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Biochemical Investigation of the Effect of Garlic (*Allium sativum*) on 7,12-dimethylbenz[α]anthracene (DMBA) Induced Mammary Cancer in Female *Albino* Rats

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Abstract: The study was designed to investigate the pre and post chemoprotection of garlic on 7, 12-dimethylbenz[α]anthrance (DMBA) induced mammary cancer in female *albino* rats. Animals (hundred rats) where equally divided into five groups, (twenty rats each). Group I: each rat received 1 mL of 0.1% saline daily for twenty days. Group II: received 1 mL of 0.1% saline given orally once a day for twenty days prior to DMBA infusion (30 mg kg^{-1}) and these rats served as controls (cancer control) for group III. Group III : were pre treated with garlic prior to DMBA infusion. Group IV: received 1 mL of 0.1% saline given orally once a day for twenty days after DMBA infusion and these rats served as cancer control for group V. Group V: Post treated with garlic after DMBA infusion. Biochemical measurement were carried out on liver tissues and serum of experimental animals. Circulating levels of lactate dehydrogenase (LDH), Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyrovate Transaminase (GPT) were elevated, while the activities of glutathione-s-transferase (GST) and superoxide dismutase (SOD) and the levels of reduced glutathione (GSH) were reduced in liver tissues in DMBA infused rats as compared to normal. Garlic treatment altered the above mentioned biochemical parameters towards normal values. Present findings indicate that garlic got both pre and post effect on DMBA infused rats, where post treatment is more effective.

Key words: Dimethylbenz[α]anthracine (DMBA), mammary cancer, garlic, liver function, antioxidant enzymes, protective effect

Introduction

The importance of garlic (*Allium sativum*) was recognized many centuries ago in early Egyptian, Chinese and Indian civilizations as a herbal or traditional medicine today in many parts of the world both for prophylaxis and for the cure of variety of diseases including acute and chronic infections, gastritis, dysentery, typhoid fever, cholera, tuberculosis, pneumonia, diabetes mellitus, heart disease and hypertension (Wargovich *et al*., 2006; Gilani and Rahman, 2005).

Prevention of cardiovascular disorders and retardation of hyperglycemia by and antiseptic activities of garlic have been documented by a number of researchers (Borek, 2006; Ahmad and Ahmed, 2006). One of the most important biological effects observed recently with garlic is prevention of cancer which is reported in various experimental systems with mouse and rats (Wargovich *et al*., 2006; Lin *et al*., 2006; Milner, 2006). Diet is an important factor in the development of almost 40% of all human neoplasias (O'Hanlon, 2006; Kraunz *et al*., 2006). Epidemiological studies indicated that

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dietary factors influence the development of breast cancer and experimental analysis suggests that natural or synthetic constituents of diet can act as an anticancer agents to inhibit breast cancer (Wang *et al*., 2006; Veena *et al*., 2006; Garcia-Solis and Aceves, 2005). Garlic (*Allium sativum*), fresh garlic extracts, garlic compounds or synthetically prepared substitutes may be another food item that significantly affects human health (Leelarungrayub *et al*., 2006; Bunowes and Van Houten, 2006; Tattelman, 2005). Although not all epidemiological studies support the theory that consumption of garlic reduces the risk of cancer (Dorant *et al*., 1993; Milner, 1996). Malekzadeh *et al*. (2005) and Bespalov *et al*. (2004) shown that breast cancer risk was decrease as consumption of garlic increase. However, Dorant *et al*. (1995) showed that garlic supplement was not associated with breast cancer. In contrast to epidemiologic studies, laboratory investigations have provided adequate evidence that garlic-containing substances inhibits a variety of chemically-induced tumors in animals (Xiao and Singh, 2006; Park *et al*., 2005). Literature survey revealed that no study have been reported on the chemoprotective effect of pre and post garlic treatment on cancer. Therefore, the present study was initiated to investigate the pre and post effect of garlic treatment on serum and liver tissue parameters in DMBA- induced cancer in female rats.

Materials and Methods

We conducted this study from June to December 2005, in the Laboratories of Medical Technology Department, Applied Science University.

Hundred female spraque-Dawley rats weighing approximately 40 g and 40±5 days old each, were randomly divided into five equal groups designated as group I, II, III, IV and V.

