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Chemopreventive Effect of Oil, Extracted from *Coriandrum sativum* on Mice Bearing Solid Ehrlich Tumor

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In a large number of epidemiological studies investigating relationship between diet and cancer, a protective effect of the consumption of vegetables and fruits on various types of cancer has been found. This protective effect has been generally attributed to the antioxidative capacities of the constituents present in these foods. The search for natural agents that protect against tumorigenesis is important to those at risk by virtue of environmental pollution or health-related treatment as well as for scientific study of the mechanism of the potential therapeutic application of these agents. The present research is a trial to evaluate the chemopreventive effect of oral administration of oil extracted from the leaves of *Coriandrum sativum* (1 mg/kg) in mice inoculated with Ehrlich Ascites Carcinoma with or without exposure to fractionated doses of gamma irradiation (up to 6 Gy). Significant increase in malondialdehye (MDA) level accompanied with significant decrease in catalase activity in both liver and tumor tissue in the group of mice, bearing tumor while total protein content was increased in tumor tissue. Serum iron was inversely proportional to the level of transferrin and total iron binding capacity (TIBC) levels. Application of coriander oil to mice bearing tumor caused partial amelioration in the assayed parameters. Such amelioration was pronounced in restoring the level of MDA in liver to the control level while, tumor tissue still suffered from sharp increase in MDA content. Combined treatment of coriander oil followed by irradiation exerted a protective effect on the elevation of MDA content induced by tumor growth in both liver and tumor tissue. Successful application of coriander oil in radiotherapeutic practices still awaits further investigation on the optimal to be used in clinical trial and whether or not it will be with value when used with fractionated radiotherapy.

Key words: Chemopreventive, *Coriandrum sativum*, mice, carcinoma

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Introduction

A major problem in the use of chemopreventing agents in cancer treatment is the toxicity of these drugs to normal cells. One approach to solve this problem is to employ the inhibitors of tumorigenesis, which have become a natural origin. Within the past few years, natural extracts or purified compounds have become well established means for studying varied aspects of cancer (Abdullaev and Gonzalez, 1997). It has been mentioned that the presence of tumor in the human body or in experimental animals is known to affect many functions of the vital organs in the body including liver, blood and lung even when the site of the tumor does not interfere directly with the functions of these organs (Dewys, 1982). In addition, the existence of hypoxic cells in solid tumor is the most important limiting factor in local control malignant tumors by radiation therapy. Small tumors are frequently cured by radiation alone, while the response of large solid tumors to radiotherapy differs greatly. To overcome the resistance of hypoxic cells present in tumor to radiotherapy, fractionated doses of gamma radiation have been applied in this study.

The commonly used spice and flavoring agent, coriander oil, derived from the leaves of the plant *Coriandrum sativum* displays antioxidant properties in foods and in biological systems (Chithra and Leelamma, 1999).

As a medicinal plant, coriander has been used as analgesic, antispasmodic, antioxidant, antirheumatic and carminative. It has also exhibited hypoglycemic, lipolytic and cytotoxic activity (Simon *et al.*, 1984).

Iron is an essential metal in mammals for oxygen transport by hemoglobin and for the functions of many enzymes including catalase and cytochromes (Sorenson, 1992). There is an increasing number of reports of an association between increased body iron during tumor growth.

The present study has been planned to test and evaluate the efficiency of coriander as chemopreventive agent independently or in combination with radiation exposure on solid tumor bearing animals. The concentration of iron, transferrin and total iron binding capacity were measured as indices of iron status as well as malondialdehyde and catalase as a rough index for the balance between free radical generations and scavenging.

Materials and Methods

Experimental animals: Female Swiss albino mice (18-20 g) were housed in especially designed cages and allowed cube pellets diet and water *ad libitum*.

A line of Ehrlich Ascites Carcinoma (EAC) was supplied through the courtesy of Dr. C. Benskijsen, AVL, Amesterdam, Holland. The tumor lines were maintained at the National Cancer Institute, Cairo, Egypt, in female swiss albino mice by weekly intraperitoneal transplantation of 2.5×10^6 cells.

Preparation of Coriander oil: The oil of *Coriandrum Sativum* L. was extracted by steam distillation (Balbaa *et al.*,1981). The volatile oil was kept in brown tightly closed container till used. The extracted oil was orally administered at a dose equal to 1 ml / kg body weight six times once every two days starting after 6 days of tumor inoculation.

