Brazilian Journal of Medical and Biological Research (2004) 37: 1029-1034
ISSN 0100-879X Short Communication

Tomato and garlic by gavage modulate 7,12-dimethylbenz[a]anthracene-induced genotoxicity and oxidative stress in mice

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Abstract

Chemoprotection by dietary agents is a promising strategy for cancer prevention. The aim of the present study was to evaluate the combined effect of tomato and garlic against 7,12-dimethylbenz-[a]anthracene (DMBA)-induced genetic damage and oxidative stress in 12-14-week-old male Swiss albino mice. The animals were randomized into experimental and control groups and divided into eight groups of five animals each. Group 1 animals were injected intraperitoneally with 35 mg/kg body weight DMBA suspended in peanut oil as a single dose. Groups 2-4 animals received tomato (500 mg/kg body weight), garlic (125 mg/kg body weight) and a combination of tomato and garlic for 5 days by gavage, respectively, followed by DMBA 1.5 h after the final feeding. The doses of tomato and garlic correspond to the average human daily consumption. Animals in groups 5, 6 and 7 received tomato alone, garlic alone and tomato + garlic combination, respectively, for 5 days. Group 8 animals received the same volume of water and served as control. The incidence of bone marrow micronuclei and the extent of lipid peroxidation and the concentrations of antioxidants glutathione, glutathione peroxidase and glutathione-S-transferase were measured in the liver, 48 h after DMBA exposure. Increased frequency of micronuclei and enhanced lipid peroxidation accompanied by compromised antioxidant defenses were observed in DMBA-treated animals. Although pretreatment with tomato or garlic significantly reduced the frequency of DMBA-induced bone marrow micronuclei, the combination of tomato and garlic exhibited more profound effect in inhibiting DMBA-induced genotoxicity and oxidative stress. We suggest that a broad spectrum of antimutagenic and anticlastogenic effects can be achieved through an effective combination of functional foods such as tomato and garlic.

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Research supported by the Department of Science and Technology, New Delhi, India.

Received September 12, 2003
Accepted April 8, 2004

Key words
- Antioxidants
- DMBA
- Garlic
- Lipid peroxidation
- Micronuclei
- Tomato
- Lycopene

Environmental factors are recognized to play a major role in the etiology of various cancers that account for over 80% of human malignancies. When cells are exposed to a carcinogenic agent, the carcinogen interacts with DNA directly, or via the formation of reactive oxygen species (ROS), resulting in chromosomal abnormalities and compromised host antioxidant defense mechanisms that eventually culminate in neoplastic transformation (1). It follows that cancer control can be achieved by decreasing the rate of DNA damage and enhancing antioxidant defenses. ROS scavengers have therefore evolved as effective cancer chemopreventive agents. Recently, a great deal of attention has been focused on fruits and vegetables with potent antioxidant properties. In particular, tomato and garlic are recognized to possess a wide range of beneficial effects.

Tomatoes and tomato-based products are rich sources of lycopene, an antioxidant carotenoid reported to be a more stable and potent singlet oxygen quenching agent compared to other carotenoids. Garlic (*Allium sativum* Linn.) used for flavoring and as a medicinal herb for centuries, is known to exhibit antioxidant, antimutagenic and anticarcinogenic effects. Epidemiological studies have provided evidence that an increased intake of tomato and garlic is associated with a decreased cancer risk (2,3). In previous studies from this laboratory, we demonstrated the modulatory effects of tomato and garlic on lipid peroxidation, antioxidant and detoxification systems during chemoprevention of 7,12-dimethylbenz[a]anthracene (DMBA)-induced oral carcinogenesis (4,5).

The current focus of chemopreventive research is on intermediate biomarkers capable of detecting early changes that can be correlated with inhibition of carcinogenic progression. Short-term tests such as the mouse bone marrow micronucleus assay as well as assessment of ROS-induced lipid peroxidation and the status of antioxidants are widely used in the detection and evaluation not only of environmental carcinogens, but also of presumptive antimutagens and anticarcinogens (6,7). The present study was designed to evaluate the effect of oral pretreatment with tomato and garlic alone and in combination on DMBA-induced genotoxicity and oxidative stress. The incidence of bone marrow micronuclei as well as the changes in lipid peroxidation and the status of the antioxidants, reduced glutathione (GSH), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) in the liver were used as intermediate biomarkers of chemoprevention.

The experiments were carried out with male Swiss albino mice aged 12-14 weeks obtained from the Central Animal House, Annamalai University, India. The animals were housed five to a polypropylene cage, with free access to food and water. All animals were fed a standard mouse pellet diet (Mysore Snack Feed Ltd., Mysore, India) and maintained under standard conditions of temperature and humidity with an alternating 12-h light/dark cycle. The animals were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India, and the study was approved by the Ethics Committee of Annamalai University.

