group 4 received 0.5ml DMH dissolved in EDTA (30mg/kg b.w) injection (s.c) once a week for 18 weeks and served as DMH group. The group 5 & 6 were given DMH injections (20mg/kg b.w) and treated with diet containing 2 and 4% of *Nigella sativa* respectively and considered as treated groups The feeding diet containing the seed powder started simultaneously with DMH treatment and continued until 6 months. At the end of the experiment (6 months) the animals were anesthetized and blood was collected by heart puncture. Then, animals were scarified, liver tissues were removed and processed for biochemical assays.

Colon tumor enumeration

After a total experimental period of 6 months, the animals were sacrificed and the colons were removed, cut open along the longitudinal axis from cecum to anus and were flushed with isotonic saline. The colons were divided into three sections (section "a" as proximal colon, section "b" as middle colon and section "c" as distal colon). The incidence, inhibition, numbers, position and size of tumors were recorded. Then the colons were fixed in 10% neutral buffered formalin (Sigma) and embedded in paraffin. Sections of tissue (6 mm) were stained with hematoxylin and eosin (H&E) for histological observation. Tumors were classified according to morphology, extent of invasion and differentiation.

Preparation of tissue homogenate and plasma

After experimental period of 6 months, heparinated blood samples were collected by heart puncture and centrifuged at 3000×G for 10 min to obtain plasma. Liver samples were immediately transferred to ice-cold containers and homogenized (20% w/v) in the appropriate buffer using a homogenizer (Heidolph Diax 600).

Biochemical assays: Lipid peroxidation

A weighed portion of liver was homogenized in phosphate buffer (100 mM, pH 7.0) and used to measure the concentration of thiobarbituric acid reacting substances (TBARS) as an indicator of lipid peroxidation. The concentration of TBARS was measured spectrophotometrically according to the instruction of the kit purchased from Enzo Life Sciences, Inc., UK.

Glutathione (GSH) estimation

GSH was estimated in liver homogenate based on the protocol of the purchased kit from BioVision, Inc., USA.

Table 1: Treatment Groups

Group	Treatment	EDTA	DMH	Dietary Nigella sativa	
				2%	4%
1	Control	+	–	–	–
2	2% (Sham group)	+	–	+	–
3	4% (Sham group)	+	–	–	+
4	DMH	–	+	–	–
5	DMH+ 2% powder	–		+	–
6	DMH+ 4% powder	–		–	+

Determination of Superoxide Dismutase (SOD) and Catalase (CAT) enzyme activities

The activities of SOD and CAT were estimated in liver homogenate using commercial kits (BioVision, Inc., USA) following instructions given by the company.

Glutathione S-transferase (GST) activity

Liver cytosolic GST activity were measured spectrophotometrically using CDNB as substrate as described in the instruction of the kit bought from Biovision, USA.

Cytochrome P450 (CYP450) activity

CYP activity was performed on liver preparations according to the procedure described in the kit purchased from the Enzo Life Sciences, Inc., UK.

Ferric reducing ability of plasma (FRAP) assay

This assay was performed using TPTZ reagent as described by Benzie and Strain (1996). FRAP level was calculated by plotting a standard curve of absorbance against μmol/l concentration of Fe (II) standard solution.

Statistical analysis

Data are presented as means±Standard Error of Mean (SEM). The results were subjected to One-way ANOVA followed by Tukey's HSD using SPSS (version 19.0) software. Significant levels were defined as $P<0.05$.

RESULTS

Effects of *Nigella sativa* on tumor number and size in DMH-induced colon carcinogenesis

As shown in Table 2, after 6 months of treatment, 125 tumors were identified in all groups except the negative control and sham groups. Out of, 4 tumors were devoted to section "a" as proximal colon, 33 tumors to section "b" as middle colon and 88 tumors to section "c" as distal colon of treated groups. The average tumor number and size in total length of the colon were significantly decreased in groups feeded with diet containing 2 and 4% of *Nigella sativa* powders in compare to DMH treated rats ($P<0.05$). These criteria also showed significant reduction in section "a" as proximal colon, section "b" as middle colon and section "c" as distal colon of treated groups ($P<0.05$), although, the difference of average number was not significant in section "b" and "c" of 2% *Nigella sativa* treated group in compare to positive group.

