PROTECTIVE EFFECTS OF GARLIC EXTRACT ON CYCLOPHOSPHAMIDE INDUCED GENOTOXICITY IN SWISS MICE

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ABSTRACT

In the present investigation the antimutagenic effects of Garlic extract has been evaluated against cyclophosphamide induced genotoxicity in bone marrow cells of mice. Single IP administration of garlic extract at various doses i.e. 125, 250 and 500 mg /kg. When treated individually did not induce chromosomal aberrations in somatic cells of mice. A single Intra peritoneal injection of 50mg/kg of cyclophosphamide induced significant increase in the percentage of micronuclei in bone marrow cells of mice. However after co administration of three doses of garlic extract there was a dose dependent decrease in the % of chromosomal aberrations was observed. Thus the results clearly indicate the protective effects of garlic extract against cyclophosphamide induced genotoxicity in bone marrow cells of mice. Therefore the data indicate that Garlic extract is a safer dietary component in cancer chemo preventive strategy.

KEYWORDS: Garlic extract, Cyclophosphamide, chromosomal aberrations.

INTRODUCTION

A number of antineoplastic drugs are in common use to combat various types of cancers. These are shown to be mutagenic in different test systems and these antineoplastic drugs such as Cyclophosphamide, Cisplatin, Tamoxifen, Gemcitabine and Paclitxel etc., have shown clastogenic effects in various test systems. Potential genetic damage due to drugs and other chemicals is well recognized. Extensive studies have been carried out on mutagenicity of various drugs in microorganisms, insects, mammals and in exposed population.[1-3]
Cyclophosphamide (CPM) is a well-known bifunctional alkylating agent widely used in cancer chemotherapy and expresses its genotoxicity when metabolically activated.\(^4\) It is extensively used for the treatment of various cancers as well as an immunosuppressant in organ transplantation, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and other benign diseases.\(^5-6\) According to the International Agency for Research on Cancer (IARC), CPM is widely used as reference mutagen and has been classified as carcinogenic for animals and humans (IARC, 1987).

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.\(^7-8\) Recent studies have shown the antigenotoxic and antimutagenic effects of garlic for various drugs and chemicals.\(^9-11\) 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.\(^12\) 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. Hence in the present investigation studies were carried out on the protective effects of garlic extract on cyclophosphamide induced micronuclei in bone marrow erythrocytes of mice.

**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.

**Preparation of Garlic Extract**

Fresh garlic cloves were purchased from the local market and made into coarse powder with mortar and pestle. The powder (about 250gm) was soaked in 500ml of ethanol for 72hrs. The solvent was runned through rotavapour to separate solvent and concentrated through Soxhlet apparatus. Garlic extract were selected in the present study.
Dosage schedule

In the present study two experiments were conducted. The animals were fed orally with cyclophosphamide and garlic extract and categorized into following groups:

- **Group I**: controls
- **Group II**: garlic extract 150mg/kg
- **Group III**: garlic extract 200mg/kg
- **Group IV**: garlic extract 250mg/kg

In the second experiment for modulation studies all the three groups as follows:

- **Group I**: controls
- **Group II**: Cyclophosphamide 50 mg/kg
- **Group III**: garlic extract 150mg/kg + Cyclophosphamide 50 mg/kg
- **Group IV**: garlic extract 200mg/kg + Cyclophosphamide 50 mg/kg
- **Group V**: garlic extract 250mg/kg + Cyclophosphamide 50 mg/kg

**METHODOLOGY**

**Analysis of chromosomal aberrations in bone marrow cells of mice**

In the present study the air drying technique of Preston et al. (1987) was employed with slight modifications to study the effect of test compounds on somatic cells of mice. Sampling times were ranged from 48 hrs to cover short and long term effects on cells at different stages of cell cycle at the time of exposure to the test compound.

The animals were sacrificed at appropriate time intervals of 48 hrs. 2 hours prior to sacrificing, 0.2 ml of 0.05 % colchicine was injected to all the animals to inhibit spindle formation in order to get well spread metaphases. All the animals were killed by cervical dislocation and hind limbs were dissected out for femur bones and freed from connective tissue and muscles with the help of gauge and immediately suspended in hypotonic solution (0.56% KC1).