Tomatoes and garlic used for the experiment were obtained from the local market. Fresh peeled and deseeded tomatoes were pulped well to a smooth consistency, diluted in water and administered at a concentration of 500 mg solids/kg body weight. An aqueous extract of garlic was prepared by homogenizing the required amount of freshly peeled cloves in an appropriate volume of water to give a concentration of 125 mg solids/kg body weight. The homogenate was centrifuged at 3120 *g* for 10 min to remove the particulate matter and the supernatant fraction was used for the experiment. For the combination, equal volumes of each preparation were mixed and used. The tomato and garlic doses used in the present study corre-

spond to the average daily dietary intake by a person weighing 65-70 kg (2,8).

The animals were randomized into experimental and control groups and divided into eight groups of five animals each. The smaller number of animals used per group is in keeping with the regulations of the Ethics Committee to minimize the number of animals. Animals in group 1 were given DMBA (35 mg/kg body weight) intraperitoneally (9). Animals in groups 2, 3 and 4 received an intragastric administration of tomato (500 mg/kg body weight), garlic (125 mg/kg body weight) and a combination of tomato and garlic (500 + 125 mg/kg body weight), respectively, for 5 days followed by DMBA 1.5 h after the final feeding (8,10). Animals in groups 5, 6 and 7 were given tomato alone, garlic alone and the tomato + garlic combination, respectively, for 5 days. Group 8 animals received the same volume of water and served as control. The animals were sacrificed by cervical dislocation 48 h after DMBA exposure. Both femurs were removed immediately and used for the analysis of micronucleated polychromatic erythrocytes (MnPCEs). The liver tissues were immediately removed and placed in cold 0.9% NaCl solution.

The mouse bone marrow micronucleus test was carried out by the method of Schmid (11) for the evaluation of chromosomal damage in experimental animals. Bone marrow from the femur was flushed in the form of a fine cell suspension into a centrifuge tube containing fetal calf serum. The cell suspension was centrifuged at 500 *g* for 10 min and the supernatant was discarded. The pellet was resuspended in a drop of serum and used for preparing slides. The air-dried slides were stained with May-Grünwald and Giemsa. A total of 2,500 polychromatic erythrocytes were scored per animal from a single slide to determine the frequency of MnPCEs. All slides were scored by the same observer.

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was assayed in the liver tissues by the method of Ohkawa et al. (12). The reaction mixture corresponding to a total volume of 10 ml containing 0.2 ml tissue homogenate, 0.2 ml 8.1% sodium dodecylsulfate, 1.5 ml 20% acetic acid, 1.5 ml distilled water, and a 5-ml n-butanol and pyridine mixture, was heated at 95ºC for 60 min. The pink-colored chromogen formed by the reaction of 2-thiobarbituric acid with the breakdown products of lipid peroxidation was read at 535 nm.

The concentration of GSH was determined by the method of Anderson (13) based on the development of a yellow color when 5,5'-dithiobis(2-nitrobenzoic acid) is added to compounds containing sulfhydryl groups. The reaction mixture contained equal volumes of 4% sulfosalicylic acid and tissue samples homogenized in 4 volumes of ice-cold 0.1 M sodium phosphate buffer, pH 7.4. GPx activity was assayed by the method of Rotruck et al. (14) with modifications. The reaction mixture corresponding to a total volume of 1.0 ml containing 0.2 ml of 0.4 mM sodium phosphate buffer, pH 7.0, 0.2 ml of 0.4 mM EDTA, 0.1 ml of 10 mM sodium azide, and 0.2 ml tissue homogenate was incubated with 0.1 ml H_2O_2 and 0.2 ml GSH for 10 min. The amount of H_2O_2 utilized was determined by estimating GSH content by the method of Anderson (13). GST activity was determined as described by Habig et al. (15). The reaction was started by the addition of 0.1 ml of 30 mM GSH to the reaction mixture containing 2.0 ml of sodium phosphate buffer, 0.1 ml of 30 mM 1-chloro-2,4-dinitrobenzene (CDNB), 0.1 ml tissue homogenate, and 1.7 ml distilled water. The reaction mixture was preincubated at 37ºC for 5 min. The increase in absorbance at 340 nm was read using CDNB as substrate. The protein content was estimated by the method of Lowry et al. (16) using bovine serum albumin as standard.

Data are reported as means ± SD. Bone marrow micronucleus incidence was ana-

lyzed by the Student *t*-test. The data for lipid peroxides and antioxidants were analyzed by analysis of variance (ANOVA) and the group means were compared by the least significant difference (LSD) test. The results were considered statistically significant when $P < 0.001$.

