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Protective Effect of *Allium sativum* and *Nigella sativa* Against NO-Mediated Alterations in Dimethylhydrazine-Induced Colon Cancer rats.

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Abstract

Plasma levels of nitric oxide (NO) and α-tocopherol as well as catalase activities in colon and liver tissues were assessed in 1, 2 dimethylhydrazine-induced colon cancer rats. Five groups of male Sprague-Dawley rats were fed the experimental diets supplemented with *Allium sativum* powder and *Nigella sativa* seeds (2.5%, 5%) or a mixed dose of both plants (5% of each) for 24 weeks, experimental period. At the fifth week rats were subcutaneously injected with dimethylhydrazine dihydrochloride (DMH) at a dose of 20mg/kg body weight for 20 weeks. Another two groups of rats were fed the basal diet for the same period, the first group designed as negative control group and injected with saline solution while the second group was injected with DMH at the same dose and designed as positive control group. Colon carcinogenesis was accompanied by a significant increase in the level of NO as well as catalase activity and significant decrease in plasma levels of α-tocopherol. Only the 5% *Allium sativum* powder fed group exhibited a significant decrease in NO level. Administration of *Allium sativum* powder and the mixed dose caused significant decrease in colonic and hepatic catalase activities and significant increase in α-tocopherol levels. On the other hand, the effects of *Nigella sativa* seeds on the measured parameters were non significant. These results were confirmed by the histopathological results that showed low incidence of colon tumors in rats fed 5% Allium *sativum* powder (17%) and the mixed dose (56%) fed groups. It could be concluded that the promising effect of garlic in DMH-induced colon cancer rats may be mediated through modulation of plasma levels of nitric oxide and α-tocopherol as well as tissue catalase activity.

Keywords: Colon carcinogenesis, DMH, Histopathology, *Allium sativum* (garlic), *Nigella sativa,* Nitric oxide, α-tocopherol, catalase, Rat.

Introduction

Colon cancer is one of the leading causes of cancer morbidity and mortality worldwide (Parkin *et al.,* 1999). Surgical is the principle method of treatment, but the surgical success rate, for patients with recently diagnosed colon cancer, is less than 40% (Bond, 1993). The antitumor drugs have been found to be toxic to some organs in the body (Thabrew *et al*., 2000). Thus, the lack of an effective treatment has underlined the importance of developing a better understanding of the role of diet in preventing colon cancer.

The preventive actions of garlic, garlic extracts and its organo-sulfur compounds against colon cancer have been demonstrated in animal models (Hatono *et al*., 1996) and in human colon cell lines

(Hirsch *et al*., 2000; Bottone *et al*., 2002). Epidemiologic studies also showed an inverse association between garlic consumption and reduced risk of colorectal carcinoma (Witte *et al*., 1996).

Pharmacological investigations of *Nigella sativa* L., seed extract revealed a wide spectrum of activities including anti-inflammatory (Houghton *et al*., 1995) and antioxidant effects (Kruk *et al*., 2000; Mansour *et al*., 2002). There have been very few studies on the anticarcinogenic effects of *Nigella sativa.* Supplementation of *Nigella sativa* seeds to the diet of methylnitrosourea-induced mammary cancer rats was reported to protect against oxidative stress, inflammatory response and carcinogenesis (Mabrouk *et al*., 2002).

Excessive production of nitric oxide during inflammation is thought to cause cellular injury and in long term cancer (Szabo and Ohshima, 1997). It has been also reported that development of colon cancer is frequently followed by inflammation of the colon and rectum (Seven *et al*., 1999). Inflammatory neutrophils produce free radicals including nitric oxide that may generate nitrating agents, which deaminate DNA bases, causing mutation (Weisiman and Halliwell, 1996).

The relation between serum α-tocopherol concentration and colorectal cancer has been examined in several studies. It was found that high plasma levels of α-tocopherol were associated with decreased incidence of colorectal cancer (Ingles *et al*., 1998) and that colon cancer incidence was accompanied by decreased levels of serum α-tocopherol (Badawy *et al*., 2000; Nair *et al*., 2001).

Catalase (CAT) is a tetrameric enzyme consisting of four identical tetrahedrally arranged subunits of 60 kDa that contains a single ferriprotoporphyrin group per subunit, and has a molecular mass of about 240 kDa (Aebi, 1980). Catalase reacts very efficiently with H_2O_2 to form water and molecular oxygen; and with H donors (methanol, ethanol, formic acid, or phenols) with peroxidase activity. In animals, hydrogen peroxide is detoxified by CAT and by GPX. Catalase protects cells from hydrogen peroxide generated within them. Even though CAT is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells. Survival of rats exposed to 100% oxygen was increased when liposomes containing SOD and CAT were injected intravenously before and during the exposure (Turrens *et al.*, 1984). The increased sensitivity of transfected CAT-enriched cells to some drugs and oxidants is attributed to the property of CAT in cells to prevent the drug-induced consumption of O_2 either for destroying H_2O_2 to oxygen or for direct interaction with the drug (Speranza *et al.*, 1993).