The bone marrow was flushed out into clean glass Petri dishes with a hypodermic syringe fitted with a 22-gauge needle and dispersed well in hypotonic solution (0.56% KC1 i.e. 0.75M KC1) to get a homogeneous cell suspension. The suspension was collected in clean centrifuge tubes and incubated at 37°C for 45 minutes. After the incubation the tubes were centrifuged for 10 minutes at 1000rpm. The supernatant was removed carefully with the help of Pasteur pipette leaving a small volume over the pellet. To the pellet 5 ml of pre chilled...
fresh fixative (3:1 absolute methanol: glacial acetic acid; prepared freshly before use and preserved in refrigerator for chilled condition) was added drop wise from the sides of the centrifuge tubes and immediately dispersed the cell suspension by aspirating several times with a Pasteur pipette. The tubes were left undisturbed for 10 minutes at room temperature. After 10 minutes the suspension was centrifuged again and the supernatant was removed carefully leaving a small volume of the supernatant over the pellet and 5ml of chilled fresh fixative was added carefully and kept for 10 minutes undisturbed. This process was repeated for 4 to 5 times to ensure proper fixation. In the final change the cells were resuspended in 0.5ml of fresh fixative.

Two to three drops of cell suspension were dropped on clean grease free, prechilled slides. The slides were coded and stored in dust free chambers. The staining was done within 24 hours after the slide preparation. The slides were stained with 2% Giemsa

**Scoring:** Finally the slides were soaked in Xylene for overnight and mounted in DPX. For each mice 100 well spread metaphases were examined randomly using Leica CW 4000 Image analyzer

**RESULTS**

The data on the incidence of chromosomal aberrations in somatic cells of mice are depicted in table 1-3 and graphs 1-3.

Various doses of garlic 125, 250 and 500 mg/kg body wt. were selected for the mutagenic effects of and the observations were illustrated in Tables 1. The frequencies (%) of chromosomal aberrations are 1.60, 1.80 and 2.00 in 125, 250 and 500 mg/kg garlic extract treated animals when compared with that of control 1.40%. There was no significant increase in the gaps, breaks. No increase is seen in Fragments in garlic extract treated groups. When compared with that of controls. No significant changes are seen in Exchanges. The frequency of breaks were 1.00, 1.20 and 1.20 and the percentages of fragments were 0.40 and frequency of chromatid separations were 0.20 in 125, 250 and 500 mg/kg garlic treated groups, when as the frequency of chromatid separations were 0.80, 0.20 and 0.40 in garlic extract treated animals. (Table 1). The differences in the frequencies of chromosomal aberrations between controls and the garlic treated mice for 48 hrs were analyzed using $X^2$ test and the results were found to be insignificant ($P>0.05$, Table- 1).
Various doses of garlic 125, 250 % 500 mg/kg body wt. were primed to mice prior to the administration of the drug cyclophosphamide 50mg/kg body wt. The modulatory effects of the garlic against the drug were analyzed by observing the frequency of chromosomal aberrations in somatic cells of mice at 48 hrs respectively. The observations were recorded and tabulated (Table- 2-3) and illustrated graphically in Graphs–3-4.

The frequency of chromosomal aberrations was found to be 15,60,13.60,12.00 in garlic extract +CP primed mice against 18.40 in CP alone administered mice (Table 2). The frequency of breaks were 8.20,7.40 &6.40 in 125+ 50, 250 +50, 500 + 50 mg/kg garlic extract + cyclophosphamide treated mice where as 9.20 was observed in cyclophosphamide alone treated group. The frequency of fragments were decreased to 5.80, 5.00, 4.40 in 125+ 50, 250 +50, 500 + 50 mg/kg garlic extract + cyclophosphamide treated mice. No exchanges were noted in controls and 1.00, 0.80 &0.80 were observed in 125+50, 250 +50, 500 + 50 mg/kg garlic extract + cyclophosphamide treated mice. And 1.20 % was observed in cyclophosphamide alone treated groups. No chromatid seperations were noted in controls where as in cyclophosphamide treated mice it was 0.80 and decreased to 0.60, 0.40 & 0.40 in 125+50, 250 +50, 500 + 50 mg/kg garlic extract + cyclophosphamide treated mice.