Table 1 summarizes the effect of pretreatment with tomato and garlic alone and in combination on the frequencies of DMBA-induced MnPCEs. A significant increase ($P < 0.001$) in the frequency of MnPCEs was found in DMBA-treated animals (group 1) compared to the untreated control (group 8). Pretreatment with tomato and garlic alone and in combination significantly reduced ($P < 0.001$) the incidence of MnPCEs compared to group 1. However, the combination of tomato and garlic exhibited greater protection (75%) against DMBA-induced bone marrow micronuclei than either agent used alone. The frequency of MnPCEs in animals of groups 5-7 was not significantly different from that observed in group 8.

Figure 1 shows the effect of pretreatment with tomato and garlic alone and in combination on the levels of TBARS, GSH and the activities of GPx and GST in the liver of control and experimental animals. Administration of DMBA (group 1) significantly increased ($P < 0.001$) the extent of hepatic lipid peroxidation and decreased GSH and GSH-dependent enzymes compared to control. Pretreatment with combined dose of tomato and garlic significantly decreased ($P < 0.001$) hepatic lipid peroxidation and enhanced the antioxidant levels compared to group 1. Among groups 5-7, the combination of tomato and garlic significantly decreased lipid peroxidation and increased GSH and GSH-dependent enzymes compared to either agent alone.

DMBA, a member of the polycyclic aromatic hydrocarbons, is present in the environment as a product of incomplete combustion of complex hydrocarbons. Being an indirect carcinogen, DMBA requires metabolic activation to become a carcinogen. DMBA is metabolized by cytochrome P4501A1 in liver microsomes and by cytochrome P4501B1 in primary bone marrow stromal cells to form diol epoxides and other toxic ROS. The toxic metabolites of DMBA, including diol epoxides, are capable of binding to adenine residues of DNA causing chromosomal damage (17).

ROS formed during DMBA metabolism can diffuse from the site of generation to

Table 1. Inhibitory effects of pretreatment with tomato and garlic alone and in combination on DMBA-induced bone marrow micronucleated polychromatic erythrocytes.

Group	Treatment	MnPCEs/2500 PCEs	PCEs/NCEs	% Inhibition
1	DMBA	34.5 ± 2.29*	1.09	–
2	DMBA + tomato (500 mg/kg body weight)	16.5 ± 2.05+	1.16	52
3	DMBA + garlic (125 mg/kg body weight)	17.5 ± 1.19+	1.24	49
4	DMBA + tomato (500 mg/kg body weight) + garlic (125 mg/kg body weight)	8.75 ± 1.63+	1.12	75
5	Tomato (500 mg/kg body weight)	9.4 ± 1.21	1.24	–
6	Garlic (125 mg/kg body weight)	7.8 ± 0.66	1.23	–
7	Tomato (500 mg/kg body weight) + garlic (125 mg/kg body weight)	8.8 ± 1.52	1.26	–
8	Control	9.0 ± 1.14	1.25	–

Data are reported as means ± SD for 5 mice in each group. DMBA = 7,12-dimethylbenz[a]anthracene. MnPCEs = micronucleated polychromatic erythrocytes; NCEs = normochromatic erythrocytes; PCEs = polychromatic erythrocytes. +$P < 0.001$ compared to group 1. *$P < 0.001$ compared to group 8 (Student *t*-test).

other targets within the cells or even propagate the injury outside to intact cells. These ROS produce deleterious effects by initiating lipid peroxidation directly or by acting as second messengers for the primary free radicals that initiate lipid peroxidation (1). Thus, enhanced hepatic lipid peroxidation in DMBA-treated animals may be attributed to excessive generation of ROS exacerbated by decreased efficiency of host antioxidant defense mechanisms.

The liver, which is rich in GSH, supplies it to various extrahepatic tissues via a distinct GSH transport system. GSH maintains the integrity of the liver when the organ is challenged by a wide variety of xenobiotics, ROS and toxic compounds. GSH, in conjunction with GPx and GST, detoxifies reactive intermediate species generated during DMBA metabolism, thereby enhancing resistance against oxidative stress (7). The depletion of GSH and GSH-dependent enzymes in the liver resulting from increased utilization to scavenge lipid peroxides may shift the redox status of the liver towards oxidative stress. Oxidative stress arising due to ROS overproduction coupled with deficiency of host antioxidant defense mechanisms observed in the present study may be an important factor contributing to the increase in bone marrow micronuclei. Mayer et al. (18) have also demonstrated a positive correlation between lipid peroxidation status and genotoxicity as reflected by increased micronucleus formation in lymphocytes. These findings confirm reports by other workers on the clastogenic effects of DMBA (17).