Animal Treatment: Mice have been sorted into two groups: one as untreated control and the other was inoculated with Ehrlich Carcinoma. The later group was divided into 4 subgroups:

1 Mice -inoculated with 2.5×10^6 tumor cells, locally in the thigh of the left lower limb.
2 Mice treated with coriander oil alone (1 mg/kg of body weight).
3 Tumor inoculated mice, treated with coriander oil.
4 Tumor inoculated mice, treated with coriander oil and subjected to fractionated doses of gamma irradiation 2 Gy day $^{-1}$ after day upto total dose of 6 Gy.

Irradiation of Animals: Irradiation was performed one day after the last dose of oil administration. Irradiation process was performed using Gamma Cell-40, which is a Caesium 137 facilitated by National Centre for Radiation Research and Technology. The dose rate was 0.666Gy / min. at the time of experimentation.

Biochemical assays: Iron and total iron binging capacity were determined as previously described (Harper, 1974), while transferrin was calculated using the following expression:

TIBC (μg/100 ml) : 1.2 = Transferrin (mg/100 ml):

Assuming 1 mg of Transferrin may bind 1.2 μg of iron as a maximum.

Malondialdehyde (MDA) as one of the main products of lipid peroxidation was measured in liver and tumor tissues (Yoshioka *et al.*,1979). Catalase activity and total protein were also determined (Chance and Maehly 1955) and (Lowery *et al.*, 1951) respectively.

Experimental animals were sacrificed 30 days after tumor inoculation or one day after the last dose of gamma irradiation exposure. The animals were dissected and serum samples were collected for iron and TIBC assays. Liver and tumor were removed for MDA, catalase and total protein, homogenized in bidistilled water using Potter-Elvehjem homogenizer.

Results

As illustrated in Table 1, serum iron was significantly increased by 60.5 % ($p < 0.001$) in mice bearing tumor. The data also showed significant decrease in serum TIBC and tranferrin amounted 24.8 and 23.6 % (0.05) respectively, while significant increase was detected in total protein of the tumor tissue in mice bearing tumor in comparison with control groups.

Partial improvement was observed in serum iron of mice inoculated with tumor and treated with coriander oil, while total protein restored its control value. On the other hand, administration of coriander oil could not improve the decreased level of TIBC and transferrin in mice bearing tumor. Treatment of mice bearing tumor with coriander oil and irradiation showed marked amelioration in serum iron even though, the level was still higher than the control value. The same group showed significant decrease in serum TIBC and transferrin accompanied with significant increase in tumor total protein. On the other hand, no significant change was detected in liver total protein of all the investigated groups.

Administration of coriander oil alone to normal mice resulted in significant increase ($p < 0.01$) of serum iron, but it had no effect on the levels of TIBC, transferrin and total protein. The data in Table 2 presents the level of MDA and catalase activity in both liver and tumor tissue to provide a rough index for balance between free radicals generation and scavenging. MDA content exhibited significant elevation in liver and tumor

tissue in the group of mice bearing tumor. The increase was more pronounced in tumor tissue (129.8 %) as compared to control group. Administration of coriander to mice bearing tumor could greatly ameliorate the level of MDA in liver and could shift its level towards normal values.

Combined treatment of coriander and irradiation improved the hepatic MDA content completely below even the normal range. Restoration in MDA content in tumor tissue was also observed in comparison with control values. On the other hand, no significant change was detected in liver and tumor tissue MDA level after administration of coriander alone to normal mice.

The results given in Table 2 also showed that mice bearing tumor exhibited highly significant decrease ($p < 0.001$) in catalase activity in liver and tumor tissue by 36.2 and 28.0 % respectively as compared with control values. The same trend in catalase activity could be observed in other groups with different degrees of significance except for mice treated with coriander oil alone that recorded normal values of catalase activity in tumor tissue.

Discussion

The present data revealed that inoculation of Ehrlich carcinoma tumor into the thigh of left leg of female Swiss albino mice let the animal suffering from deleterious changes in serum iron, TIBC, tranferrin and tumor total protein as well as significant increase of MDA in liver and tumor tissue accompanied with significant decrease of catalase activity in the tested tissues.