Group I (20 rats): In this group, each rat received 1 mL of 0.1% saline daily for twenty days, they where kept at laboratory conditions till the termination of the experiment. Group II (20 rats): 1 mL 0.1% saline were given orally once a day for twenty days prior to single dose (30 mg kg^{-1} body weight) infusion with DMBA. These rats served as control (cancer control) for group III.

Groups III (20 rats): one milliliter of garlic extract (25 g 100 mL in 0.1% saline) were administered orally once a day for twenty days prior to cancer induction with DMBA infusion.

Groups IV (20 rats): one milliter of 0.1% of saline was given orally twenty days after DMBA infusion and these rats served and cancerous control for group V.

Group V (20 rats): Garlic (same dose as given to group III animals was administered orally once a day for 20 days after 24 h to an oral infusion of DMBA (30 mg kg^{-1} body weight).

All Animals were Given Food and Drinking Water Ad libitum

All the five different groups were sacrificed after hundred days of the commencement of the experiment. Blood was collected in tubes without anticoagulant and the separated serum samples were kept frozen at -30°C. Liver tissues were collected too and kept frozen with normal saline.

Serum samples were used for the assay of GOT, GPT, LDH, while liver tissues were subjected for the estimation of GST, SOD and total, free and protein bound GSH.

Throughout the study, student t-test was used for statistical analysis, a value of $p<0.05$ was considered significant difference.

Results

DMBA Induced Mammary Cancer in Female Rats

Groups II and IV (cancerous groups) showed same pattern and significance of alteration (as DMBA infused rats) compared to normal controlled group, therefore, a reading of only one group is mentioned in Table 1-3 as cancerous group. The serum levels of GOT, GPT and LDH were significantly elevated ($p<0.001$) as compared to control rats (Table 1).

Table 1: Effect of pre and post treatment of garlic on serum LDH, GOT and GPT levels in DMBA induced cancerous rats (Mean±SEM)

			DMBA induced cancer with garlic treatment	
	Normal (10)	DMBA induced cancer (10)	Pre-treated (10)	Post treated (10)
LDH (U mL^{-1})	117.17±12.00	189.00±13.29	160.40±9.14	141.60±7.86
SGOT (IU mL^{-1})	13.55±1.867	49.26±1.581	50.85±1.085	56.06 ±1.03
SGPT (IU mL^{-1})	8.86±1.195	40.89±1.453	42.48±1.011	48.05±1.450

Table 2: The levels of GST and SOD in liver tissues of normal, DMBA induced cancer only and pre and post garlic treated rats (Mean±SEM)

			DMBA induced cancer with garlic treatment	
	Normal (10)	DMBA induced cancer (10)	Pre-treated (10)	Post treated (10)
SOD (Units/mg tissues)	2.640±0.111	1.723±0.026	1.936±0.030	2.233±0.043
SGT (Units/mg protein)	169.30±1.440	109.30±0.732	139.06±0.893	151.58±0.639

Table 3: The liver tissue levels of total, free and protein bound GSH in normal, DMBA induced cancer and cancerous rats with pre and post garlic treated rats (Mean±SEM)

			DMBA induced cancer with garlic treatment	
	Normal (10)	DMBA induced cancer (10)	Pre-treated (10)	Post treated (10)
Total GSH (μ mole/min/g of tissues)	8.613±0.264	6.047±0.245	6.791±0.070	7.265±0.094
Free GSH (μ moles/g of tissues)	1.480±0.061	1.042±0.037	1.157±0.028	1.255±0.033
Protein bound GSH (μ mole/g of tissues)	7.115±0.230	5.054±0.039	5.631±0.059	6.020±0.061

As compared to the normal control values, the activities of GST, SOD and the levels of total, free and protein bound GSH were significantly decreased ($p<0.001$) in liver tissues of DMBA treated rats (Table 2).

Effect of Pre and Post Treatment with Garlic on DMBA Induced Cancer

When animals were (pre) treated with garlic before the induction of cancer with DMBA, the changes recorded in the above mentioned biochemical parameters were less marked than in untreated animals except in the activities of GOT and GPT in serum were elevated further (Table 1-3). Even though the cancer was developed but the conditions of rats were better than those garlic untreated DMBA infused cancerous rats.