The objective of the present study is to look at how NO production would affect colon as well as liver tissues in DMH-induced colon cancer rats and to elucidate the protective effect that might be exerted by *Allium sativum* powder and *Nigella sativa* seeds against the noxious effect of free radicals.

Materials And Methods

Chemicals

1, 2 Dimethylhydrazine dihydrochloride (DMH), standards of α-tocopherol and vitamins used for vitamins mixture were purchased from Sigma chemical company, USA, while minerals used for salt mixture were purchased from ADWIC Company Egypt. Nitric oxide Kit (NO) (Cat No. 22110) was obtained from OXIS international, Inc USA.

Plants

Nigella sativa seeds were obtained from the Agriculture Research Center Giza, Egypt. Seeds were crushed into powder at time of diet preparation. However, dehydrated *Allium sativum* (garlic) powder was obtained from the local market.

Experimental animals

A total of 140 growing male Sprague-Dawley rats weighing between 60–70 gm were obtained from the animal house of The National Research Center Giza, Egypt.

Rats were housed individually in mesh bottomed metallic cages under healthy environmental conditions. Water and diets were provided *ad-libitum*.

Experimental design

Rats were divided into 7 groups (20 rat/group). Group 1 and 2 were fed the basal diet (table 1) for 24 weeks, at the fifth week group 1 was subcutaneously injected with saline solution (negative control), while group 2 was injected with DMH at a dose of 20 mg/kg body weight for 20 consecutive weeks (positive control) (Bandara *et al*., 1975). The other five groups were fed the basal diet supplemented with garlic or *Nigella sativa* (2.5%, 5%) or a mixed dose of both (5% of each) for 24 weeks (table 1), at the fifth week they were subcutaneously injected with DMH and follow the same schedule as the positive control group.

Samples collection

At the end of the experimental period (24 weeks), rats were anesthetized with diethyl ether, after overnight fasting and sacrificed. A Blood samples were collected from the hepatic portal vein in heparinized tubes. Plasma was separated by centrifugation at 3000 r.p.m for 20 minutes and kept frozen for the determination of α-tocopherol, nitric oxide. Immediately after sacrificing rats, colon and liver were plotted free from adhering blood, washed with cold saline and dried between filter papers. A tiny portion of each colon and liver was used for determination of catalase activity.

Biochemical assay

α-tocopherol was determined by High pressure liquid chromatography (HPLC) according to the method of Bieri *et al*. (1979). Nitric oxide was rapidly degraded in aqueous solution to nitrite and nitrate, the nitrite was measured colorimetrically using Griess reagent as described by Schmit (1995). Catalase activity was determined as mentioned by Maehly and Chance (1954).
Because of the long term of study and drastic effect of cancer, some animals were died through the study. The least number of survival animals (10 rats) was chosen for statistical analysis.

Histopathological examination:

Suitable sections of each colon and liver were fixed in 10% buffered formal saline and processed for preparation of 5mm-thick paraffin sections. These sections were sequentially stained with Hematoxylin (HX) and Eosin (E) and examined under the light microscope to identify tumor types and grades (McGraw, 1966). Grading was used to compare the severity of tumors.

Statistical analysis:

The data were presented as the mean ±SE. Statistical differences were determined by one-way analysis of variance (ANOVA) with Schiff's Multiple Range test at $p < 0.05$ (Zar, 1984).

Results

I. Biochemical results

Plasma nitric oxide content figure (1) was significantly elevated by 27.4% ($F_{1,19}$= 12.3, p< 0.05) in the positive control group as compared with the negative control group. Dietary intervention used in this study resulted in non significant depression in nitric oxide content with the exception of 5% *Allium sativum* fed rats that exhibited a significant decrease with a value of 20.4% ($F_{1,19}$=5.54 , p< 0.05) as compared with the positive control group.

As illustrated in figure (2), colorectal cancer induction caused significant decrease in α-tocopherol with a percentage of 26.6% ($F_{1,19}$= 18.9 , p< 0.05) as compared with the negative control group. However, α-tocopherol level was elevated in all treated rats. The elevation was significant in the 2.5% 5% *Allium sativum* powder and the mixed dose fed groups with percentage of change equal to 18.7% ($F_{1,19}$=5.1, p< 0.05), 30%($F_{1,19}$= 20.0, p< 0.05) and 22%($F_{1,19}$= 9.3, p< 0.05) respectively.

