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GARLIC EXTRACT PREVENTS GENOTOXIC DAMAGE INDUCED BY CHROMIUM IN BONE MARROW CELLS OF MICE

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ABSTRACT

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 was selected for modulation and were administered to animals after priming with chromium all the animals were killed after 72 hrs of treatment and the mitotic preparations were made. 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.

Number of Tables: 2 Number of References : 28

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 immunomodualtion 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 Lau, 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

Elemental chromium was first discovered and characterized by a French chemist Nicolas – Louis vanquelin in Siberian red lead ore Crocoites in 1797 (Katz et al1993, Costa et al 2008) It is naturally elements in crustal abundance and is found virtually in all phases including air, water, soil and biota. Themajor industries using chromium are the metallurgical, chemical and refractory brick industries (Losi et al, 1994). 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). Chromiumand 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).

MATERIALS AND METHODS:

Animal treatment:

The study was conducted after taking the approval of Institutional Ethical Committee on twenty adult male swiss albino mice 30 to 50 days 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±2ºC) fed with mice feed and were given ad libitium access to water.

Garlic extract preparation:

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 24 hrs the solvent was run through rotavapour to separate solvent and concentrated through Soxhlet apparatus. The obtained extract was run through rotavapour for futhur concentration. The Final extract was lyophilized to powder and stored at 4ºc 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.

In the present study two experiments were conducted the animals were feed orally with chromium and garlic extract and categorized in to following groups:

Group I : controls
Group II: garlic extract150mg/kg
Group III: garlic extract 200mg/kg
Group IV: garlic extract 250mg/kg

In the second experiment for modulation studies for all the three groups chromium primed with garlic extract of three different doses for seven days along with the mitomycin C and chromium 60 mg/kg groups.

Analysis of chromosomal aberrations in somatic cells of mice:

The animals were killed after 72 hrs of 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 37ºC for 45 minutes. Four slides for each were prepared from control and experimental animals. The staining was done within 72 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

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 24th hr exposure the percentage of total chromosomal aberrations was 3.20, 3.40 and 4.20, after the administration of 150, 200 and 250 mg/kg of garlic respectively as against 2.60 in control group of animals.The statistical analysis of data showed insignificant differences in the frequency of chromosomal aberrations in somatic cells of mice $P>0.05$.

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 (1984) studied the acute toxicity test of garlic extract in wistar rats and mice. The LD50 values of garlic extract by IP and SC administration were estimated over 30 ml/kg respectively in male and female of both rodants. In 30 ml/kg of IP group, five of ten in male rats and one of ten in female rats died within a day after administration, however no specific signs due to influence of garlic extract were observed survivals for 7 days. Sumiyoshi et al (1984) investigated the influence of garlic extract on the chronic toxicity test orally in Wistar rats for 6months. There were no toxic symptoms due to the garlic extract even at dose level of 2000 mg/kg for 5 times a week during 6months. High dose of garlic extract did not inhibit the body for 5 times a week during 6months. High dose of garlic extract did not inhibit the body weight gain, while the food consumption decreased slightly for the nutritional effects of it in both male and female rats. There were no significant differences in toxic signs were observed on any of the tissues and organs examined.

The antioxidant nature of garlic has been attributed to the presence of organosulphur compounds such as S-allylcysteine, dailylsulphide, allylmethylsulphide, S-methylcysteine (Chandra Mohan et al, 2004). 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 orgnosulfur compounds, S-allylcysteine and S-allylmercaptocysteine, found in aged garlic extract, showed radical scavenging activity in both chemiluminescence and 1,1-diphenyl-2picrylhydrazyl assays, indicating that these compounds may play an important role in the antioxidative acidity of aged garlic extract.

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.

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.