Treatment of rats with garlic after DMBA infusion (post treatment) brought about changes in all above parameters to bring these a little closer to the values in control animals except in serum, the activities of GOT and GPT were further elevated as compared to DMBA infused rats (Table 1-3).

The effectiveness of garlic post treatment is more marked on DMBA-induced cancer as compared to pre treatment with garlic.

Discussion

present findings, indicate that garlic has an anticarcinogenic effect apparent in the alteration of the biochemical parameters (LDH, GSH, GST and SOD) towards normal values.

The efficacy of garlic was evaluated in terms of the extent of reversion of mentioned biochemical parameters from untreated DMBA infused toward normal values. Thus the post treatment with garlic was found more effective in preventing DMBA carcinogenesis. The efficacy of various drugs or compounds on inhibition or promotion of experimental cancer was evaluated in terms of number of observed tumors per animal and the rate of tumor development, which have their own limitations

(Kokoska *et al*., 1993; Dunson and Dinse, 2000). On the other hand, in the present study the effect of the indigenous drugs on DMBA induced mammary carcinogenesis is evaluated in terms of altered biochemical parameters in cancer.

The preventive or curative effects of garlic residues in its water and oil soluble organo-sulfur components reported by Aggrawal and Shishodia (2006), which are shown to markedly inhibit tumor growth. The results of Xiao and Singh (2006), Dorai and Aggrawall (2004) and Gunadharini *et al*. (2005) are mainly in agreement with present results.

To explain the mechanism of garlic action, one the following explanations could be a possible answer. The action of these compounds seems to be mediated through alteration of neurohumoral and free radical metabolizing enzymes and thereby changing the levels of neurohumors and free radicals. The increase in glutathione level by garlic treatment could be due to the selenium present in garlic (Sundaram and Milner, 1996). Glutathione treatment (Chinthalapally *et al*., 2001; El-Bayoumi *et al*., 2003; Steele *et al*., 2002) and chemoprevention (Schwartz and Shklar, 1996; Rao *et al*., 2001) or increased levels of glutathione due to selenium treatment (Schwartz and Shklar, 1996) is shown to reduce tumor burden.

Moreover, the antioxidants are also shown to enhance the immune response in tumor (Schwartz *et al*., 1990). Similar to present findings the levels of glutathione and GST have been shown to be enhanced following consumption of garlic or sulfur compounds (Sumiyoshi and Wargovich, 1990). Selenium probably decreased the binding of DMBA metabolites to mammary cell DNA and thus reduce incidence of chemically induced tumor (Liu *et al*., 1991). Similarly, garlic as well because of the selenium content reduces the binding of DMBA metabolites to mammary cell DNA (Liu *et al*., 1992; Amagase and Milner, 1993). The dietary selenium is also reported to enhance the efficacy of garlic as an anti-carcinogenic agent (Peters *et al*., 2006). Garlic powder inhibits the incidence of DMBA induced mammary tumor. However, cancer prevention studies in preclinical models have not revealed any significant differences between the inorganic form of selenium and those naturally occurring forms of selenium (Rao *et al*., 2001; El-Bayoumy *et al*., 2003; Peters *et al*., 2006). The enhanced GST and SOD and reduced glutathione (GSH) levels after treatment with garlic extracts may help in management of H_2O_2 produced by macrophages in responses to altered immune system. The level of LDH, the tumor marker is also decreased towards normal values after garlic treatment (Sheen *et al*., 1999), showing a decreased lactate metabolism. Thus, by garlic treatment both the immune and free radical metabolizing responses are enhanced and the condition of rats is improved, which is also evident by a revert in the level of GST and GSH and by a decrease in the levels of LDH towards normal after treatment, while a further increase in GOT and GPT levels was observed. Similar to present findings, Pal *et al*. (1995) has also reported that some clinical conditions induce increases in serum activities of LDH, GOT and GPT and after treatment the LDH reverted toward normal values with a slight increase in GOT and GPT levels. At the end of the experiment all of the post garlic treated animals were still alive. Thus it can be concluded that garlic may prevent both initiation and promotion of cancer, but its preventive effect on promotion of DMBA carcinogenesis is more marked and because of garlic pharmacological safety, can be used to prevent cancer and in combination with current cancer therapies (Dorai and Aggrawal, 2004). Further study can be done for elucidating the exact mechanism of action and identifying the most active compound in garlic that produces the anticarcinogenic effect.

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