The inhibitory effects of garlic extract groups against cyclophosphamide induced chromosomal aberrations in somatic cells of male mice were 48hrs 15.21, 26.08, 34.78 in which were grouped as III, IV & V and depicted in Table-3 and Graph-3. The results clearly indicate the protective effects of garlic extract groups in CP induced cytotoxicity in mice.

The differences is frequency of CA between control and primed groups were found to be significant (Table 3 p<0.01)
Table 1: Frequency of various types of chromosomal aberrations recorded in somatic cells of mice analyzed after 48 hrs treatment with various doses of garlic extract.

<table>
<thead>
<tr>
<th>Dose (mg/kg) and duration</th>
<th>Normal metaphases</th>
<th>Breaks</th>
<th>Fragments</th>
<th>Exchanges</th>
<th>Chromatid separations</th>
<th>Total No. of aberrations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48hrs Control- II</td>
<td>497(98.60)</td>
<td>4(0.80)</td>
<td>2(0.40)</td>
<td>0(0.00)</td>
<td>1(0.20)</td>
<td>7(1.40)</td>
</tr>
<tr>
<td>125 mg/kg GE</td>
<td>498(98.40)</td>
<td>5(1.00)</td>
<td>2(0.40)</td>
<td>0(0.00)</td>
<td>1(0.20)</td>
<td>8(1.60)*</td>
</tr>
<tr>
<td>250 mg/kg GE</td>
<td>491(98.2)</td>
<td>6(1.20)</td>
<td>2(0.40)</td>
<td>0(0.00)</td>
<td>1(0.20)</td>
<td>9(1.80)*</td>
</tr>
<tr>
<td>500 mg/kg GE</td>
<td>490(98.00)</td>
<td>6(1.20)</td>
<td>2(0.40)</td>
<td>1(0.20)</td>
<td>1(0.20)</td>
<td>10(2.00)*</td>
</tr>
</tbody>
</table>

The values in the parenthesis are percentages.

Gaps and polyploids are not included in total aberrations.

*p>0.05

Table 2: Protective effects of garlic extract in CP induced chromosomal aberrations in somatic cells of mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Normal metaphases %</th>
<th>Abnormal metaphases %</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group - I</td>
<td>Control</td>
<td>492(98.40)</td>
<td>8(1.60)</td>
<td></td>
</tr>
<tr>
<td>Group – II</td>
<td>Cyclophosphamide 50 mg/kg</td>
<td>408(82.60)</td>
<td>92(18.40)</td>
<td></td>
</tr>
<tr>
<td>Group - III</td>
<td>50+125mg/kg GE</td>
<td>422(84.40)</td>
<td>78.15(60)</td>
<td>15.21</td>
</tr>
<tr>
<td>Group - IV</td>
<td>50+250mg/kg GE</td>
<td>432(87.40)</td>
<td>68.13(60)</td>
<td>26.08</td>
</tr>
<tr>
<td>Group - V</td>
<td>50+500 mg/kg GE</td>
<td>440(88.00)</td>
<td>60.12(60)</td>
<td>34.78</td>
</tr>
</tbody>
</table>

Table 3: Frequency of CA recorded in somatic cells of mice with CP & primed with garlic extract for 48 hrs time period.

<table>
<thead>
<tr>
<th>Dose mg/kg</th>
<th>Structural</th>
<th>Total Aberration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breaks</td>
<td>Fragments</td>
</tr>
<tr>
<td>Control- I</td>
<td>6(1.20)</td>
<td>2(0.40)</td>
</tr>
<tr>
<td>Cyclophosphamide 50 mg/kg</td>
<td>46(9.20)</td>
<td>36(6.20)</td>
</tr>
<tr>
<td>50+125mg/kg GE</td>
<td>43(8.20)</td>
<td>29(5.80)</td>
</tr>
<tr>
<td>50+250mg/kg GE</td>
<td>37(7.40)</td>
<td>25(5.00)</td>
</tr>
<tr>
<td>50+500 mg/kg GE</td>
<td>32(6.40)</td>
<td>22(4.40)</td>
</tr>
</tbody>
</table>

The values in parentheses are percentages.

