Original Article

Protection of *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine-induced *in vivo* clastogenicity by aqueous garlic extract

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The modulatory effects of garlic extract on the *in vivo* clastogenicity of *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG), a carcinogenic nitrosamine, were evaluated by quantification of micronuclei and chromosomal aberrations in metaphase cells from the bone marrow of male Wistar rats. A single intraperitoneal injection of MNNG (40 mg/kg bodyweight) was found to be clastogenic as revealed by the increased frequency of micronucleated polychromatic erythrocytes and chromosomal aberrations. Pretreatment with aqueous garlic extract (250 mg/kg bodyweight) for 5 days significantly reduced the frequencies of MNNG-induced micronuclei and chromosomal aberrations. The results demonstrate that administration of garlic extract protects against the clastogenic effects of MNNG.

Key words: chemoprevention, chromosomal aberrations, clastogen, garlic, gastric cancer, India, micronuclei, *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG).

Introduction

Gastric cancer, one of the most common malignant neoplasms worldwide, is a major cause of morbidity and mortality in India.[1] Chemoprevention provides a practical approach to identifying potentially useful inhibitors of stomach cancer development.[2] Accumulating evidence supports the hypothesis that several naturally occurring dietary constituents may offer chemoprotection during experimental gastric carcinogenesis induced by *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG).[3–5]

Garlic (*Allium sativum* Linn.), used by humans for centuries as a spice and medicinal herb, has come under extensive study in the light of its anticancer effects both *in vitro* and *in vivo*.[6,7] In particular, garlic is known to inhibit infection by *Helicobacter pylori*, an important aetiological agent in gastric carcinogenesis.[8] Recently, we demonstrated the inhibitory effects of garlic on 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis and MNNG-induced rat forestomach carcinogenesis using biochemical end-points.[9–11]

The current focus of chemoprevention is on biomarkers capable of detecting early subtle changes that can be correlated with carcinogenic progression as well as reversal. Cytogenetic biomarkers have assumed significance as reliable, early indicators of the biological effects of carcinogen-induced DNA damage owing to the strong association between specific chromosomal alterations and tumorigenesis.[12] We therefore evaluated the effects of garlic on MNNG-induced genetic damage by quantification of micronuclei and chromosomal aberrations as cytogenetic end-points of chemoprevention.

Materials and methods

Animals

All the experiments were carried out with male Wistar rats aged 7–8 weeks obtained from the Central Animal House, Annamalai University, India. The animals were housed in polypropylene cages and provided with food and water ad libitum. They 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, Annamalai University.

Chemicals

N-Methyl-*N′*-nitro-*N*-nitrosoguanidine was obtained from Fluka-Chemika-Biochemika, Buchs, Switzerland. All other reagents used were of analytical grade.

Preparation of garlic extract

An aqueous extract of fresh garlic was prepared by homogenising the required amount of freshly peeled cloves in an

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appropriate volume of double distilled water to give a concentration of 25 mg/mL.[13] The homogenate was centrifuged at 3120 *g* for 10 min to remove the particulate matter and the supernatant fraction was used for the experiment. At this stage of preparation, 96% of the extract was remaining.

Treatment schedule

The animals were randomised into control and experimental groups and divided into four groups of 10 animals each. Animals in group 1 were injected with MNNG (40 mg/kg bodyweight) intraperitoneally.[14] Group 2 animals received 250 mg/kg bodyweight garlic extract by intragastric intubation for 5 days followed by injection of MNNG (40 mg/kg bodyweight) 90 min after the final feeding. Rats in group 3 were given garlic extract alone for 5 days. Group 4 received the same amount of distilled water and served as control. All animals were injected colchicine 90 min prior to death. The animals were killed by cervical dislocation 27 h after carcinogen exposure.

Cytogenetic analysis

The micronucleus test was carried out according to the method described by Schmid.[15] The bone marrow from the femurs 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 1000 polychromatic erythrocytes were scored per animal to determine the frequency of micronucleated polychromatic erythrocytes (MnPCE).

Bone marrow cells from control and experimental animals were processed for analysis of chromosomal aberrations by the method of Sharma and Sharma.[16] The bone marrow from the femurs was flushed into a centrifuge tube containing 0.9% saline and centrifuged at 500 *g* for 5 min. The supernatant was removed and hypotonic KCl was added to the sediment. After incubation for 20 min at 37°C, the contents were centrifuged for 5 min and the sediment was fixed in methanol–acetic acid (3:1 v/v). Three changes of fixative were given prior to slide preparation. The slides were air-dried, stained in Giemsa solution and scored blindly. One hundred well-scattered metaphase plates were scored for each animal, giving a total of 1000 metaphases per group.