The anticlastogenic acitivity fo crude extract of garlic (Allium sativum L.) was studied in bone marrow cells of mice. Male laboroatory-bred Swiss albino mice were given one of three concentrations for 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 endpoints studied were 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 give garlic extract as dietary supplement. The extent of reduction was different for the three clastogens and may be attributed to the interaction with the different components of the extract (Das et al., 1993)

The endpoints scored were frequencies of chromosomal aberrations and damaged cells induced in bone marrow preparations. These parameters were found to be directly dose dependent and after an initial enhancement at 7 days, were reduced following prolonged exposure for 24hr to the low level observed at 24 hr. Therefore, administration of a low concentration of garlic extract daily is suggested for at least 30 days to obtain the maximum benefit of the extract in protecting against the clastogenic effects of known genototoxicants (Das et al 1996).

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 clastogeinc 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 and mitra, 2007).

The results are in agreemen

t 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 theirbone marrow tissue was analyzed for chromosomal damage. The animals of the positive control group (CP alone) show a significant increase in chromosomal aberrations both at 24 and 48h

sampling time. GE, alone did not significantly induced 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 cytogentic 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. Thus results of the present investigations revealed that GE has chemopreventive potential against CP induced chromosomal mutations in swiss albino mice (Shukla et al., 2002).

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 [Isqb]DMBA [rsqb] 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. It is proposed that one or other of many constituents' garlic and tomato may be responsible for the definite protective effect on chromosomal aberrations (Archana, et al 2002)

Further in another study Kumaraguruparan, et al (2005) studied the protective effect of pretreatment with tomato and garlic against N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced genotoxicity and oxidative stress was investigated in male swiss mice. In vivo bone marrow micronucleus test was performed to assess the antigenotoxic effect of tomato and garlic. Oxidative stress was monitored by estimating the extent of lipid peroxidation and the status for the glutathione redox cycle antioxidants. Increased frequency of bone marrow micronuclei with enhanced lipid peroxicdation was associated with compromised antioxidant defenses in MNNG treated animals. Although pretreatment with tomato and garlic significantly reduced the frequencies of MNNG-induced bone marrow micronuclei, the combination of tomato and garlic exerted a greater protective effect. This was associated with modulation of lipid peroxidation as well as reduced glutathione (GSH) and the GSH-dependent enzymes glutathione peroxidase (GPx) and glutathione-S-transferase (GST). These findings suggest that a diet containing even low levels of different naturally occurring compounds is effective in exerting antigenotoxic effects by modulating oxidative stress.

Saffron is a well-known spice and food colorant commonly consumed in different parts of the world. Recently, much attention has been focused on the biological and medicinal properties of saffron. In the present study the interactive effects of saffron with two commonly consumed

dietary agents, garlic and was evaluated for antigenotoxic effects against cyclophosphamide (CPH) in the mouse bone marrow micronucleus test experimental animals were orally pretreated with saffron (100 mg/kg body weight), garlic (250 mg/kg body weight) in combination for five consecutive days, 2h prior to the administration of CPH. Maximum reduction in the frequencies of micronucleated polychromatic erythrocytes (Mn PCEs) induced by CPH was observed when all the three test compounds were administered together. Furthermore, the protective effects were more pronounced in the garlic-administered groups compared to curcumin and/or saffron administered groups. (Prem Kumar et al., 2004)

The anticlastogenic activity of crude extract of garlic (Allium sativum L.) was studied in bone marrow cells of mice. Male laboratory-bred swiss albino mice were given one of three concentrations of the freshly prepared extract (100 mg, 50 mg, and 25 mg/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 (1.5 mg/kg) and cyclophosphamide (25 mg/kg) or sodium arsenite (2.5 mg/kg), simultaneously with garlic extract. After 24 hr, chromosome preparation was made from the bone marrow cells. The endpoints studied were chromosomal aberrations and damaged cells. Garlic extract alone induced a low level of chromosomal damage. The clatogenicity of all three mutagens were reduced significantly in the animas which had been given garlic extract as dietary supplement. The extent of reduction was different for the three clastogens and may be attributed to the interaction with the different components of the extract (Tandras das et al., 2006)