Catalase activity figure (3) was significantly decreased in the colon of 2.5%, 5% *Allium sativum* powder and the mixed dose fed rats by 16.0% ($F_{1,19}$=8.2, p<

0.05), 30.4% ($F_{1,19}$= 31.7, p< 0.05) and 19.5% ($F_{1,19}$=14.9, p< 0.05) respectively as compared with the positive control group. Similarly, hepatic catalase activity figure (4) was significantly decreased in 2.5%, 5% *Allium sativum* powder and the mixed dose fed rats by 10.3% ($F_{1,19}$=10.2, p< 0.05), 15.5% ($F_{1,19}$= 21.0, p< 0.05) and 13.4% ($F_{1,19}$=12.9, p< 0.05)respectively.

II-Histopathological findings:

In this study, colon and liver sections were examined microscopically to detect the histopathological changes, neoplastic changes as well as identification of the type and grade of the neoplasm if present.

Control groups:

-The results revealed that colon and liver sections of rats from the negative control group showed no histopathological abnormalities (fig. 5).

-The tumor incidence in the positive control group (DMH) was 100%, in the form of insitu carcinoma (20%) and invasive carcinoma of different grades (80%) as shown in (table 2 & fig. 6). Also, liver sections from animals of this group showed hepatotoxic manifestations in the form of necrosis, hyperactive nuclei, anisonucleosis, hydropic degeneration and fatty changes (fig. 7).

***Allium sativum* fed groups:**

-Microscopic examination of 2.5% *Allium sativum* fed rats revealed the presence of colonic carcinoma in 64% of rats (9/14) in the form of insitu carcinoma (21%) and invasive carcinoma (43%), (table 2). Also liver sections of this group showed toxic manifestations without any hepatic malignancy. While examination of colonic sections of rats fed diets supplemented with 5% *Allium sativum* showed the lowest tumor incidence (17%) among all groups, (table 2& fig. 8). The microscopic examination of liver section of this group revealed less toxicity comparing to the positive control group (fig 9).

Nigella sativa fed groups:

-The positivity for malignancy in 2.5% *Nigella sativa* group was 67% and it was in the form of invasive carcinoma of different grades (table 2). On the other hand histopathological examination of liver sections of rats of this group revealed presence of metastatic carcinoma in three cases (table 2 & fig. 10). Regarding 5% *Nigella sativa* fed group, the induction of colon cancer was evidenced in 90% of the examined colons (table 2 & fig 11). Livers of these rats showed three cases with liver metastasis, (fig. 12).

Mixed dose group:

-Rats fed mixed dose (5% *Allium sativum* +5% *Nigella sativum*) showed colonic tumor incidence in 56% of the examined colons (8/18) in the form of invasive carcinomas of different grades (6 cases of grade 1 carcinoma, 1 case of grade 3 carcinoma and 1 case of grade 4 carcinoma (table2).

Examination of Liver sections revealed toxic hepatitis changes without any metastatic tumors (fig. 13).

From the above data and data recorded in table (2), it was found that the preventive role of garlic 5% is the highest (83%) comparing to the other groups, followed by the mixed dose (5% garlic + 5% *Nigella sativum*) which resulted in 44% prevention of induction of colon cancer in rats followed by garlic 2.5% which gave 36% prevention. So we can conclude that garlic have an antimetastatic effect which was evident by the absence of liver metastasis in garlic treated groups.

Table (1): Composition of different experimental diets.

Ingredients (gm %)	Basal diet[1]	Experimental diets				
		3	4	5	6	7
Casein	13	13	13	13	13	13
Sunflower oil	10	10	10	10	10	10
Salt mixture[2]	4	4	4	4	4	4
Vitamin mixture[3]	1	1	1	1	1	1
Cellulose	5	5	5	5	5	5
Nigella seeds	-	2.5	5	-	-	5
Garlic powder	-	-	-	2.5	5	5
Corn starch	67	64.5	62	64.5	62	57

1: Basal diet was prepared according to Campbell (1963) and was fed to negative (group1) and positive (group 2) control groups.
2: Salt mixture was prepared according to Hegsted *et al*. (1941).
3: Vitamin mixture was prepared according to AOAC (1995).

Table (2): the preventive role of *Allium sativum* and /or *Nigella sativa* on colon cancer induced in rats.

Groups	No. of survived rats	Positive cases	% of prevention	Insitu Ca.	Invasive Ca.	G1 Ca.	G2 Ca.	G3 Ca.	G4 Ca.	Liver metastasis
N.C	10	-	-	-	-	-	-	-	-	-
P.C.	10	10	Zero	2	8	2	5	1	-	-
2.5% A.S	14	9	36%	3	6	5	-	1	-	-
5% A.S	12	2	83%	-	2	1	-	-	1	-
2.5% N.S	12	8	33%	-	8	2	2	-	4	3
5% N.S	10	9	10%	-	9	2	3	2	2	3
Mixed dose	18	8	44%	-	8	6	-	1	1	-

Note: N.C: Negative control, P.C: Positive control, A.S: *Allium sativum,* N.S: *Nigella sativa.* (garlic). Ca. = Carcinoma.