Statistical analysis

The data were transformed using Arcsine table and analysed by two-way analysis of variance (ANOVA) followed by Neuman–Keuls' multiple range test to compare the significance of differences among the different experimental sets.

Results

The data presented in Table 1 show the influence of pretreatment with garlic on the frequency of MnPCE induced by MNNG. A significant increase in the frequency of MnPCE was observed in group 1 compared with group 4. Pretreatment with garlic extract significantly reduced the frequency of MnPCE induced by MNNG.

Table 2 shows the influence of pretreatment with garlic on the frequency of chromosomal aberrations induced by MNNG. A marked increase in the frequency of chromosomal aberrations was observed in group 1. Pretreatment with garlic extract significantly reduced MNNG-induced chromosomal aberrations. Metaphases from bone marrow cells showing normal chromosomes and different types of chromosomal aberrations are presented in Fig. 1.

Discussion

N-Nitroso compounds, the predominant aetiological agents in gastric carcinogenesis are genotoxic agents that induce gastric mucosal mutagenesis leading to intestinal metaplasia, dysplasia and finally carcinoma.[17] The nitroso compound, MNNG, a potent gastric carcinogen, has been widely used as a mutagen.[14,18] It been found to induce single- and double-strand breaks in DNA.[19] Free radicals, primarily hydroxyl radicals, have been suggested to play a key role in the mutagenic effects of MNNG. The enhanced frequencies of micronuclei and chromosomal aberrations in MNNG-treated animals in the present study confirm reports by other workers on the clastogenic effects of MNNG.[20,21]

Table 1. Effect of pretreatment with garlic extract on MNNG-induced micronucleated polychromatic erythrocytes (MnPCE) in rats

Group	Treatment	MnPCE/1000 PCE† Mean ± SEM
1	MNNG	45.3 ± 5.6*
2	MNNG + Garlic	21.4 ± 2.5* **
3	Garlic	16.5 ± 2.2*
4	Control	16.1 ± 2.6

MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. †Transformed values are presented as mean ± SEM for groups of 10 rats.
A total of 1000 cells were counted/animal and averaged over the number of animals. Values are statistically significant at $P < 0.05$.
*Significantly different from group 4; **significantly different from group 1.

Table 2. Effect of pretreatment with garlic extract on MNNG-induced chromosomal aberrations in rats

Group	Treatment	Types of aberrations							Chromosomal aberrations† Mean ± SEM
		G′	G″	B′	B″	F	M	Total	
1	MNNG	9	11	14	12	116	6	168	16.8 ± 1.2*
2	MNNG + Garlic	10	8	11	6	40	4	79	7.9 ± 0.9* **
3	Garlic	6	5	6	2	35	2	56	5.6 ± 0.6**
4	Control	9	9	6	4	28	3	59	5.9 ± 0.7

G′, chromatid gap; G″, isochromatid gap; B′, chromatid break; B″, chromosome gap; F, fragment; M, minute; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.
†Transformed values are presented as mean ± SEM for groups of 10 rats. A total of 1000 metaphases from six animals per treatment were analysed.
Values are statistically significant at $P < 0.05$. * Significantly different from group 4; ** Significantly different from group 1.