Many studies have indicated that tumor growth can cause antioxidant disturbances and acceleration in lipid peroxidation in liver and tumor hosts (Burlankova and Molochkina, 1973). A dramatic increase in lipid peroxide level was reported in patients with malignant tumor (Changdao *et al.*,1989).

The hepatic injury due to tumor growth appeared to be associated with oxidative stress –mediated mechanisms as evidenced by increased lipid peroxidation and decreased catalase activity in liver, both indices of oxidative stress. Similar observations reported occurrence of unbalance in lipid peroxidation level and antioxidant system in patients with Hodgkins disease (Muravskaya *et al.*, 1994).

The significant decline in catalase activity in mice bearing Ehrlich tumor as reported in the present study in agreement with previous studies of Sun *et al.* (1989) and Marklund *et al.* (1982) who found a depression in catalase activity in seven lines of neoplastic tumor cells. The decrease of catalase activity as a result of tumor growth may be attributed to its inactivation by superoxide radical through converting it to the ferroxy and ferryl states of the enzyme (Kono and Fridovich, 1982).

Iron is normally conserved and re-utilized vigorously. It is supplied to the bone marrow by the plasma protein transferrin, subsequently reappears in haemoglobin, and is eventually recycled to transferrin after the processing of senescent red cells by macrophages. Iron within circulation is in equilibrium with the iron in all body tissues (Pippard and Heppleston, 1996). In addition, the small amount of iron present in all tissues is essential for many vital enzymes, including the cytochromes involved in oxidative energy production, catalase acting as free radical scavenger and cyclooxygenase, which converts arachidonic acid to PGE2 (Sorenson, 1992). Iron balance is maintained by regulation of iron absorption. It has been postulated that the need of iron by the haemopoietic system is increased during tumor growth to remain within the normal range. Therefore, iron absorption is accelerated by the intestinal mucosa (Fairbanks and Klee, 1994).

Excess body iron may result in hemochromatosis in which tissue is damaged. The hepatic injury occurred during tumor growth causes the release of many constituents into plasma; among these is iron, released from liver stores (Goldberg and Gornall, 1980). TIBC is a measure of maximum concentration of iron that serum proteins principally transferrin, can bind. About one third of the iron binding sites of transferrin are occupied by Fe^{+3} and hence, serum tranferrin has considerable

Table 1: Iron, TIBC, transferrin and total protein in mice bearing, Ehrlich tumor treated with Coriander oil and exposed to gamma irradiation.

Group Parameters	Control	Tumor alone	Corind. alone	Corind. + Tumor	Corind. + Tumor + Rad.
Iron (μg/dl)		***	**	**	**
mean ± SE	94.11 ± 1.46	151.09 ± 5.79	118.33 ± 4.49	138.94 ± 7.19	116.11 ± 4.43
% Change		60.5	25.7	47.6	23.3
TIBC (μg/dl)		*	NS	***	***
mean ± SE	326.96 ± 12.51	245.89 ± 21.89	306.94 ± 17.32	228.43 ± 4.01	227.72 ± 4.73
% Change		-24.8	-6.1	-30.1	-30.4
Transferrin (mg/dl)		*	NS	**	**
mean ± SE	268.29 ± 13.82	204.91 ± 18.25	251.61 ± 12.19	186.19 ± 2.46	189.76 ± 3.94
% Change		-23.6	-6.2	-30.6	-29.3
T. Protein In liver		NS	NS	NS	NS
#mean ± SE	231.17 ± 23.99	242.25 ± 3.71	239.92 ± 4.97	229.48 ± 1.37	226.46 ± 5.99
% Change		4.7	3.7	-0.7	-2.0
T. Protein In tumor		*	NS	NS	**
#mean ± SE	180.43 ± 3.77	204.95 ± 6.18	185.65 ± 2.91	190.95 ± 2.56	206.38 ± 4.99
% Change		13.5	2.9	5.8	14.3

Each value represents mean ± SE of 4 rats. # Each value represents mean ± SE (mg/g tissue) of 4 rats.
* Significant at > 0.05 ** Significant at > 0.01 ***Significant at > 0.00 NS non-significant

Table 2: Malondialdehyde (MDA) level and catalase activity in mice bearing, Ehrlich tumor treated with Coriander oil and exposed to gamma irradiation.