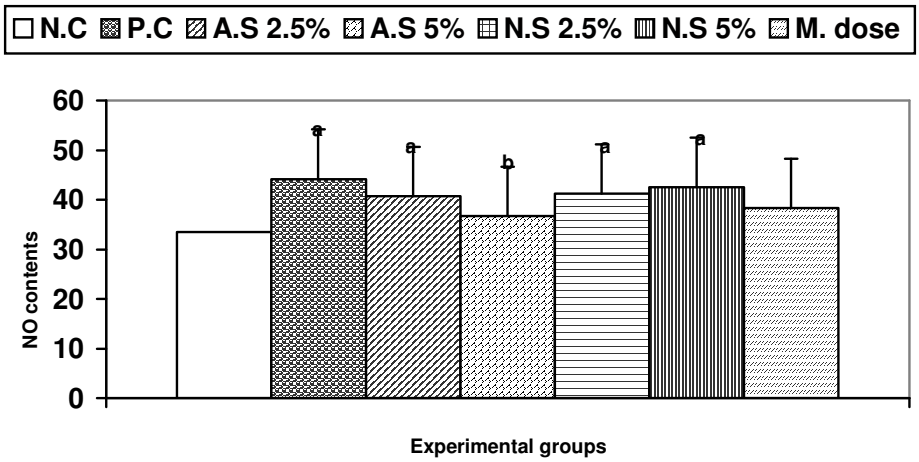

Figure (1): Plasma nitric oxide contents of colon cancer-induced rats fed *Allium sativum* powder and *Nigella sativa* seeds.
a: Significant difference from the negative control.
b: Significant difference from the positive control.
P< 0.05.
Note: N.C: Negative control, P.C: Positive control, N.S: *Nigella sativa*, A.S: *Allium sativum* (garlic), M: Mixed dose, NO: Nitric oxide.

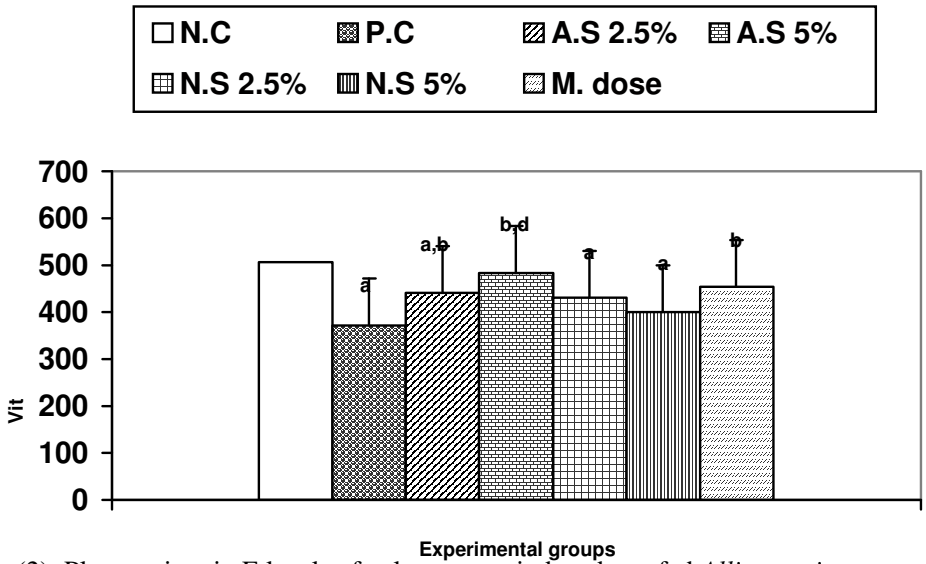

Figure (2): Plasma vitamin E levels of colon cancer-induced rats fed *Allium sativum* powder and *Nigella sativa* seeds.
a: Significant difference from the negative control.
b: Significant difference from the positive control.
d: Significant difference between the same level in different plants (between plants).
P< 0.05.
Note: N.C: Negative control, P.C: Positive control, N.S: *Nigella sativa*, A.S: *Allium sativum* (garlic), M: Mixed dose.