The results are comparable with genotoxic effects of herbal drops of garlic and pasipy were evaluated using the micronucleus test. Maximum Tolerated Dose (MTD) was determined by a dose-response test. For each medicine three treatment groups were considered with doses of MTD, ½ MTD and ¼ MTD according to the CSGMT protocol (1995 Japan). Drugs were administered orally to mice (test groups). Mitomicin C was used as a known genotoxic agent in positive control group. The peripheral blood samples before treatment (zero time samples) were considered as negative control. The appearance of a micronucleus is used as an index for genotoxic potential. The results obtained indicated that the herbal drops showed genotoxicity effect and it was dose-dependent compared to the negative control group. This genotoxicity was significant ($p<0.05$) but the genotoxic effects of garlic and pasipy were “not significant” compared to the historical negative control group ($p<0.05$). (Kalantari, et al 2007).

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 (hgCI(2)) 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 HgCI (2) injection and on the next 15 successive days simultaneously with HgCI (2) injection; and the last group of rats was injected IP with HgCl(2) for 15 days and at the end of the experiment (which lasted 30 days). The cytometric results revealed that injection of HgCl(2) 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 I 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 HgCl(2) injection, but garlic appears to be more protective (El Shenawy, et al 2008). The antioxidant nature of garlic has been attributed to the presence of orgnosulphur compounds such as s-allylcysteine, daillylsulphide, allylmethylsulphide, s-methylcysteine (Amagase, 1997; Ide and Lau, 1997; Imai et al, 1994; Wei and Lau, 1998; Chandra Mohan et al, 2004). 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 the garlic preparations and orgnosulfur 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-dipheny-2-picrylhydrazyl assays, indicating that these compounds may play an important role in the antioxidative activity of aged garlic extract

Table 1: *Classification of various types of chromosomal aberrations recorded in somatic cells of mice analyzed after 72h 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	
72 hrs							
Control III	3 (0.60)	6 (1.20)	1 (0.20)	1 (0.20)	1 (0.20)	2 (0.40)	10 (2.00)
150 mg/kg	5 (1.00)	7 (1.40)	2 (0.40)	1 (0.20)	2 (0.40)	2 (0.40)	12 (2.40)
200 mg/kg	5 (1.00)	8 (1.60)	2 (0.40)	1 (0.20)	3 (0.60)	2 (0.40)	13 (2.60)*
250mg/kg	6 (1.20)	9 (1.80)	3 (0.60)	1 (0.20)	3 (0.60)	2 (0.40)	14 (3.00)*

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

*P>0.05

Table2: *Classification of various types of chromosomal aberrations in somatic cells of mice analyzed after 72h chromium treated animals primed with various doses of garlic*

Dose (mg/kg) and duration of treatment	Structural aberrations				Numerical aberrations		Total no. of aberrations (%)
	Gaps	Breaks	Fragments	Exchanges	Polyploidy	Chromatid separations	
72 h							
Control III	3 (0.60)	5 (1.00)	0 (0.00)	0 (0.00)	1 (0.20)	3 (0.60)	8 (1.60)
Mytomycin c	8 (1.60)	30 (6.00)	5 (1.00)	4 (0.80)	3 (0.60)	18 (3.60)	57 (11.40)*
60 mg/kg-chromium	19 (3.80)	26 (5.20)	7 (1.40)	4 (0.80)	3 (0.60)	7 (1.40)	44 (8.80)*
60+150 mg/kg	8 (1.60)	22 (5.40)	5 (1.00)	2 (0.40)	2 (0.40)	12 (2.40)	41 (8.20)*
60+200 mg/kg	8 (1.60)	19 (3.80)	5 (1.00)	2 (0.40)	2 (0.40)	11 (2.20)	37 (7.40)*
60+250 mg/kg	7 (1.40)	17 (3.40)	4 (0.80)	2 (0.40)	1 (0.20)	8 (1.60)	31 (6.20)*

Gaps and polyploids are not included in total aberrations. The values in parenthesis are the percentages.
*P<0.05

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