

Full Length Research Paper

Garlic extract prevents chromium induced cytogenetic damage in somatic cells of mice

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The protective effect of garlic extract in chromium induced genotoxicity was evaluated using analysis of chromosomal aberrations in somatic cells of mice in *in vivo* animal model. Three doses of garlic extract were selected for modulation and were administered to animals after priming with chromium. All the animals were killed after 48 hrs of treatment and the mitotic preparations were made in somatic cells of mice. A significant decrease was observed in the percentage of chromosomal aberrations when animals primed with garlic extract. The present results clearly indicate the protective nature of garlic extract against heavy metal genotoxicity.

Keywords: Chromium, Chromosomal aberrations, Protection, Garlic extract.

INTRODUCTION

Garlic is readily available medicinal herb known for its health benefits. It has a wide range of medicinal properties like antiviral, antifungal, antihelmintic, anti-inflammatory, antidote, anticancer, antimutagenic, hepatoprotective and immunomodulation etc. (Banerjee et al., 2003; Khanum et al., 2004). Recent studies have shown the antigenotoxic and antimutagenic effects of garlic for various drugs and chemicals (Shukla and Taneja, 2002; Bhuvaneswari et al., 2004, Siddique and Afzal, 2005; Belloir et al., 2006). Studies of the anticarcinogenic effects of garlic on several carcinogens were found to be effective in different ways such as direct inhibition of tumor cell metabolism, inhibition of initiation and promotion phases of carcinogenesis and modulating the post immune response and besides all these garlic acts as a strong antioxidant by its ability to scavenge free radicals, (Wei and Law, 1998). Sulfur rich constituents of garlic such as Diallyl Sulfide (DAS) and Diallyl Disulfide (DADS) are known to induce activities of phase II enzymes, which in turn reduce the genotoxicity of several carcinogens (Guyonet et. al., 2002).

Elemental chromium was first discovered and characterized by a French chemist Nicolas-Louis vanquelin in Siberian red lead ore Crocoites in 1797

(Katz et al 1993, Costa et al 2008) It is naturally elements in crustal abundance and is found virtually in all phases including air, water, soil and biota. The major industries using chromium are the metallurgical, chemical and refractory brick industries (Losi et al, 1994). When animals administered with garlic extract (GE) the percentage of chromosomal aberrations were found to be slightly higher than controls. Such results indicate that garlic extract is non-mutagenic in somatic cells of mice.

Major uses of hexavalent chromium compounds include metal plating, manufacture pigments and dyes, corrosion inhibitors chemical synthesis, refractory production leather tanning and wood preservation (Blade et al, 2007, Arun et al 2005). Chromium has been studied for its potential genotoxicity in rats and mice (Devi et al, 2001) in chromium exposed population (Wu et al, 1996). It induced DNA damage by suppressing both DNA replication and transcription. It induces tumor in experimental animals and chromosomal aberrations, sister chromatid exchanges, cell transformations, gene mutations in mammalian cell cultures (Tsou et al, 1996). Chromium and its intermediates interact directly with DNA to form DNA complexes that results in DNA protein and DNA amino acids crosslink (Gibbs et al, 2000). Workers occupationally exposed to chromium are considered to be an elevated risk for developing cancer through Inhalation (De Flora et al, 2000, Aggarwal et al, 2003).

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Table 1. Classification of various types of chromosomal aberrations recorded in somatic cells of mice analyzed after 48th hr treatment with various doses of garlic.

Dose mg/kg and duration	Structural aberrations				Numerical aberrations		Total No. of aberrations (%)
	Gaps	Breaks	Fragments	Exchanges	Polyploidy	Chromatid separations	
48 hrs							
Control II	3	6	1	1	0	2	10
	(0.60)	(1.20)	(0.20)	(0.20)	(0.00)	(0.40)	(2.00)
150 mg/kg	4	7	1	1	2	2	11
	(0.80)	(1.40)	(0.20)	(0.20)	(0.40)	(0.40)	(2.20)*
200 mg/kg	5	8	2	1	3	2	13
	(1.00)	(1.60)	(0.40)	(0.20)	(0.60)	(0.40)	(2.60)*
250mg/kg	5	8	3	1	3	2	14
	(1.00)	(1.60)	(0.60)	(0.20)	(0.60)	(0.40)	(2.80)*

*P>0.05

Gaps and polyploids are not included in total aberrations.

The values in parenthesis are percentages.