Pretreatment with tomato and garlic effectively reduced the frequency of occurrence of DMBA-induced bone marrow micronuclei as well as the extent of hepatic lipid peroxidation and enhanced the antioxidant status. Moreover, our data demonstrate that the administration of tomato plus garlic exhibited a stronger effect in reducing DMBA-induced genotoxicity and oxidative stress than either agent used alone. This synergistic interaction between tomato and garlic in inhibiting the clastogenic effects of DMBA has been reported recently by Sengupta et al. (19). In this study, concomitant administration of a combined dose of tomato and garlic by gavage was found to attenuate bone marrow chromosomal aberrations induced in Swiss mice by topical application of DMBA. In the present study, pretreatment with tomato and garlic combination exerted significant protective effects against increased fre-

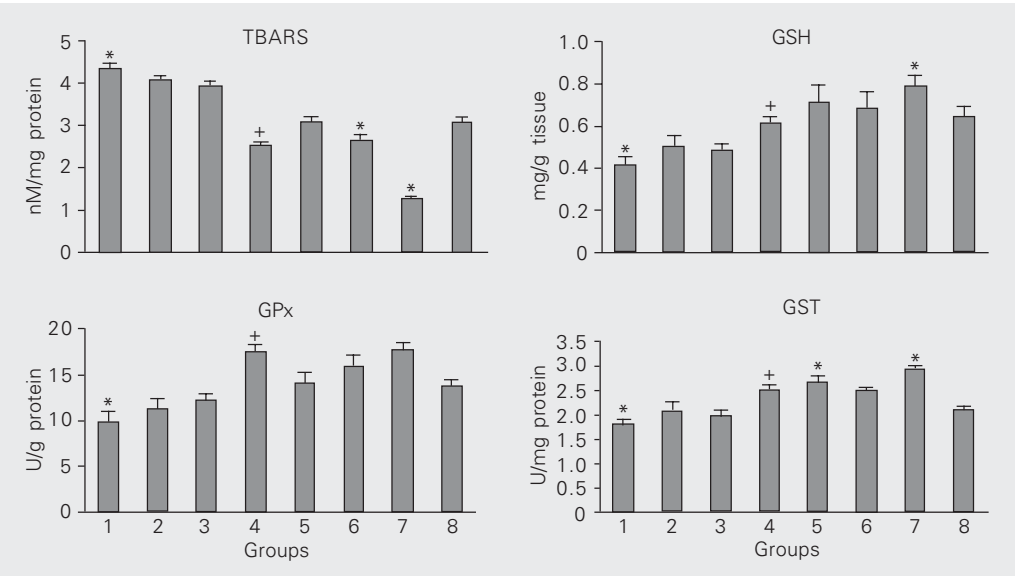

Figure 1. Effect of pretreatment with tomato and garlic alone and in combination on TBARS and GSH levels and on GPx and GST activities in the liver of DMBA-treated mice. Data are reported as means ± SD for 5 mice in each group. CDNB = 1-chloro-2,4-dinitrobenzene; DMBA = 7,12-dimethylbenz[a]anthracene; GPx = glutathione peroxidase (units = µmol GSH utilized/min); GSH = glutathione; GST = glutathione-S-transferase (units = µmol CDNB-GSH conjugate formed/min); TBARS = thiobarbituric acid reactive substances. Group 1 = DMBA; group 2 = DMBA + tomato; group 3 = DMBA + garlic; group 4 = DMBA + tomato + garlic; group 5 = tomato; group 6 = garlic; group 7 = tomato + garlic; group 8 = control. *P < 0.001 compared to group 8; $^{+}$P < 0.001 compared to group 1 (ANOVA followed by LSD test).

quency of bone marrow micronuclei and oxidative stress induced by intraperitoneal injection of DMBA, potentiating the antigenotoxic and antioxidant properties of these agents.

The protective effects of tomato and garlic observed in the present study may be related to the antioxidant and antimutagenic properties of their constituents such as lycopene and organosulfur compounds, respectively. Being lipophilic, lycopene is absorbed in the intestine, taken up by the liver and from there transported to various tissues (2). Lycopene has been reported to protect the liver against T2 toxin by reducing lipid peroxidation and modulating GSH metabolism *in vivo* (20). Recently, we have reported dose-dependent protection of tomato paste against DMBA-induced genotoxicity and oxidative stress in mice as well as the incidence of hamster buccal pouch tumors (4,9). The antigenotoxic and anticarcinogenic effects of garlic and its organosulfur constituent SAC have also been reported by us as well as by others (3,5,9).

The results of the present study clearly indicate that a broad spectrum of antimutagenic and anticlastogenic effects can be achieved through an effective combination of functional foods such as tomato and garlic which would be more cost effective than supplementation of individual antioxidants for protection against oxidative stress.

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