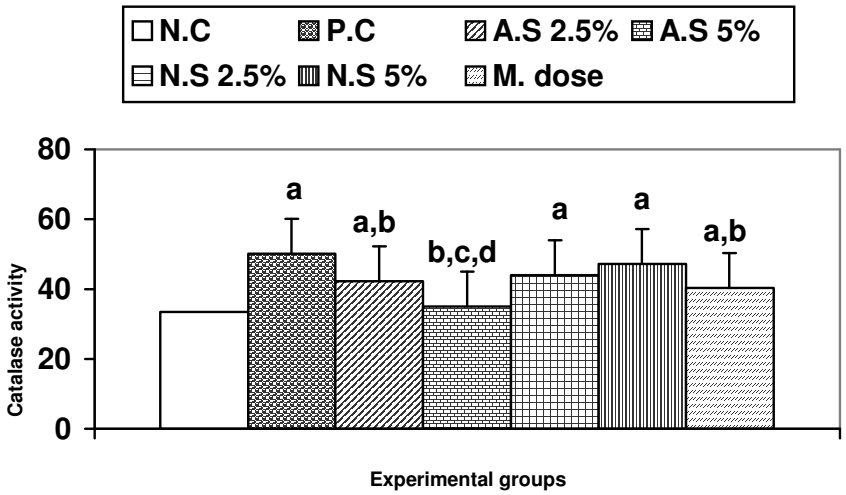

Figure (3): Catalase activity in colon of colon cancer-induced rats fed *Allium sativum* powder and *Nigella sativa* seeds.
a: Significant difference from the negative control.
b: Significant difference from the positive control.
c: Significant difference between the two levels of each plant (within plant) .
d: Significant difference between the same level in different plants (between plants).
P< 0.05.
Note: N.C: Negative control, P.C: Positive control, N.S: *Nigella sativa*, A.S: *Allium sativum* (garlic), M: Mixed dose.

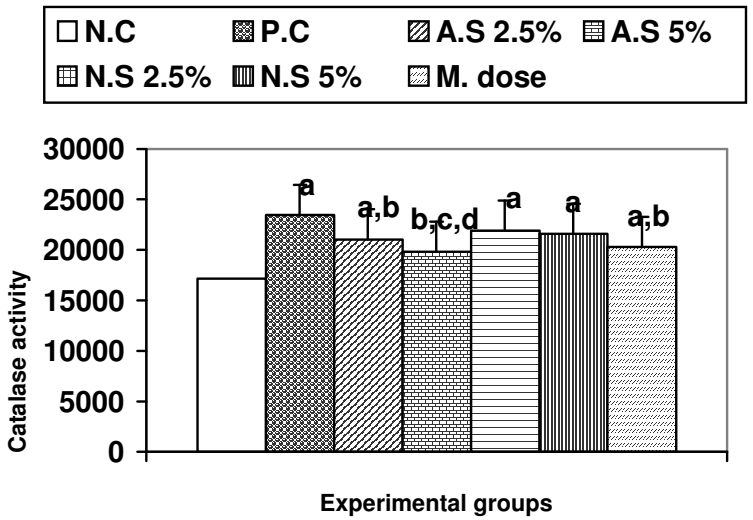

Figure (4): Hepatic catalase activity of colon cancer-induced rats fed *Allium sativum* powder and *Nigella sativa* seeds.
a: Significant difference from the negative control.
b: Significant difference from the positive control.
c: Significant difference between the two levels of each plant (within plant) .
d: Significant difference between the same level in different plants (between plants).
P< 0.05.
Note: N.C: Negative control, P.C: Positive control, N.S: *Nigella sativa*, A.S: *Allium sativum* (garlic), M: Mixed dose.

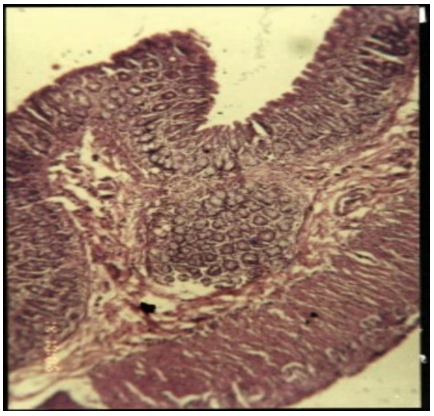

Fig. 5: Section in rat colon of the negative control group showing normal structure. (H & E X100)

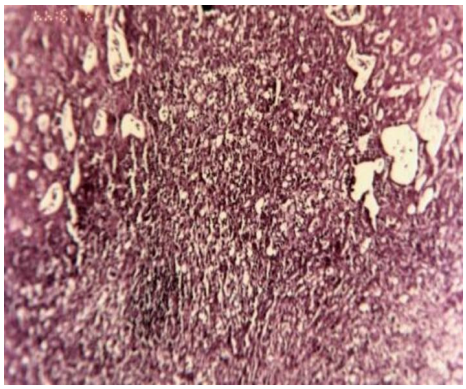

Fig.7: Section in rat liver showing hepatotoxic manifestations in the form of hyperactive nuclei, anisonucleosis, as well as areas of necrosis. Positive control (H&E X 100)