MATERIALS AND METHODS

Animal treatment

A study was conducted after taking the approval of Institutional Ethical Committee on twenty adult male swiss albino mice 30 to 50days old and weighing around to 30 to 40 g were maintained in plastic cages under controlled lighting conditions (12:12 light and dark cycle) relative humidity (50±5%) and temperature (37±2°C) fed with mice feed and were given ad libitum access to water. A group of 5 mice per experiment were taken and treated with Chromium and garlic extract. The doses were prepared daily in distilled water and were administered by gastric gavage method for PEF and 26G needle intraperitoneal injection for ADR treatment.

Preparation of garlic extract

Fresh garlic bulbs (*Allium Sativum*), were obtained from the local market and dried. The dried part of the bulb was made in to coarse powder with motor and pestle. The powder (250g) was soaked in 500 ml of ethanol for 48 hrs. The solvent was runned through rotavapour to separate solvent and concentrated through Soxhlet apparatus. The obtained extract was run through rotavapour for futhur concentration. Final extract was lyophilized to powder and stored at 4°C until use (Khalid and Al- Numair, 2009). The dose was selected based on therapeutic concentration, after thorough review of litterateur and mortality tests conducted in our laboratory. Hence doses in the range of 150, 200 and 250 mg/kg of garlic extract were selected in the present study.

Analysis of chromosomal aberrations in somatic cells of mice

The animals were killed two days after administration of the last dose. The bone marrow was flushed into clean glass Petri dishes with hypertonic solution (0.56% KCl) were used to get a homogeneous cell suspension. It was then collected in clean centrifuge tubes and incubated at 37°C for 45 minutes. Four slides for each were prepared from control and experimental animals. The staining was done within 48 h of preparation according to the method Preston et al, 1981. The slides were screened for 50 well spread metaphases per animal for the presence of various types of chromosomal aberrations like gaps breaks, fragment, chromatid separations and polyploids in control and treated group of animals. The differences in the frequencies of chromosomal aberrations between control and treated groups were analyzed using Chi-Square test. For calculating mitotic index (MI) a minimum of 1000 cells were counted for each animal.

Treatment

For genotoxic studies

The animals were divided into ten groups and were administered orally with different doses of garlic extract in split doses. The control I and II were fed intra peritoneal with 1 ml of physiological saline. 5 animals were kept for each concentration. The control and treated group of animals were sacrificed after 48hr for mitotic preparations. The schedule of the experiments is as follows:

Group 1 mice – control – I

Table 2. Classification of various types of chromosomal aberrations in somatic cells of mice analysed after 48th hr chromium treated animals primed with various doses of garlic.

Dose (mg/kg) and duration of treatment 48 h	Structural aberrations				Numerical aberrations		Total no. of aberrations (%)
	Gaps	Breaks	Fragments	Exchanges	Polyploidy	Chromatid separations	
Control II	3 (0.60)	5 (1.00)	0 (0.00)	0 (0.00)	1 (0.20)	3 (0.60)	8 (1.60)
Mytomyacin c	9 (1.80)	27 (5.40)	5 (1.00)	3 (0.60)	13 (2.60)	15 (3.00)	50 (10.00)
60 mg/kg	18 (3.60)	24 (5.80)	6 (1.20)	3 (0.60)	2 (0.40)	7 (1.40)	40 (8.00)
60+150 mg/kg	8 (1.60)	18 (3.60)	5 (1.00)	2 (0.40)	2 (0.40)	11 (2.20)	36 (7.20)*
60+200 mg/kg	8 (1.60)	16 (3.20)	4 (0.80)	2 (0.40)	1 (0.20)	10 (2.00)	32 (6.40)*
60+250 mg/kg	8 (1.60)	15 (3.00)	3 (0.80)	2 (0.40)	1 (0.20)	6 (1.20)	27 (5.40)*

*P<0.05

Gaps and polyploids are not included in total aberrations. The values in parenthesis are the percentages.