Effects of *Nigella sativa* on tumor incidence and inhibition in DMH-induced colon carcinogenesis

After 6 months of treatments, colorectal tumors were identified in each group except the negative control group (Fig. 1) (Table 3). *Nigella sativa* treatments in both

Table 2: Effect of Dietary Nigella sativa on Number and Size of the Tumors in Proximal, Middle, Distal and Total Colon of DMH-treated Rats

Treatment groups	No. rats examined	No. rats with tumor	Total No. tumor				No. tumor/rat				Tumor size (mm)			
			a	b	c	T	a	b	c	T	a	b	c	T
Control	8	0	0	0	0	0	0	0	0	0	–	–	–	–
2% (Sham group)	8	0	0	0	0	0	0	0	0	0	–	–	–	–
0.4% (Sham group)	8	0	0	0	0	0	0	0	0	0	–	–	–	–
DMH	8	8	4	15	34	53	0.5±0.3*	1.9±0.7*	4.2±0.9*	6.6±1.1*	3.6±2.8*	58.3±21*	102.3±26.2*	164.2±34.6*
DMH+ 0.2% powder	8	8	0	10	29	39	0**	1.3±0.7	3.5±0.7	4.8±0.6**	0**	24.8±10.9**	62.6±10.7**	87.4±8.3**
DMH+ 0.4% powder	8	7	0	8	25	33	0**	1±0.6**	3.1±0.6**	4.1±1**	0**	6.14±4**	34.8±7.2**	40.9±9.7**

The rats in group 1 received 0.5ml of EDTA- the vehicle of DMH- (s.c) once a week for 18 weeks and considered as control group. The rats in groups 2 & 3 received only pellet diet containing 2 and 4% of Nigella sativa respectively for 6 months, and served as sham groups. Rats in group 4 received 0.5ml DMH dissolved in EDTA (30mg/kg b.w) injection (s.c) once a week for 18 weeks and served as DMH group. The group 5 & 6 were given DMH injections (20mg/kg b.w) and treated with diet containing 2 and 4% of Nigella sativa respectively for 6 months and considered as treated groups. The colons were divided into three sections (section "a" as proximal colon, section "b" as middle colon, section "c" as distal colon and T means the total length of colon). Values are mean± S.E.M. obtained from 8 animals in each group. *P<0.05 is considered significantly different from control group within each parameter. **P<0.05 is considered significantly different from DMH-treated group within each parameter.

Table 3: Effect of Dietary Nigella Sativa on Tumor Incidence and Inhibition in DMH-Treated Rats in Proximal, Middle, Distal and Total Colons

Treatment groups	No. rats examined	No. rats with tumor	Tumor incidence (%) No. rats with tumor/ rats examined				Tumor inhibition (%)			
			a	b	c	T	a	b	c	T
Control	8	0	0	0	0	0	–	–	–	–
2% (Sham group)	8	0	0	0	0	0	–	–	–	–
4% (Sham group)	8	0	0	0	0	0	–	–	–	–
DMH	8	8	38	75	100	100	0	0	0	0
DMH+ 2% powder	8	8	0	50	100	100	100	33	15	26
DMH+ 4% powder	8	7	0	87	87	87	100	46	26	38

The rats in group 1 received 0.5ml of EDTA- the vehicle of DMH- (s.c) once a week for 18 weeks and considered as control group. The rats in groups 2 & 3 received only pellet diet containing 2 and 4% of Nigella sativa respectively for 6 months, and served as sham groups. Rats in group 4 received 0.5ml DMH dissolved in EDTA (30mg/kg b.w) injection (s.c) once a week for 18 weeks and served as DMH group. The group 5 & 6 were given DMH injections (20mg/kg b.w) and treated with diet containing 2 and 4% of Nigella sativa respectively for 6 months and considered as treated groups. The colons were divided into three sections (section "a" as proximal colon, section "b" as middle colon, section "c" as distal colon and T means the total length of colon).

