

Original Article

Garlic exerts hepatoprotective effects during 4-nitroquinoline 1-oxide-induced oral carcinogenesis in rats

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The modifying effects of garlic (*Allium sativum* Linn.) on hepatic lipid peroxidation and antioxidant status during 4-nitroquinoline 1-oxide (4NQO)-induced oral carcinogenesis were investigated in male Wistar rats. Enhanced lipid peroxidation in the liver of tumour-bearing animals was accompanied by significant decreases in the activities of glutathione peroxidase (GPx), glutathione S-transferase (GST) and reduced glutathione (GSH) levels. Administration of aqueous garlic extract significantly lowered lipid peroxidation and enhanced the levels of hepatic antioxidants and detoxifying enzymes. The results of the present study suggest that garlic exerts its hepatoprotective effects by influencing hepatic biotransformation enzymes and antioxidants.

Keywords: antioxidants, chemoprevention, garlic, glutathione, lipid peroxidation, liver, 4-nitroquinoline 1-oxide, oral cancer.

Introduction

Epidemiological and experimental studies have shown that increased consumption of fruits and vegetables is associated with decreased cancer risk.¹ Modification by dietary agents has therefore evolved as a cost-effective approach to control the incidence of cancer. Garlic (*Allium sativum* Linn.) has been used as a spice and medicinal herb for centuries. Garlic and its constituents have come under extensive study in light of their anticancer effects both *in vitro* and *in vivo*.² Previously, we demonstrated the inhibitory effects of garlic against 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis and N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric carcinogenesis.^{3–5}

The oral cavity is an excellent target for chemoprevention studies due to its easy accessibility for examination and follow-up of the lesions. Oral squamous cell carcinomas induced by 4-nitroquinoline 1-oxide (4NQO) in rats, which show morphological and histological similarities to human oral tumours, have been extensively used to test a wide variety of synthetic and natural agents for chemopreventive potential.⁶ Previously, we demonstrated the chemopreventive potential of neem leaf and turmeric against 4NQO-induced oral carcinogenesis.⁷

The liver of tumour-bearing animals has evolved as a reliable model for studying malignant transformation and intervention by chemopreventive agents. Chemopreventive agents are known to intercept quantitative changes in hepatic enzymes and metabolites induced by the presence of an extrahepatic tumour.⁸ In previous reports from this laboratory, the importance of host liver changes in monitoring the chemopreventive potential of plant products in experimental oral and gastric carcinogenesis has been demonstrated.^{9–12}

The present study was undertaken in order to investigate the effect of garlic on hepatic lipid peroxidation, reduced glutathione (GSH) and the GSH-dependent enzymes glutathione

peroxidase (GPx) and glutathione S-transferase (GST) during 4NQO-induced oral carcinogenesis.

Materials and methods

Animals

All the experiments were carried out with male Wistar rats aged 6–8 weeks and obtained from the Central Animal House, Annamalai University, India. They were housed six to a polypropylene cage and provided with food and water *ad libitum*. The animals were maintained in a controlled environment under standard conditions of temperature and humidity with an alternating light/dark cycle. All animals were fed standard pellet diet (Mysore Snack Feed, Mysore, India). The animals used in the present study were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India and approved by the ethical committee of Annamalai University.

Chemicals

4NQO was obtained from Fluka-Chemika-Biochemika, Buchs, Switzerland. All other reagents used were of an analytical grade.

Preparation of garlic extract

An aqueous extract of fresh garlic was prepared by homogenizing the required amount of freshly peeled cloves in an appropriate volume of double distilled water to give a concentration of 25 mg/mL.¹³ The homogenate was centrifuged

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at 3120 g for 10 min in order to remove the particulate matter and the supernatant fraction was used for the experiment. At this stage of preparation, 96% of the extract remained.

Treatment schedule

The animals were randomized into experimental and control groups and divided into five groups of six animals each. At 7 weeks of age, animals in groups 1–3 were given 20 p.p.m. 4-NQO in drinking water for 8 weeks.¹⁴ Group 1 received no other treatment. Group 2 animals were intragastrically administered aqueous garlic extract at a dose of 250 mg/kg bodyweight starting at 6 weeks of age until 1 week after the final exposure to the carcinogen and were switched to the basal diet and maintained on this diet for 22 weeks. Group 3 animals received garlic extract as in group 2 starting 1 week after the cessation of 4NQO treatment and continued for 22 weeks. Group 4 animals received garlic extract for 32 weeks. Group 5 animals were given a basal diet and tap water throughout the experiment and served as the untreated control.

The experiment was terminated at the end of 32 weeks and all animals were sacrificed by cervical dislocation after an overnight fast. Fresh tissues were used for estimations.

Estimations

Thiobarbituric acid reactive substances (TBARS) released from endogenous lipid peroxides reflecting the lipid peroxidation process were assayed in tissues as described by Ohkawa *et al.*¹⁵ Reduced glutathione was determined by the method of Ellman.¹⁶ Glutathione peroxidase activity was assayed by following the utilization of hydrogen peroxide according to the method of Rotruck *et al.*¹⁷ The activity of glutathione S-transferase was determined by the method of Habig *et al.* using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate.¹⁸ Tissue protein was estimated by the method of Lowry *et al.*¹⁹

Statistical analysis

Statistical analysis on the incidence of lesions was performed using Fisher's exact probability test. The data for TBARS, antioxidants and detoxifying enzymes were analysed using analysis of variance (ANOVA) and the group means were compared by Duncan's multiple range test (DMRT). Values were considered statistically significant when $P < 0.05$.