Group Parameters	Control	Tumor alone	Corind. alone	Corind. + Tumor	Corind. + Tumor + Rad.
MDA		***	NS	NS	*
In liver					
#mean ± SE	190.56 ± 2.47	253.42 ± 12.22	177.96 ± 5.39	211.69 ± 9.58	160.77 ± 9.32
% change		32.9	-6.6	11.08	-15.6
MDA		***	NS	***	NS
In tumor					
#mean ± SE	110.66 ± 4.31	254.31 ± 8.74	114.27 ± 7.40	194.09 ± 5.88	118.84 ± 5.85
% change		129.8	3.3	75.3	7.4
Catalase		***	*	***	**
In liver					
$mean ± SE	29.04 ± 0.59	18.52 ± 1.21	20.95 ± 2.72	18.30 ± .82	19.95 ± 1.52
% change		-36.2	-27.9	-37.0	-31.3
Catalase		***	NS	***	***
In tumor					
$mean ± SE	29.27 ± 1.20	21.07 ± 0.63	30.54 ± 1.98	19.95 ± 0.65	10.39 ± 0.57
% change		-28.0	4.3	-31.9	-64.5

Each value represents mean ± SE (u mol / g wet tissue) of 4 rats.
$ Each value represents mean ± SE (units / mg wet tissue) of 4 rats.
* Significant at > 0.05 ** Significant at > 0.01 ***Significant at > 0.00 NS non-significant

reserve iron binding capacity. The serum TIBC varies in disorders of iron metabolism. TIBC is often decreased in chronic inflammatory disorders or malignancies (Fairbanks and Klee, 1994) and transferrin as well (Heininger and McNeely, 1980). It is proved that all tumors contain certain percentage of hypoxic cells (Adams *et al.*, 1976). Disruption of intracellular iron and its release has been reported earlier in hypoxic cells. It is established that hypoxia is known to increase the iron content as well as release of iron in the extracellular spaces in many tissues of the body (Jamindar and Dawson, 1995).

Small tumors are frequently cured by radiation alone, while the response of large solid tumors to radiotherapy differs greatly. To overcome the resistance of hypoxic cells present in tumor to radiotherapy, fractionated doses of gamma radiation have been applied in this study.

In the present study, coriander administration to mice bearing tumor with and without fractionated doses of gamma irradiation has the tendency to normalize MDA content in liver accompanied by inhibition in catalase activity in tumor tissue, which means that coriander oil enhances radiation effect through increasing cytotoxicity and decreasing protection factors. In this respect, it has been elucidated that it is required to use cytotoxic and drug scheduling that do not enhance the radiation injury to normal tissues (Steel and Peckham, 1979). This means that combined treatment of coriander with radiation exposure revealed an improvement in the therapeutic gain, which may be an enhancement of tumor response and minimization of normal tissue damage.

Previous studies demonstrated the antiperoxidative effect of *Coriandrum sativum* as well as increasing activities of antioxidant enzymes (Chithra and Leelamma, 1999).

Coriander is generally recognized safe, for human consumption as a spice, natural flavoring and for use as an essential oil. The essential oil content of dried leaves is mainly linalool (55.57 %) camphor, borneol, geraniol, carvone, terpinene and limonene and other constituents (Simon *et al.*, 1984). Isoquercitrin and rutin are also identified as flavonoid constituents in coriander (Kunzemann and Herrmann, 1977). Carvone and limonene when given three times (at 5 and 10 mg / animal) every two days, induced the detoxifying enzyme glutathione-S-transferase in the target tissues of female mice (Zheng *et al.*, 1992; Lam and Hasegawa, 1989). The inhibition of tumor growth by antioxidant free radicals has been implicated in both the initiation and promotion stages of carcinogenesis. In the majority of studies, the natural chemopreventive agents must be present at the time of tumor growth in order to observe the inhibition or a delay in the onset of tumorigenesis. Efforts should be made to develop a standardized protocol for evaluating the chemopreventive agents as inhibitors of both initiation and progression stages of tumor development. It is difficult to propose a common mechanism for the protective effects of oils extracted from natural origin. All the results suggest that the protective mechanisms are complex and may well include protection and enhancement of cellular immune response.

Nevertheless, it should be emphasized that successful application of coriander in radio therapeutic practice still awaits further investigation on the optimal to be used in clinical trial and whether or not it will be with value when used with fractionated radiotherapy.

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