Group 2 mice – 150mg/kg

Group 3 mice – 7.5mg/kg

Group 4 mice – 10mg/kg

For modulating studies

In the present study the highest dose of 60mg/kg was selected genotoxicity. Garlic extract were given in split doses orally for 7 consecutive days and 60mg/kg of chromium was given on the 7th day, one hour after regular exposure to antimutagen as a single intraperitoneal dose. 5 animals were kept for each concentration. Control group of animals were also maintained simultaneously as:

Group 1 mice – control – I

Group 2 mice – 70mg/kg mitomycin C

Group 3 mice – 60mg/kg of chromium

Group 4 mice – 60+150mg/kg

Group 5 mice – 60+200mg/kg

Group 6 mice – 60+250mg/kg

RESULTS AND DISCUSSION

The results clearly indicate that there was a gradual increase in the percentage of various types of chromosomal aberrations with dose and duration of exposure but they are insignificant when compared with the control group. At 48th exposure the percentage of total chromosomal aberrations was 3.40, 4.00 and 4.40, after the administration of 150, 200 and 250 mg/kg of garlic respectively as against 2.60 in control. The above

findings clearly indicate that there was no significant increase in the frequency of chromosomal aberrations in somatic cells of garlic treated mice when compared to controls. So it is clear indication that garlic does not exhibit any mutagenic effects in somatic cells of mice may be due to the presence of allicin in garlic which is responsible for its antioxidant property.

Our results are comparable with that of Abraham and Kesavan (1984) who reported the genotoxic effects of orally administered garlic in bone marrow cells of mice by performing the micronucleus test. Results of the micronucleus test with garlic were not significantly different from control values. In another study Nakagawa et al (1980) studied the acute toxicity test of garlic extract in wistar rats and mice.

The antioxidant nature of garlic has been attributed to the presence of organosulphur compounds such as S-allylcysteine, diallylsulphide, allylmethylsulphide, S-methylcysteine (Wei and Lau, 1998). These volatile compounds are generally considered to be responsible for most of the pharmacological properties of garlic (Chandra Mohan et al, 2004). Imai et al 1994 reported the antioxidant properties of three garlic preparations and organosulfur compounds in garlic. Among the variety of organosulfur compounds, S-allylcysteine and S-allylmercaptocysteine, found in aged garlic extract, showed radical scavenging activity in both chemiluminescence and 1,1-diphenyl-2-picrylhydrazyl assays, indicating that these compounds may play an important role in the antioxidative activity of aged garlic extract.

The results showed that there was a gradual decrease in the frequency of various types of chromosomal

aberrations with increasing dose and time intervals in somatic cells of chromium+garlic treated animals. Thus as a result of various type of chromosomal aberrations the percentage so total chromosomal aberrations at 24h exposure to chromium+ garlic were 2.20 in control and were 9.60 in 60mg/kg of chromium treated animals to 8.40, 7.00 and 5.60 of chromium +garlic treated animals respectively. Thus as a result of various types of chromosomal aberrations the percentages of total chromosomal aberrations at 48h exposure to chromium+garlic were 2.40 in control and were 12.00 in 60mg/kg of chromium treated animals to 10.20, 8.20 and 7.20 of chromium+garlic treated animals respectively.

The present results are comparable with that of Das et al, 1993. Male laboratory-bred Swiss albino mice were given one of three concentrations of the freshly prepared extract (100mg, 50mg, and 25mg/kg body weight) as a dietary supplement by gavage for 6 consecutive days. On the seventh day the mice were administered a single acute dose of two known clastogens, mitomycin C (1.5mg/kg) and cyclophosphamide (25 mg/kg) or sodium arsenite (2.5 mg/kg), simultaneously with garlic extract. After 24hr, chromosome preparations were made from the bone marrow cells. The endpoint studied was chromosomal aberrations and damaged cells. Garlic extract alone induced a low level of chromosomal damage. The clastogenicity of all three mutagens were reduced significantly in the animals which had been given garlic extract as dietary supplement.

Previous studies have shown that the anticlastogenic properties of two dietary supplements, garlic and mustard oil, were screened against the clastogenic activity of sodium arsenite, since diet may contain factors which affect the process of mutagenesis and carcinogenesis. Aqueous extract of garlic (100 mg/kg b.w.) and mustard oil (0.643 mg/kg b.w.) were fed to *Mus musculus* for 30 consecutive days either singly or simultaneously. Sodium arsenite (0.1 mg/kg b.w.) was injected subcutaneously on days 7, 14, 21 and 30 of experiment, singly and together with the dietary supplements. The animals were sacrificed 24h after the last exposure to sodium arsenite and clastogenic effects were observed in the bone marrow cells. The degree of modulation of sodium arsenite-induced chromosomal aberrations was more pronounced in mustard oil than in garlic extract and simultaneous administration of both the dietary supplements reduced the clastogenic effects of sodium arsenite closer to the level of the negative control. The greater efficacy could be due to the interaction of the two dietary supplements and its radical scavenging property. (Choudhry et al., 1997).