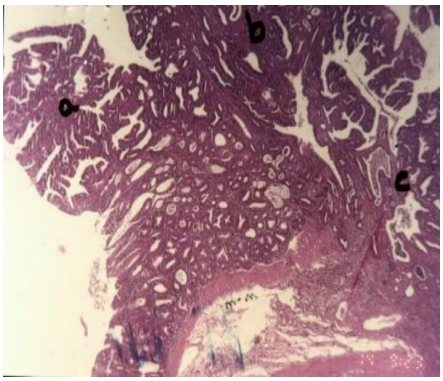

Fig.6: Section in rat colon of the positive control group showing adenomatous hyper-plastic changes (a) with areas of insitu carcinoma (b) as well as area of invasive carcinoma(c). (H & E X100)

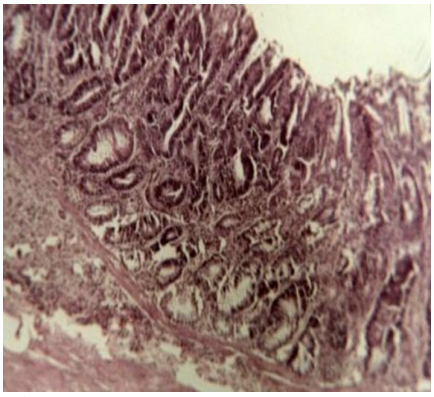

Fig. 8: Section in rat colon showing area of dysplastic changes, no evidence of frank malignancy. Treatment**:** *Allium sativum* (Garlic) 5% and DMH . (H & E X100)

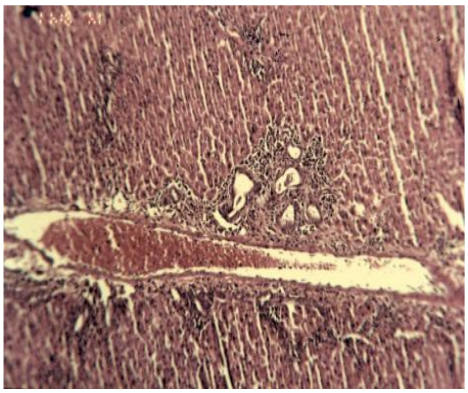

Fig.9: Section in rat liver showing hydropic degeneration, fatty changes and chronic inflammatory reaction, no evidence of malignancy. Treatment**:** *Allium sativum* (Garlic) 5% and DMH. (H & E X100)

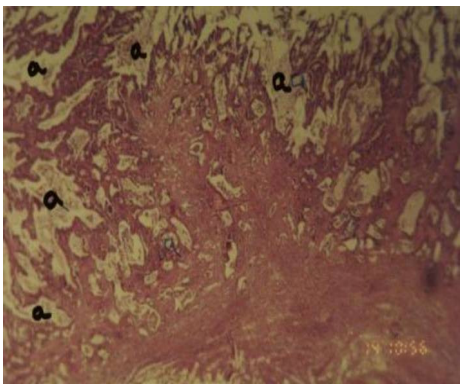

Fig. **11:** section in rat colon showing evidence of induced colon cancer (invasive carcinoma grade II). Treatment: *Nigella sativa* 5 % and DMH. (H&E X100)

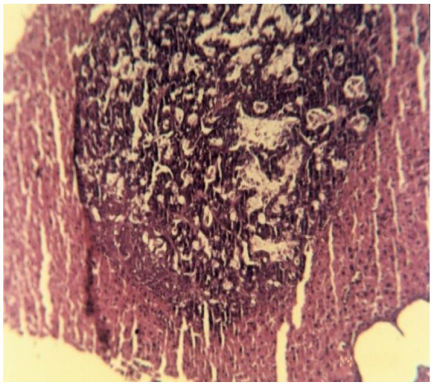

Fig. 10: Section in rat liver showing metastatic carcinoma GΠ and necrosis. Treatment: *Nigella sativa* 2.5%and DMH. (H&E X100)

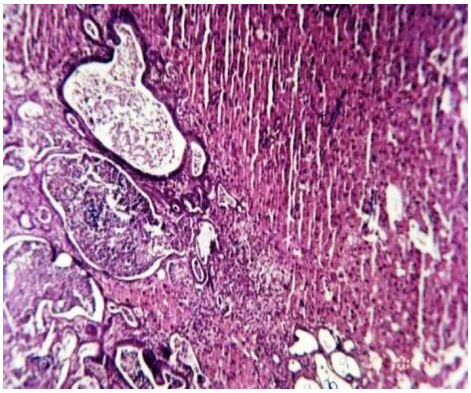

Fig. 12 Section in rat liver showing metastatic colonic carcinoma GΠ and necrosis. Treatment: *Nigella sativa* 5% + DMH. (H & E X100)