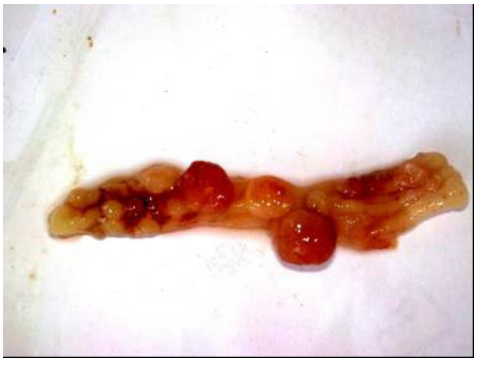

Figure 1: Representative macroscopic view of the colon tumor.

doses could inhibit the formation of tumors. The tumor incidence in *Nigella sativa* treated group at the dose of 4% in diet was reduced to 87%. In both treated groups (5&6), the tumor inhibition rates were also 26 and 38%, respectively. As shown in the Table 3, the tumor incidence in the colon tissue of all groups was in section"c" >"b">"a", whereas, the highest inhibition rate were seen in section "a".

Effects of *Nigella sativa* on tumor classification in DMH-induced colon carcinogenesis

Effect of dietary *Nigella sativa* on tumor total number in DMH-treated rats based on the tumor classifications in proximal, middle, distal and total colon are shown in Table 4. In total length of the colon, after 6 months of treatment, 11 tumors were assigned to tubular adenoma, 20 tumors to carcinoma in situ and 94 tumors to adenocarcinoma invasive in all groups except the negative control and sham groups. The tubular adenoma tumors were only identified 3 in section "a" as proximal colon and 8 in section "b" as middle colon of treated groups. 20 tumors as carcinoma in situ is applied 1, 5 and 14 to sections "a", "b" and "c", respectively. 20 and 74 tumors also recognized as adenocarcinoma invasive in sections "b" and "c", respectively. It seems that adenocarcinoma invasive especially in section "c" had a higher rate of tumor incidence in the colon of treated rats. In addition, the adenocarcinoma invasive tumors which was 45 in DMH treated rats, reached to 28 and 21 in seed powder treated groups (Group 5&6), respectively.

Histopathological observation in DMH-induced colon tumor treated with *Nigella sativa*

The colon of the control rats (Group 1) and *Nigella sativa* treated groups without DMH injection (Groups 2 and 3) showed normal Lieberkuhn's glands with normal mucosal, normal submucosal layers and typical colonic architecture with no signs of apparent abnormality (Fig. 2A-C). The colons of these groups (Groups 1–3) were observed to be similar and there was no microscopically observable changes, including tumor, in colonic morphology. There were no histological evidences of neoplasia or toxicity in rats of these groups. The histopathological study showed that tumors were observed only in the colons of animals of the group that received DMH. These changes revealed the presence of tumor with histological features of invasive adenocarcinoma. Dysplasia and abnormal structures in the Lieberkuhn's glands were also observed. In this group, neoplastic cells have invaded muscularis layers and organized gland like structures accompanying cystic dilution (Fig. 2D). In DMH-treated rats followed by 2% *Nigella sativa* seed powder (Group 5) invasive adenocarcinoma was observed. Proliferation of tumor cells and organized gland like structures with cystic dilation were also detected. The tumor cells have invaded submucosa through the muscularis mucosa and organized the gland