Gaps and polyploids are not included in total number of aberrations.
DISCUSSION

The present results are comparable to Santos Renato et al.,[14] who reported that Cyclophosphamide at 135mg/kg dose induced a significant increase in the frequency of micronuclei in polychromatic erythrocytes of male mice. Further, the percentage of chromosomal aberrations was 59.33 in 50mg/kg body wt. Cyclophosphamide treated mice.[15]

The results indicate non-mutagenic nature of garlic extract in bone marrow cells of mice. Similar data was observed when animals treated with phyllanthus emblica Carrot juice, Aegelus marmelus fruit extract showed lower frequency of chromosomal aberration in bone marrow erythrocytes of mice.[16-18]

Our results are comparable with that of Abraham and Kesavan.[19] 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. Sumiyoshi et al.[20] investigated the influence of garlic extract on the
chronic toxicity test orally in Wistar rats for 6 months. There were no toxic symptoms due to the garlic extract even at dose level of 2000 mg/kg for 5 times a week during 6 months. High dose of garlic extract did not inhibit the body for 5 times a week during 6 months. 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 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 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 cyclophosphamide+garlic treated animals. Thus as a result of various type of chromosomal aberrations the percentage so total chromosomal aberrations at 24h exposure to cyclophosphamide+ garlic were 2.20 in control and were 9.60 in 60mg/kg of cyclophosphamide treated animals to 8.40, 7.00 and 5.60 of cyclophosphamide +garlic treated animals respectively. The anticlastogenic activity of crude extract of garlic (Allium sativum L.) was studied in bone marrow cells of mice.

Male laboratoary-bred Swiss albino mice were given three concentrations from 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. 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 genotoxicants.[21]

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 24 h 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.\[22\]

The result 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) showed a significant increase in chromosomal aberrations both at 24 and 48 h sampling time.

GE, alone did not induce aberrations at either sampling time, confirming its non-mutagenicity. However in the GE pre-treated and CP posttreated 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. Thus results of the present investigations revealed that GE has chemopreventive potential against CP induced chromosomal mutations in swiss albino mice.\[9\]

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 of garlic and tomato may be responsible for the definite protective effect on chromosomal aberrations.\[^{23}\]

In the another 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.\[^{24}\] 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 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 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.\[^{25}\] 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).\textsuperscript{[26]}

The antioxidant nature of garlic has been attributed to the presence of organosulfur compounds such as s-allylcysteine, daillylsulphide, allylmethylsulphide, smethylcysteine.\textsuperscript{[27,28]} These volatile compounds are generally considered to be responsible for most of the pharmacological properties of garlic. Imai et al.\textsuperscript{[29]} reported the antioxidant properties of garlic preparations and organosulfur compounds in garlic. Among the variety of organosulfur compounds, sallylcysteine 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.

The protective nature of garlic oil, pipergine, cassia, ausiculata, phylanlhus emblica, tomato fruit extract, Solanum Lycopersicum fruit extract against drug induced cytogenetic damage has been reported.\textsuperscript{[3,30,33]}

**CONCLUSIONS**

Animals when treated with various doses of garlic extract showed non mutagenic nature and the percentage of chromosomal aberration in bone marrow cells of mice.cells of mice were equivalent with that of control values. There was an increase in the incidence of chromosomal aberrations in cyclophosphamidtreated group when compared to the control Group. There was a significant decrease in the percentage of chromosomal aberration in bone marrow cells of mice when cyclophosphamidewas primed with various doses of garlic extract. Thus garlic extract showed protective effects against the cyclophosphamide induced genotoxicity in bone marrow cells of mice. Hence garlic extract supplementation is a safer dietary component in chemotherapeutic strategy.

**ACKNOWLEDGMENT**

The author Srivani is thankful to Prof. Sugita Mathur, Head, Department of Zoology, University College of Science, Osmania University for providing for Laboratory facilities.
REFERENCES

16. Shoba Rani M and Rudrama Devi K. Trends in life science, 2006; 21(1,2).