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PROTECTIVE EFFECTS OF *SOLANUM LYCOPERCICUM* FRUIT EXTRACT IN CYCLOPHOSPHAMIDE INDUCED GENOTOXICITY IN SOMATIC CELLS OF MICE

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ABSTRACT

In the present investigation the antimutagenic effect of *solanum lucopercicum* fruit extract has been evaluated against cyclophosphamide induced genotoxicity. Single IP administration of *Solanum lycopercicum* fruit extract at various doses i.e. 250, 500 and 1000 mg /kg. When treated individually did not induce chromosomal aberration in bone marrow cells of mice. A single I P 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 *Solanum lycopercicum* fruit extract there was a dose dependent decrease in the % of chromosomal aberrations was observed. Thus the results clearly indicate the preventive effects of *Solanum lycopercicum* fruit extract against CP induced genotoxicity in bone marrow cells of mice. Therefore the data indicate that SL fruit extract is a safer dietary component in cancer chemo preventive strategy.

KEYWORDS: *Solanum lycopercicum* fruit extract (SLFE), Cyclophosphamide, bone marrow cells.

INTRODUCTION

Cyclophosphamide (CPM) is a well-known bifunctional alkylating agent widely used in cancer chemotherapy and expresses its genotoxicity when metabolically activated.[1] 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.[2,3] 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). Reactive metabolites of CPM chemically alkylate DNA as well as protein, producing cross-links which are responsible for its cytotoxic effect. Normal tissues injury or damage is the major limitation of using CPM, which gives rise to numerous side effects, CPM treatment also results in the production of reactive oxygen species (ROS), which cause peroxidative damage to kidney and other vital organs. Antineoplastic and toxic effects like necrosis, apoptosis, and oncosis of CPM are linked with two active metabolites, i.e., phosphoramide and acrolein. Further Tripathi and Jena[4] reported CPM to be toxic in germ cells of mice, it has also been reported that CPM treatment for non-Hodgkin's lymphoma leads to the induction of secondary cancers in bladder and kidney. The important factor for therapeutic and toxic effects of CPM is the requirement of metabolic activation by hepatic microsomal cytochrome P mixed functional oxidase system.[5] On activation, CPM generates active alkylating metabolites such as 4-hydroxycyclophosphamide, acrolein, and aldophosphamide mustard, which hamper with cellular DNA synthesis in fast dividing cells and ultimately lead to cell death.[6&7] Further cyclophosphamide induced chromosomal aberrations in somatic and germ cells of mice.[8] Solanum lycopersicum (tomato) is an important vegetable in India, Several epidemiological and experimental studies suggested the preventive role of lycopene, a active constituents of Solanum lycopersicum reduction in the risk of several different types of cancer. Such as cancers of the lung, stomach, prostate gland, cervix, breast, oral cavity, pancreas, colorectum, and esophagus.[9-16] Dietary lycopene comes primarily from tomatoes, although apricots, guava, watermelon, papaya, and pink grapefruit are also significant sources. Tomatoes are the best source of lycopene. A population-based case control study found that lycopene from Solanum lycopersicum (tomato) based foods was associated with a small reduction in risk for prostate cancer. High concentration of lycopene in prostate tissues resulted in a nearly three-fold increase in programmed cell damage among cancer cells. It has been suggested that lycopene supplements may benefit those with prostate cancer [10]. In animal studies the antitumour effect of Lycopene was reported in S180 tumor which inhibited the growth of S180 tumor.[17] The antitumor effect may be related to its immune function and antioxidative effect. Smoking modifies associations between nutrients and mortality.[18] Lycopene did not caused direct maternal or developmental toxicity in rats or rabbits at dosages as high as 2000 or 3000 mg/kg/day.[19] Therefore, we have made to study the anti mutagenic effect of Solanum lycopersicum fruit extract using the analysis of chromosomal aberrations in mouse bone marrow cells.

MATERIALS AND METHODS

Selection of animals

Among the various dose groups tested to study the toxic effects of Cyclophosphamide, the percentage of chromosomal aberrations was high at highest dose of Cyclophosphamide. Hence the highest dose of Cyclophosphamide of 50mg/kg body wt.,selected for modulation of its toxic effects with antioxidants enriched *tomato fruit extract* (250, 500 & 1000mg/kg body wt.) were selected to study *in vivo*test systems. *Tomato fruit extract* ((250, 500 & 1000mg/kg body wt.) extractions were given in split doses orally for 7 consecutive days (to cover the seven cell divisions of erythropoiesis (Adler, 1984) and I0mg/kg body wt. of Cyclophosphamide. Cyclophosphamide was administrated on day 7, one hr after regular exposure to antimutagen as a single intraperitonial dose. The animals on 8^{th}, 9^{th}, 10^{th}day which corresponds to 24, 48 & 72hrs exposure to mutagen respectively. Control group of animals were also maintained simultaneously.

METHODOLOGY

The animals were killed by cervical dislocation and femur bones were obtained and hypotonic solution has been added.Two to three drops of cell suspension were dropped on clean grease free, pre-chilled slides. The slide was blown once across and allowed to dry on aslide warmer. Two slides from each animal were prepared by air drying technique from control and treated animals. The slides were coded and stored in dust free chambers. The staining was done within 24hrs after the slide preparation. The slides were stained with 2% Gieitisa (2ml of Giemsa in 46ml of double distilled water plus 2ml of phosphate buffer* pH 6.8) for 7-8 minutes and later they were rinsed in double distilled water and allowed to dry. Rinsed in distilled water and again stained for 10 mts in Giemsa stain diluted with distilled water (1:6 ratio). ificed at appropriate time intervals of 48 & hrs. 2hrs prior to sacrificing, 0.2ml 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) according to the method of Preston et al, 1980.[20] The bone marrow was flushed out into clean glass Petri dishes with a hypodermic syringe fitted with a 22-guage 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 1000 rpm. 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. The slides with perfect morphology of the nucleated cells were selected for screening. The slides were screened for the presence of micronuclei in polychromatic erythrocytes and norm chromatic erythrocytes and micro photographed (Fig. 1a & b). Finally the slides were soaked in Xylene for overnight and mounted in DPX. For each mouse 100 well spread metaphases were examined randomly using Leica CW 4000 Image analyzer. A total of 100 metaphases were screened for the presence of various types of chromosomal aberrations such as breaks, gaps, acentric fragments and polyploids etc., in control and treated group of mice.