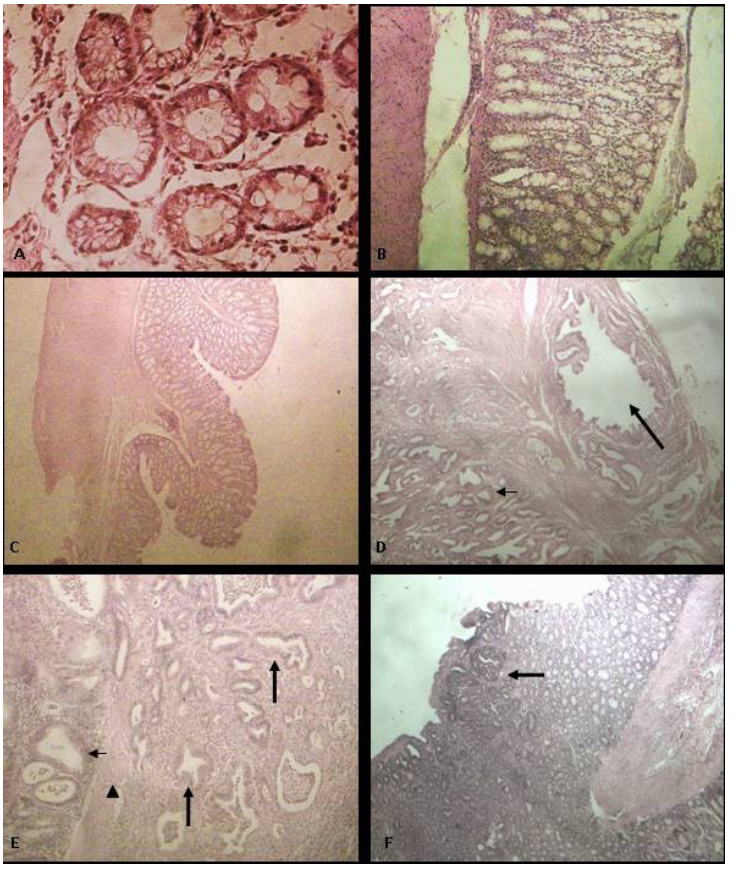

Figure 2: Histopathological changes in the colon of tumor bearing rats.: (A): A histological section of normal colon shows normal epithelial cells which lay liberkuhn's glands and limited by basement membrane with no signs of abnormality (H&E , 400*). (B&C) Histological sections of colons of the sham groups (groups 2 and 3) are present. They are similar to control group. There was no microscopically observable change. The mucosa, structure and epithelial cells of the glands are quite normal (H&E 40*). (D) A microscopic section of colon from a DMH-treated rat represents invasive adenocarcinoma. Dysplasia and abnormal structures are seen in the lieberkuhn's glands in the lower left side of the Figure (small arrow). Neoplastic cells have invaded muscularis layers and organized gland like structures accompanying cystic dilution (Big arrow) (H&E 40*). (E) Histological sections of Nigella sative supplemented rat colons (2% in diet). Proliferation of tumor cells and organized gland like structures with cystic dilation (small arrow) were observed (Invasive adenocarcinoma). The tumor cells have invaded submucosa through the muscularis mucosa (arrow head) and organized the gland like structures with cystic dilation (big arrows) (H&E 40*). (F) Histological sections of Nigella sative supplemented rat colons (4% in diet). Hyperplastic epithelium of the glands which organized tubular structures limited by basement membranes can be observed clearly (Tubular adenoma). Dysplastic cells with the signs of pleomorphism , hyperchromasia, bigger nuclei and nucleoli revealed carcinoma insitu, can be observed in a part of the tumor (arrow) (H&E 40*).

Table 4: Effect of Dietary Nigella Sativa on Tumor Total Number in DMH-treated Rats based on the Tumor Classifications in Proximal, Middle, Distal and Total Colon

	Tumor stage											
Treatment group	**Tubular adenoma**				**Carcinoma in situ**				**Adenocarcinoma invasive**			
	a	b	c	T	a	b	c	T	a	b	c	T
Control	–	–	–	–	–	–	–	–	–	–	–	–
2% (Sham group)	–	–	–	–	–	–	–	–	–	–	–	–
4% (Sham group)	–	–	–	–	–	–	–	–	–	–	–	–
DMH	3	1	0	4	1	3	0	4	0	11	34	45
DMH + 2% powder	0	4	0	4	0	2	5	7	0	4	24	28
DMH + 4% powder	0	3	0	3	0	0	9	9	0	5	16	21