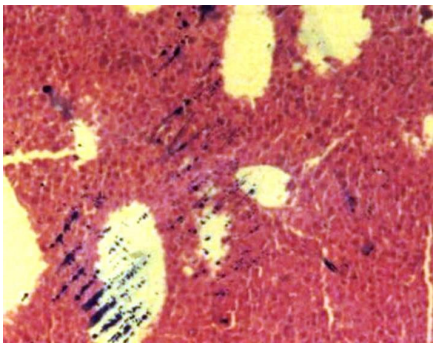

Fig. 13 Section in rat liver showing Toxic hepatitis and necrosis without evidence of malignancy. Mixed dose. (H&E, X100)

Discussion

Several studies have been reported that the levels of inducible nitric oxide synthase, iNOS were increased in colon tumor tissues of azoxymethane-induced colon cancer rats (Rao *et al*., 1998) and in human colon carcinoma cell lines (Lagares-Garcia *et al*., 2001). These studies may support the results of the present study which showed an increase in plasma nitric oxide of DMH-induced colon cancer control rats. However, Moochhala *et al*. (1996) have found that human colorectal adenocarcinomas exhibited a marked reduction of constitutive nitric oxide synthase, cNOS and iNOS. Bakan *et al*. (2002) reported that the increased production of NO in plasma of patients with gastric cancer can be related to an alteration in oxidant-antioxidant status, and that high concentration of NO for a long period could result in DNA damage leading to mutations and cancer. With regard to the possible relationship between overexpression of iNOS and colon carcinogenesis, the interaction of $NO^{\cdot}$ with cyclooxygenase pathways seems to be important. Nitric oxide has been found to enhance the activity and expression of cyclooxygenase-2 in a variety of cell types (Salvemini *et al*., 1994). Cyclooxygenase-2 (COX-2) is in fact known to be increased in colorectal tumors in men and rodents (Liu *et al*., 2003). Overexpression of COX-2 has been demonstrated to render tumor cells resistant to apoptosis and growth advantage ((Dubois *et al*., 1996). Because NO plays an important role in the regulation of vascular tone and blood flow, it is possible that the production by endothelial nitric oxide synthase in endothelial cells of the neovasculature may cause vasodilation and increase blood flow to the tumor tissues, supporting their growth (Takahashi *et al*., 1997). This assumption is confirmed by reduction of blood flow in tumor-associated neovasculature with the use of NOS inhibitors (Fukumura *et al*., 1997).

Administration of *Allium sativum* powder to DMH-induced colon cancer rats significantly reduced the elevated levels of

plasma nitric oxide, especially in 5% *Allium sativum* fed group. These results are in accordance with previous reports indicating that ajoene and allicin (Dirsch *et al*., 1998) as well as garlic extract and S-allylcysteine (SAC) (Kim *et al*., 2001) inhibited nitric oxide production in lipopolysaccharides-stimulated macrophage cell lines, through suppression of iNOS mRNA and protein expression in the activated macrophages.

The observation that DMH-induced colon cancer positive control rats exhibited a marked increase in plasma α-tocopherol is in agreement with those obtained by Antosiewicz *et al*.(2002) in rats, and in patients with colorectal cancer (Nair *et al*., 2001). However, Saygili *et al*. (2003) have reported that plasma levels of vitamin E in colon cancer patients were similar to those of healthy subjects. They suggested that, this could be related to the previous nutritional status of the studied population.

Vitamin E supplementation was associated with decreased incidence of colon tumors in experimental animals (Cook and Mc Namara, 1980; Sumiyoshi, 1985) and increased level of α-tocopherol in patients with advanced colorectal cancer (Malmberg *et al*., 2002) and adenomatous polyps (Simone *et al*., 2002). However, other epidemiologic studies failed to find an association between vitamin E intake and colorectal cancer.

The increase of plasma α-tocopherol level in *Allium sativum* fed group in the present work could be attributed to the antioxidant effect of garlic against DMH-induced colon cancer, manifested by the significant decrease in MDA and the significant increase in glutathione content as well as the activity of cellular scavenging enzymes observed in our study (unpublished data). This may have sparing effect on α-tocopherol levels.

Catalase reacts very efficiently with H_2O_2 to form H_2O and $O_2^{\cdot}$ In this way catalase can maintain the concentration of $O_2^{\cdot}$ Catalase becomes more efficient in protection against severe oxidant stress (Yan and Harding, 1997).