The results are in agreement with the studies on antimutagenic effect of garlic extract (GE) has been evaluated using 'in vivo chromosomal aberration assay' in Swiss albino mice. Cyclophosphamide (CP), a well-known mutagen, was given at a single dose of 25 mg/kg b.w. intraperitoneally. Pretreatment with 1, 2.5 and 5% of

freshly prepared GE was given through oral intubation for 5 days prior to CP administration. Animals from all the groups were sacrificed at sampling times of 24 and 48 h and their bone marrow tissue was analyzed for chromosomal damage. The animals of the positive control group (CP alone) show a significant increase in chromosomal aberrations at 48h sampling time. GE, alone did not significantly induce aberrations at either sampling time, confirming its non-mutagenicity. However in the GE pre-treated and CP post-treated groups, a dose dependent decrease in cytogenetic damage was recorded. A significant suppression in the chromosomal aberrations was recorded following pretreatment with 2.5 and 5% GE administration. The anticytotoxic effects of GE were also evident, as observed by significant increase in mitotic index, when compared to positive control group. Reduction in CP induced clastogenicity by GE was evident at 24 h and to a much greater extent at 48 h of cell cycle. Thus results of the present investigations revealed that GE has chemopreventive potential against CP induced chromosomal mutations in Swiss albino mice (Sukhla et al., 2002). Similar reports were observed with GE against CY induced genotoxicity in bone marrow cells of mice (Prem Kumar et al., 2004).

Consumption of garlic and tomato has been associated with reduced risk of many human cancers. The effects of these two dietary items were studied experimentally on carcinogen [I^{sqb}] DMBA [r^{sqb}] induced clastogenicity in Swiss mice. Chromosomal aberrations, which are predictor of cancer risk, were found to be reduced in bone marrow cells of Swiss mice exposed to carcinogens. Significant reduction of chromosomal aberrations was noted in bone marrow on day 21 and 30 ($p < 0.02$) although reduction was first evident after 96 hours. This is possibly the first report to suggest that oral administration of garlic and tomato can protect from the damaging effects of carcinogenic insult.

Kaur, et al (2007), studied the genotoxic effects of stavudine by using comet assay test. It was applied to evaluate the possible genomic damage caused by stavudine and also the ameliorating effects of garlic oil and vitamin E against its genotoxicity in different organs of mice. Two different doses of garlic oil (low and high dose) and vitamin E were administered to mice separately and in combination for six consecutive days followed by a dose of stavudine. The mice were sacrificed after 48 h of stavudine administration. Both the antioxidants (vitamin E and garlic oil) separately and in combination reduced the genotoxicity of stavudine. The protective effects of high doses of garlic oil were more pronounced as compared to vitamin E administered group.

A study was designed to compare the protective effect of selenium and garlic against liver and kidney damage induced by (ip) injection of 0.5 mg/kg mercury chloride (HgCl₂) in rats. Thirty-six Sprague-Dawley rats were used in the experiment and divided into six groups: one

group was orally given (1 ml) saline and served as a control group; two groups of rats were given either selenium (0.1 mg/kg) or garlic (63 mg/kg) alone, once daily an oral dose for 30 successive days; other two groups of rats were given either selenium or garlic alone, once daily a dose for 15 successive days prior to HgCl₂ injection and on the next 15 successive days simultaneously with HgCl₂ injection; and the last group of rats was injected ip with HgCl₂ for 15 days and at the end of the experiment (which lasted 30 days). The cytometric results revealed that injection of HgCl₂ induced an increase in the DNA density in kidney tissues with an increase in aneuploid cells and decrease in diploid cells. However, DNA density decreased in liver tissues with mild decrease in diploid cells and little percentage of aneuploid cells. We can conclude that oral administration of either selenium or garlic produces a significant protection against liver and kidney damage induced by the HgCl₂ injection, but garlic appears to be more protective (El Shenawy, et al 2008).

CONCLUSIONS

When animals treated with various doses of garlic extract. There was no increase in the frequency of chromosomal aberrations in somatic cells of mice when compared with the controls. The data indicate GE is non-mutagenic in nature.

Chromium showed clastogenic activity at cellular level.

The animals when primed with various doses of garlic extract significantly inhibit the percentage of Chromosomal aberrations induced by chromium in somatic cells of mice.

The treatment of garlic extract is a safe plant extract in cancer chemotherapeutic strategy.

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