The rats in group 1 received 0.5ml of EDTA- the vehicle of DMH- (s.c) once a week for 18 weeks and considered as control group. The rats in groups 2 & 3 received only pellet diet containing 2 and 4% of Nigella sativa respectively for 6 months, and served as sham groups. Rats in group 4 received 0.5ml DMH dissolved in EDTA (30mg/kg b.w) injection (s.c) once a week for 18 weeks and served as DMH group. The group 5 & 6 were given DMH injections (20mg/kg b.w) and treated with diet containing 2 and 4% of Nigella sativa respectively for 6 months and considered as treated groups. The colons were divided into three sections (section "a" as proximal colon, section "b" as middle colon, section "c" as distal colon and T means the total length of colon).

like structures with cystic dilation (Fig. 2E). The tubular adenoma was recognized in group 6 received DMH + 4% *Nigella sativa*. Hyperplastic epithelium of the glands which organized tubular structures limited by basement membranes can be observed clearly. Dysplastic cells with the signs of pleomorphism, hyperchromasia, bigger nuclei and nucleoli revealed carcinoma insitu, can be observed in a part of the tumor (Fig. 2F).

Effects of *Nigella sativa* on hepatic oxidative injury parameters in DMH-induced colon tumor

Data on the effects of oral administration of *Nigella sativa* seed powders on the levels of oxidative liver injury parameters of the control and experimental rats treated with DMH are given in Table 5. No difference was noticed in the levels of hepatic TBARS (measured as an index of LP) in DMH treated rats and untreated controls. DMH treatment significantly increased the levels of GSH ($P< 0.05$). However, oral administration of *Nigella sativa* seed powders (0.2 and 0.4% in diet) to DMH-treated rats significantly decreased the levels of GSH ($P < 0.05$) as compared to rats treated with DMH alone (Table 5).

The hepatic activities of antioxidant enzymes, SOD and CAT, in DMH-treated rats were significantly lower than that of control group ($P<0.05$). Whereas, *Nigella sativa* seed powder supplementation failed to alter SOD and CAT activities through out the experimental period ($P>0.05$) (Table 2).

Effects of *Nigella sativa* on ferric reducing ability of plasma (FRAP) in DMH-induced colon tumor

As shown in Table 5, the FRAP value were significantly higher in DMH-treated rats as compared to control group ($P<0.05$). Also, administration of *Nigella sativa* seed powders to DMH administered rats had no effects on FRAP level ($P>0.05$).

Effects of *Nigella sativa* on the activities of hepatic detoxification enzymes (GST and CYP450)

As shown in Fig. 3, the activity of CYP450 in liver of DMH-treated animals is increased significantly ($P<0.05$). Oral administration of *Nigella sativa* seed powders at both doses could significantly reduce the activity of hepatic CYP450 ($P<0.05$). Similarly, GST activity significantly increased in liver of DMH-treated rats when compared to untreated control ($P < 0.05$). Oral administration of *Nigella sativa* seed powders to DMH-treated rats significantly decreased the activities of hepatic GST as compared to DMH group alone ($P < 0.05$) (Fig. 4).

DISCUSSION

Previously, we reported the chemopreventive activity of caraway essential oils and also its mechanism in colon carcinogenesis (ACF formation) induced by DMH.[25–26] Present study gives an insight to the mechanism(s) of chemopreventive role of Iranian *Nigella sativa* seed powder in experimental colon tumor induced by DMH