Results

The incidence of oral neoplasms and preneoplastic lesions in different groups is shown in Table 1. In group 1, the incidences of squamous cell carcinoma and squamous cell papilloma were 80 and 100%, respectively, whereas in group 3 the

incidence of squamous cell carcinoma and squamous cell papilloma was 16%. No malignant neoplasms and pre-malignant lesions were observed in rats in groups 4 or 5.

Table 2 indicates the extent of lipid peroxidation as evidenced by the formation of TBARS and glutathione concentration, as well as the activities of GPx and GST in the liver of control and experimental animals. Lipid peroxidation levels in group 1 were significantly higher than those of group 5. In group 4, the levels were significantly decreased compared with groups 1 and 5. Glutathione and glutathione-dependent enzymes in liver tissues were markedly decreased in group 1 compared with group 5. The levels in groups 2 and 3 were increased compared with group 1. In group 4, the levels were significantly increased compared with group 5.

Discussion

Hepatic metabolism of carcinogens plays a key role in extrahepatic carcinogenesis. 4NQO, the carcinogen used in the present study, has been reported as being metabolized in the liver in addition to the tongue.²⁰ 4NQO undergoes metabolic activation to form 4-hydroxyaminoquinoline 1-oxide, which forms adducts with DNA.²¹ Enhanced lipid peroxidation in the livers of rats bearing oral tumours reflects excessive generation of free radicals during 4NQO metabolism exacerbated by decreased efficiency of host antioxidant defense mechanisms.

The liver plays a major role in the interorgan homeostasis of GSH, the major cellular non-protein thiol, and supplies it to extrahepatic tissues.²² GPx utilizes GSH as a substrate to catalyse the reduction of organic hydroperoxides and hydrogen peroxide. Glutathione S-transferase, a multigene family of detoxification enzymes, catalyse the binding of electrophiles with GSH.²³

Glutathione, in conjunction with GPx and GST, plays a crucial role in maintaining the integrity of the liver when challenged by toxic agents. Hepatic GSH depletion has been reported to enhance lipid peroxidation.²⁴ 4NQO-induced depletion of GSH and the GSH-dependent enzymes, GPx and GST may shift the redox status of the liver with consequent adverse effects on critical sulfhydryl groups of hepatic functional proteins. Previous studies have shown a decrease in the levels of GSH and GPx during neoplastic transformation and under conditions of excessive generation of lipid peroxides.^{9–12} Our results corroborate these findings.

Administration of garlic reversed the changes induced by 4NQO, supporting the hypothesis that dietary anticarcinogens are effective chemopreventive agents. Garlic has been reported to inhibit lipid peroxidation and enhance GSH levels and GST activity.^{10,12} Garlic has been reported to protect

Table 1. Incidence of preneoplastic and neoplastic lesions

Group	Treatment	No. rats examined	Precancerous lesions		Squamous cell papilloma	Squamous cell carcinoma
			Hyperplasia	Dysplasia		
1.	4NQO	6	6 (100)	6(100)	6 (100)	5 (80)
2.	4NQO + garlic (initiation)	6	2 (32) ^a	0	0	0
3.	4NQO → 74 garlic (postinitiation)	6	2 (32) ^a	2 (32) ^a	1 (16) ^a	1 (16) ^a
4.	Garlic	6	0	0	0	0
5.	Control	6	0	0	0	0

^aSignificantly different from group 1 by Fisher's exact probability test, $P < 0.05$. Parentheses represent percentage of lesions.

Table 2. Lipid peroxidation and antioxidant status in liver of control and experimental animals (mean \pm SD; $n = 6$)

Group	Treatment	TBARS (nmol/100 mg protein)	GSH (U ^A)	GPx (U ^B)	GST (U ^C)
1.	4NQO	201.3 \pm 10.1 ^a	1.21 \pm 0.12 ^a	7.3 \pm 0.9 ^a	1.31 \pm 0.06 ^a
2.	4NQO + garlic (initiation)	153.4 \pm 9.8 ^{ab}	1.62 \pm 0.14 ^{ab}	12.1 \pm 1.2 ^{ab}	1.72 \pm 0.07 ^{ab}
3.	4NQO \rightarrow garlic (postinitiation)	166.6 \pm 8.9 ^{ab}	1.48 \pm 0.11 ^{ab}	9.8 \pm 1.4 ^{ab}	1.54 \pm 0.11 ^{ab}
4.	Garlic	106.3 \pm 11.1 ^{ab}	2.46 \pm 0.13 ^{ab}	19.2 \pm 1.1 ^{ab}	2.93 \pm 0.09 ^{ab}
5.	Control	136.2 \pm 10.3	1.82 \pm 0.16	14.3 \pm 1.03	1.98 \pm 0.10

a, As compared with group 5, $P < 0.05$ (Duncan's multiple range test); b, as compared with group 1, $P < 0.05$ (Duncan's multiple range test); A, mg/g tissue; B, μ mol of glutathione (GSH) utilized/min/g protein; C, μ mol of 1-chloro-2,4-dinitrobenzene-GSH conjugate/min/mg protein.

hepatocytes against carbon tetrachloride-induced liver injury.²⁵

Despite the small sample size, the results of the present study validate the role of garlic as a putative dietary anticarcinogen as it mitigates the effects of diverse carcinogens. We feel that garlic may exert its chemopreventive effects by influencing hepatic biotransformation enzymes and antioxidants. This can alter cancer development at extrahepatic sites. However, further studies are required before establishing the chemopreventive potential of such naturally occurring dietary constituents against experimentally induced oral, as well as other, tumours. Identification of such naturally occurring dietary anticarcinogens will serve as new tools for malignancies.

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