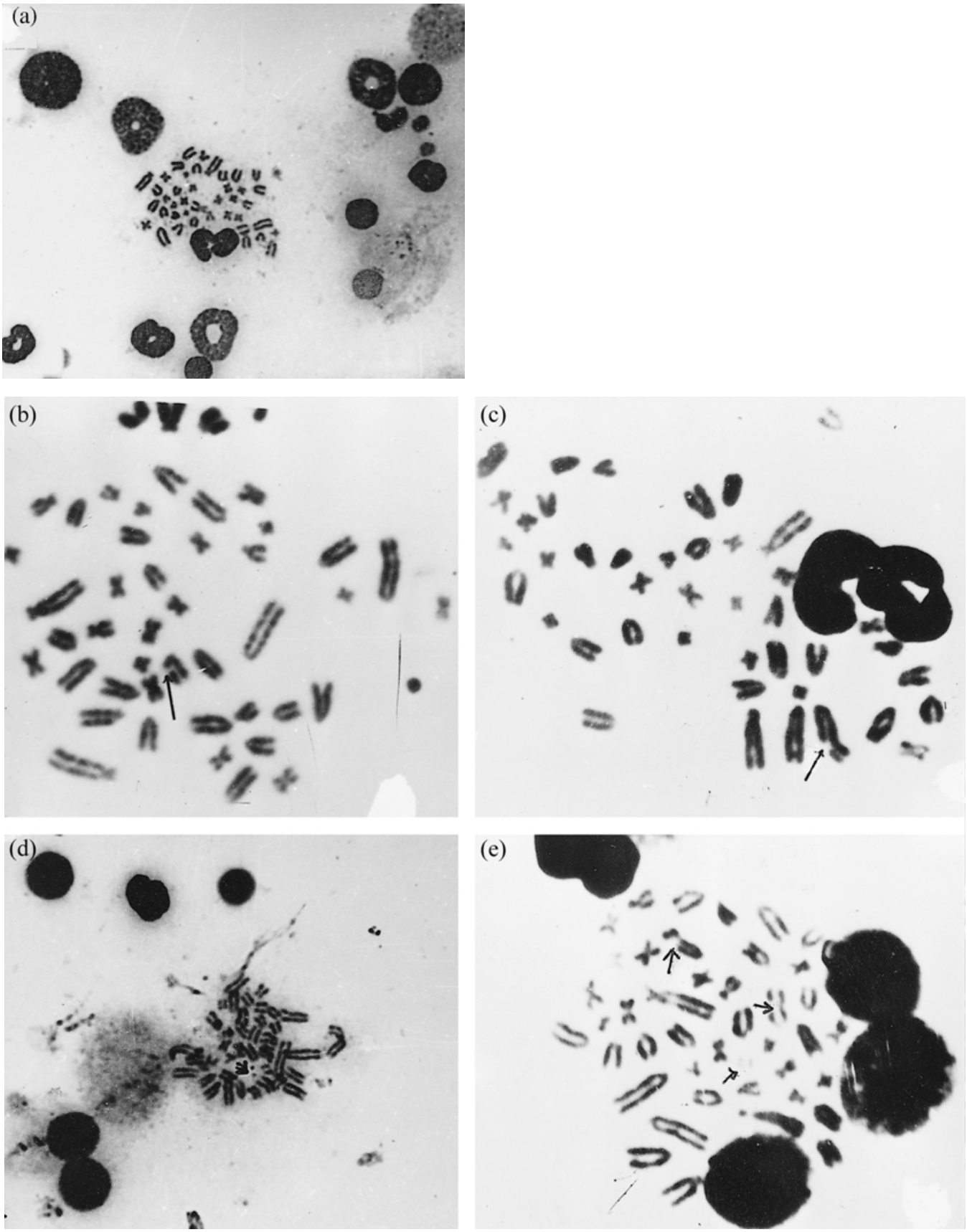

Figure 1. Metaphases from bone marrow showing normal chromosomes, and different types of chromosomal aberrations. (a) Normal chromosomes; (b) chromatid gap (arrow); (c) chromatid break (arrow); (d) double minutes (double arrow); (e) multiple aberrations (gaps, breaks, fragments).

Pretreatment with garlic significantly reduced the clastogenic potency of MNNG. The antimutagenic activities of garlic and its allium constituents have been demonstrated against γ-radiation, sodium arsenite and other clastogens.[13,22,23] Allyl sulfides, important constituents of garlic, were found to exhibit antigenotoxic effects against a variety of carcinogens. Diallylsulfide has been reported to reduce nuclear aberrations induced by MNNG.[24]

The protective effects of garlic on MNNG-induced genotoxicity may be related to its anti-oxidant properties. Recently, we demonstrated that the chemopreventive potential of garlic against MNNG-induced oxidative stress is mediated by its free radical scavenging effects.[25] Garlic contains a number of compounds that are recognised as scavengers of electrophilic intermediates of carcinogenesis including vitamin C and organosulfur compounds such as *S*-allylcysteine. Most notably, vitamin C which plays an important role in inhibiting the formation of *N*-nitroso compounds is known to ameliorate the mutagenic potency of MNNG.[26,27]

The results of the present study indicate that administration of garlic has significant protective effects against MNNG-induced chromosomal damage. These findings strengthen the observation that naturally occurring compounds of plant origin have inhibitory effects on chemical mutagenesis and carcinogenesis.[28] However, further studies are necessary before firm conclusions can be drawn on the mechanism of action of these agents.

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