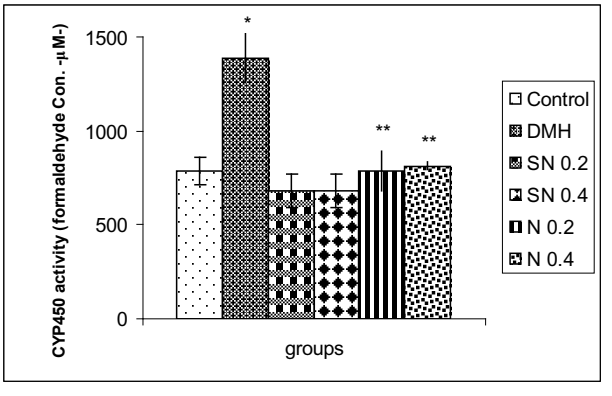

Figure 3: Effect of caraway *Nigella sativa* on CYP450 activity in tumor bearing rats. The rats in group 1 received 0.5ml of EDTA- the vehicle of DMH- (s.c) once a week for 18 weeks and considered as control group. The rats in groups 2 & 3 received only pellet diet containing 2 and 4% of Nigella sativa respectively for 6 months, and served as sham groups. Rats in group 4 received 0.5ml DMH dissolved in EDTA (30mg/kg b.w) injection (s.c) once a week for 18 weeks and served as DMH group. The group 5 & 6 were given DMH injections (20mg/kg b.w) and treated with diet containing 2 and 4% of Nigella sativa respectively for 6 months and considered as treated groups. Values are mean± S.E.M. obtained from 8 animals in each group and carried out in duplicate. * P<0.05 is considered significantly different from control group within each parameter. ** P<0.05 is considered significantly different from DMH-treated group within each parameter.

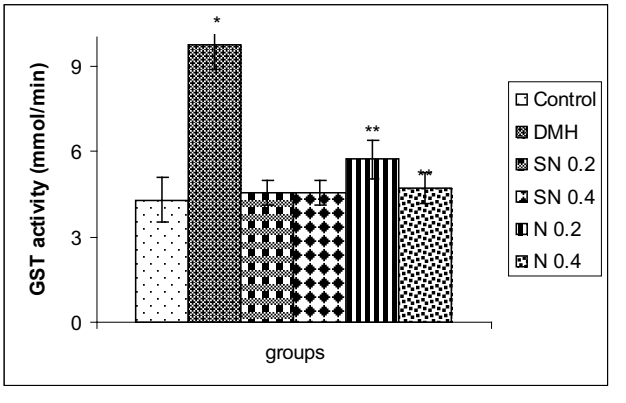

Figure 4: Effect of caraway *Nigella sativa* on GST activity in liver of tumor bearing rats. The rats in group 1 received 0.5ml of EDTA- the vehicle of DMH- (s.c) once a week for 18 weeks and considered as control group. The rats in groups 2 & 3 received only pellet diet containing 2 and 4% of Nigella sativa respectively for 6 months, and served as sham groups. Rats in group 4 received 0.5ml DMH dissolved in EDTA (30mg/kg b.w) injection (s.c) once a week for 18 weeks and served as DMH group. The group 5 & 6 were given DMH injections (20mg/kg b.w) and treated with diet containing 2 and 4% of Nigella sativa respectively for 6 months and considered as treated groups. Values are mean± S.E.M. obtained from 8 animals in each group and carried out in duplicate. * P<0.05 is considered significantly different from control group within each parameter. ** P<0.05 is considered significantly different from DMH-treated group within each parameter.

through measuring the levels of hepatic oxidative stress, antioxidant and also metabolizing enzymes.

The data in the present study showed that *Nigella sativa* seed powders at the doses of 2 and 4% in diet possessed chemopreventive activity in DMH-induced colon tumors. DMH is a known environmental carcinogen which is primarily metabolized in liver[17] which then manifest its action in the colon tissue leading to colon cancer. Natural products may protect against DMH-induced colorectal tumorigenesis by reducing the formation of the ultimate DMH carcinogen (methyldiazonium) either through stimulating detoxification pathways[27] or modulation of antioxidant status.[28,29] The liver is the major organ in which most of the toxic components such as drugs, carcinogens and pollutants are metabolized and hence are more sensitive against drugs.[30,31]

Oral administration of *Nigella sativa* seed powders to DMH-treated rats significantly inhibited colonic tumor developments (Table 2–4). Carcinoma lesions were observed in the last one third of colon in positive control group in compare with negative control one ($P<0.05$). Pleomorphism, hyperchromasia, increased nucleus cytoplasm ratio and large nucleolus were the microscopic specifications of the malignant cells in this group. Other studies also confirmed this result indicating the colon tumour induction through injection or oral administration of DMH in 23–33 weeks[32–34]. Also, in shorter period (10 weeks) after DMH administration, preneoplastic lesions such as aberrant crypt foci (ACF) were observed[35].