Hepatocarcinogenesis as well as hyperlipidemic agents may increase cellular catalase activity (Calabrese and Canada, 1989). This finding supports the present study in the positive control group and is consistent with the results of Cerutti and Trump (1991) that indicated overexpression of catalase activity in tumor cells. The increase in catalase activity could be attributed to the high levels of reactive oxygen species (ROS) in malignant cells which attack hematopoietic cells. However, *Allium sativum* powder and *Nigella sativa* seeds were found to decrease catalase activity when compared with the positive control group in an attempt to reach its normal activity as in the negative control group.

Chen *et al*. (1999) reported that treatment with diallyl sulfide (DAS) and garlic homogenates reduced the hepatic catalase levels in rats and mice. This finding may support our results. The improvement in the other antioxidant defense systems measured may partially explain the normalization of catalase in 5% garlic fed group.

Microscopic examination showed that 100% of DMH-induced colon cancer positive control rats developed colon tumors, with the majority having adenocarcinomas (80%). Cheng *et al*. (1995) noticed that colon tumor incidence of 86% in DMH-induced colon cancer rats, while Mclntosh *et al*. (2001) detected colon neoplasia in 80% of rats-treated with azoxymethane. Moreover, Melen-Mucha and Niewiadomaka (2002) found that the majority of tumors developed in DMH-treated rats were adenocarcinomas. These results seem to support the present work.

Pervious reports have shown that garlic and its organosulfer compounds significantly inhibited colon tumor incidence in DMH-treated animals (Cheng *et al*., 1995), suppressed growth of cultured human colon tumor cells (Knowles and Milner, 1997) and inhibited rat sarcoma tumor cells migration in a dose dependent manner (Hu *et al*., 2002). These findings strengthened the evidence for protection afforded by *Allium sativum* powder in the present study.

It could be concluded that, in rat model of DMH-induced colon cancer, administration of garlic may modulate the

elevated plasma NO level as well as catalase activity and restore the lowered levels of α-tocopherol. However, *Nigella sativa* failed to induce significant effect on the measured parameters.

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القدرة الوقائية للثوم و حبة البركة ضد التغيرات الناتجة عن أكسيد النيتريك فى الجرذان المستحثة بسرطان القولون بالهيدرازين ثنائى الميثيل.

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تم تقدير مستوى أكسيد النيتريك و الألفاتوكوفيرول فى بلازما الدم و كذلك انزيم الكتاليز فى أنسجة القولون و الكبد للجرذان المحدث فيها سرطان القولون بمادة الهيدرازين ثنائى الميثيل حيث تم تغذية خمس مجموعات من الجرذان على وجبات اضيف اليها الثوم و حبة البركة بجرعتين (2,5% و 5%) او على وجبة مختلطة من النباتين (5%) من كل منهما) لمدة 24 اسبوع. فى الأسبوع الخامس من التجربة تم حقن الجرذان بمادة الهيدرازين ثنائى الميثيل ثنائى الهيدروكلوريد بجرعة قدرها 20 مليجرام لكل كيلو جرام من وزن الجسم لمدة20 إسبوع كما تم تغذية مجموعتين اخرتين من الجرذان على الوجبة القياسية لنفس المدة, حيث مثلت احداهما المجموعة الضابطة الطبيعية و تم حقنها بمحلول فسيولوجى بينما مثلت المجموعة الثانية المجموعة الضابطة المصابة بالسرطان و تم حقنه بمادة الهيدرازين ثنائى الميثيل ثنائى الهيدروكلوريد. ادت الإصابة بالسرطان البإحداث إرتفاعا معنويا فى مستوى كلا من أوكسيد النيتريك و انزيم الكتاليز و إنخفاضا معنويا فى مستوى الألفاتوكوفيرول . حدث إنخفاضا معنويا فى مستوى اوكسيد النيتريك فقط فى الجرذان التى تغذت على الجرعة 5% من الثوم كما أدت التغذية على الثوم و الجرعة المختلطة من النباتين الى إحداث إنخفاضا معنويا فى إنزيم الكتاليز بأنسجة القولون و الكبد و كذلك زيادة الألفاتوكوفيرول, بينما كان تأثير التغذية على حبة البركة غير معنويا على كل القياسات السابقة. وقد تإكدت هذة التغيرات بنتائج الفحص الهستوباثولوجبو التى أظهرت إنخفاضا فى معدل الإصابة بسرطان القولون فى الجرذان التى تغذت على الجرعة 5 % من الثوم (معدل الاصابة=17%) و الجرعة المختلطة (معدل الاصابة=56%). نستنتج من الدراسة السابقة أن تأثير الثوم على الجرذان المجدث فيها سرطان القولون قد يكون ناتجا عن تعديل مستوى أوكسيد النيتريك و الألفاتوكوفيرول فى البلازما و كذلك محتوى الأنسجة من إنزيم الكتاليز.