Histopathological observation of sham groups indicated no disturbance implying no toxicological effects of *N. sativa* intake. Oral administration of whole seed of *N. sativa* especially at dose of 4% to DMH treated rats modulate the carcinogenic effects of DMH ($P<0.05$). Other studies also indicated the chemopreventive effects of *N. sativa* in colon carcinogenesis[36,37]. Salim et al., 2003 indicated the colon chemopreventive effects of *N. sativa* oils through suppressing the cellular multiplication[22].

Our biochemical data indicated that the mechanism by which *Nigella sativa* seed powders inhibited tumor formation is by modulating the DMH detoxification pathways in the liver which mediate detoxification and metabolic disposal of carcinogen leading to inhibition of carcinogenic process. In this connection, the seed powders (2 and 4% in diet) decrease the CYP450 elevated in the liver of DMH treated rats. Likewise, the increased hepatic GST activity in DMH treated rats is compensated by both doses of the seed powder.

The current findings relating to the effects of *Nigella sativa* seed powders on DMH-induced hepatic detoxification enzymes are consistent with those of several previous reports that chemopreventive agents exert their anticarcinogenic effects by modulating the reactive metabolite in the liver. Van Leishout reported that inhibitors of carcinogenesis have an enhancing effect on the carcinogen detoxification enzymes.[38–40] Cytochrome P450 (CYP) enzymes are a superfamily of heme containing proteins that catalyze xenobiotic metabolism phase I reactions.[41] Increase in CYP450 in DMH-treated rats implying its role in detoxification of DMH as a carcinogen.[27] Suppression of CYP450 by the seeds (Fig. 3) diminish the formation of DMH reactive metabolite which is leaded to lower carcinogenic effect of DMH by inhibiting the methylating DNA, RNA or protein of colonic epithelial cells.[42] Previously we have reported that caraway essential oil potentially prevented the ACF developments in colon carcinogenesis by modulating the detoxification pathways of DMH.[25,26] It is also

Table 5: Effect of Dietary Nigella Sativa on Liver and Plasma Oxidative Stress and Antioxidant Parameters in Tumor Bearing Rats

Treatment	LP (µmol/g liver)	GSH (ng/ml) liver	SOD activity (U/ml) liver	CATactivity (mU/ml) liver	FRAP (mmol/l) plasma
Control	230.9±13.5	264.7±31.5	2.6±0.3	4.9±0.5	428.1±35.7
2% (Sham group)	240.1±18.2	263.3±20.1	2.8±0.2	4.3±0.3	415±17.5
4% (Sham group)	243.3±12.3	264.8±20	2.8±0.2	4.9±0.4	410±17.6
DMH	245.5±12.2	350.3±16*	0.8±0.07*	3.1±0.4*	908.6±66.9*
DMH + 2% N. powder	239.5±14.5	288.2±17.2**	0.9±0.5	3.2±0.4	833.4±39
DMH + 4% N. powder	245±14.4	252±18**	0.8±0.08	3.2±0.4	893.1±74.5

The rats in group 1 received 0.5ml of EDTA- the vehicle of DMH- (s.c) once a week for 18 weeks and considered as control group. The rats in groups 2 & 3 received only pellet diet containing 2 and 4% of Nigella sativa respectively for 6 months, and served as sham groups. Rats in group 4 received 0.5ml DMH dissolved in EDTA (30mg/kg b.w) injection (s.c) once a week for 18 weeks and served as DMH group. The group 5 & 6 were given DMH injections (20mg/kg b.w) and treated with diet containing 2 and 4% of Nigella sativa respectively for 6 months and considered as treated groups. Values are mean±S.E.M. obtained from 8 animals in each group and carried out in duplicate. * P<0.05 is considered significantly different from control group within each parameter. ** P<0.05 is considered significantly different from DMH-treated group within each parameter.

indicated that caraway oils reversed the TCDD (2, 3, 7, 8-tetrachlorodibenzo-p-dioxin) -dependent induction in cytochrome P450 1A1 in rat hepatoma cells.[43]

On the other hand, many studies have indicated that chemoprevention can be achieved through modulation of the GST system.[44,25] The changes in GST enzyme activities might be due to the malignant state which recovery of the enzyme activities could help to reverse malignancy. Previous findings[45] firmly establish GST inhibition as one of the major mechanism to explain the chemopreventive efficacy of phytochemicals. GST is a biotransformation enzyme in phase II involved in the detoxification of xenobiotics, carcinogens, free radicals and peroxides by conjugating these toxic substances with GSH, ultimately protecting cells and organs against carcinogen-induced toxicity. Induction of GST activity in liver of DMH-treated rats may be due to its effective role in detoxification of carcinogenic metabolite of DMH.[12,46] Decreasing of GST in liver by *Nigella sativa* seed powders which use GSH as substrate (Table 5) might advice to more metabolic disposal of carcinogenic DMH metabolites, resulting in the protection of hepatocytes and simultaneous inhibition of colon tumorigenesis. On the other hand, it was found that GST and GSH is induced upon oxidative stress in cancer.[47,29] Indeed, the reduction in GST induction following seed treatments may reflect a decreased oxidative stress arising from the oxygen radical scavenging activity of *Nigella sativa* seed powder.[29] In addition, the overexpression of GST enhances the production of eicosonoids, another common attribute observed in many tumors.[48] Moreover, GST increases the capacity of the tumor cells to withstand the burden of toxicants and procarcinogens.[47,49]

In spite of these changes by DMH, the seed supplementation failed to alter the oxidative stress parameters (SOD, CAT, FRAP, LP) in DMH-treated rats (Table 5). The low activities of hepatic antioxidant parameters (SOD and CAT) in DMH treated rats with simultaneous development of colonic tumor (Table 2–4) observed in the present study indicating that in DMH treated rats, the oxidant-antioxidant homeostasis is disturbed due to DMH metabolism[17,18] and also the liver is susceptible to oxidative damage during colon carcinogenesis. No change in the TBARS level in DMH-treated rats may be due to increased FRAP level, indicating compensatory increased of plasma antioxidants which is leaded to increased resistance and/or decreased susceptibility of the liver to free radical attack.[50] On the other hand, these changes were accompanied by an increase in the levels of GSH, a co-substrate for GST, which actively in concert eliminate hydrogen peroxide and lipid hydroperoxides. Moreover, in the presence of GSH as a substrate of GST, conjugation of toxic electrophiles with GSH takes place, conferring a selective growth advantage to cancer cells. Thus, the elevated GSH level in liver accompany with increased GST activity observed in our study may be used as markers of cell proliferation involved in the pathogenesis of DMH-induced colon cancer.[49,50] In addition, studies indicated that glutathione is synthesized by tumors in response to stress. For example, infiltrating ductal breast carcinoma has more than twice the levels of glutathione found in normal breast tissue.[47,51] In this regard, this higher GSH concentration that accompanies increased GST activity seemed to be a major means of compensation in this tissue as the result of the DMH imposed oxidant stress.[52] Furthermore, the ratio among these antioxidant enzymes is important, as any imbalance will result the accumulation of toxic-free radicals that cause cell damage.[53] Parallel to our results, enzymatic scavenging via antioxidants such as SOD and CAT in the face of an increase in oxidative stress may also play a role in determining the level of GSH and GST activity as other antioxidants.[54,29] Reduction in GST activities together with GSH level on *Nigella sativa* supplementation shows that the seeds may play a role in maintaining the balance between these antioxidant enzymes, which is in harmony with the previous reports.[43,45,55]

Our results indicated that *Nigella sativa* seed powders may protect against DMH induced colorectal tumorigenesis by reducing the formation of the ultimate DMH carcinogen through inhibition of DMH-metabolizing enzyme activities, such as GST and CYP450. From this study, it is obvious that one of the mechanisms of chemoprevention of colon tumorigenesis by *Nigella sativa* seed may be the enhancement of carcinogen detoxification but not antioxidant enzyme system in the liver.

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