RESULTS

The results on the incidence of chromosomal aberrations by cyclophosphamide and tomato fruit extract and its protective effects are depicted in table 1-3 and illustrated graphically in 1 & 2. The frequencies (%) of chromosomal aberrations in the tomato extract treated mice mg/kg body wt. were 1.20, 1.60 and 2.20 in 250, 500 , 1000 mg/kg tomato fruit extract groups respectively when compared to that of controls 1.40% (Table 1-9).There was no increase in the gaps in the treated mice which recorded were the Breaks in the treated groups were recorded as 1.20, 1.00, 1.40 in 250, 500 and 1000 mg/kg tomato fruit extract when compared to that of controls with 0.80%. Fragments were 0.60, 0.40 and 0.40 after 250, 500 & 1000 mg/kg of tomato fruit extract groups. Nofragments were observed in control. No exchanges were seen in control groups. The frequency of exchanges were 0.20 in 1000 mg/kg TFE group. Chromatid separations were 0.00, 0.20 and 0.20% after administration of 250, 500 & 1000 mg/kg tomato fruit extract groups. No frequency of Chromatid separations were observed in controls (Table - 3). The differences in the frequencies of chromosomal aberrations between and tomato fruit extract treated mice for 48hrs were analyzed by X^2 test and the results were found to be insignificant (P>0.05,).

Table 1: Frequency of various types of chromosomal aberrations in somatic cells of mice analyzed after 48 hrs of tomato fruit extract.

Dose (mg/kg) and duration	Normal metaphases	Breaks	Frag-ments	Exchan-ges	Chromatid separations	Total No. of aberrations (%)
Control- II	497(98.60)	4(0.80)	3(0.60)	0(0.00)	0(0.00)	7(1.40)
250mg/kg	498(98.40)	5(1.00)	3(0.60)	0(0.00)	0(0.00)	9(1.80)*
500mg/kg	491(98.2)	6(1.20)	2(0.40)	0(0.00)	1(0.20)	8(1.60)*
1000mg/kg	490(98.00)	7(1.40)	2(0.40)	1(0.20)	1(0.20)	11(2.20)*

Gaps and polyploids are not included in total aberrations.

The values in parentheses are percentages.

*p>0.05

Various doses of tomato extract 250, 500 & 1000 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 tomato against the drug was analysed by observing the frequency of chromosomal aberrant cells. The observations were recorded tabulated (Tables –2 & 3)

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

Group	Dose	Normal metaphases %	Abnormal metaphases%	Inhibition %
Group - I	Control- II	492(98.40)	**8(1.60)**	
Group – II	Cyclophos-phamide 50mg/kg	409(81.80)	91(18.20)	
Group - III	50 +250mg/kg	428(85.60)	72(14..40)	**20.87***
Group - IV	50+500mg/kg	437(89.40)	63(12.20)	**30.76***
Group - V	50+500mg/kg	451(90.20)	49(9.80)	**46.14***

*P<0.05

The values in parentheses are percentages

Table 3: Classification of CA recorded in somatic cells of mice treated with CP & primed with tomato fruit extract.

Dose (mg/kg) and duration of treatment	Structural aberrations (%)			Numerical aberrations (%)	Total of no. of aberrations (%)
	Breaks	Frag-ments	Exchan-ges	Chromatid separations	
Control- II					8(1.60)
Cyclophos-phamide 50mg/kg	45(9.00)	35(5.00)	7(1.40)	4(0.80)	91(18.20)
50 +250mg/kg	32(6.40)	32(6.40)	6(1.20)	3(0.60)	72(14.40)*
50+500mg/kg	29(5.40)	27(5.20)	5(1.00)	2(0.40)	63(12.20)*
50+500mg/kg	22(4.40)	20(4.00)	5(1.00)	2(0.40)	49(9.80)*

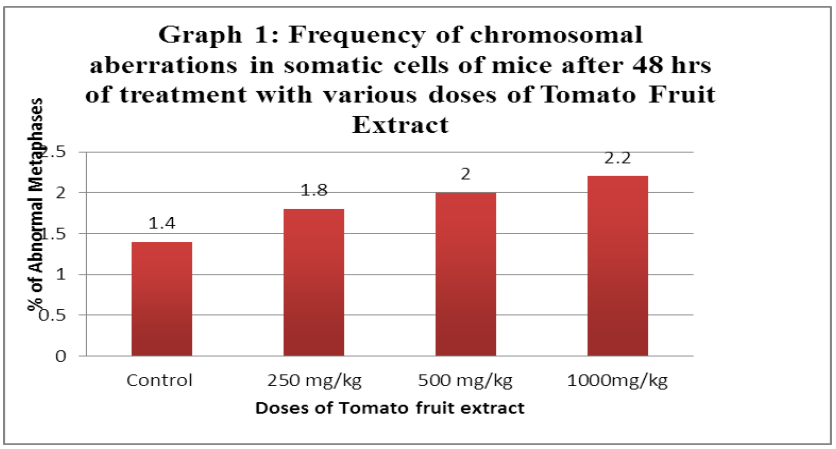
*P<0.05

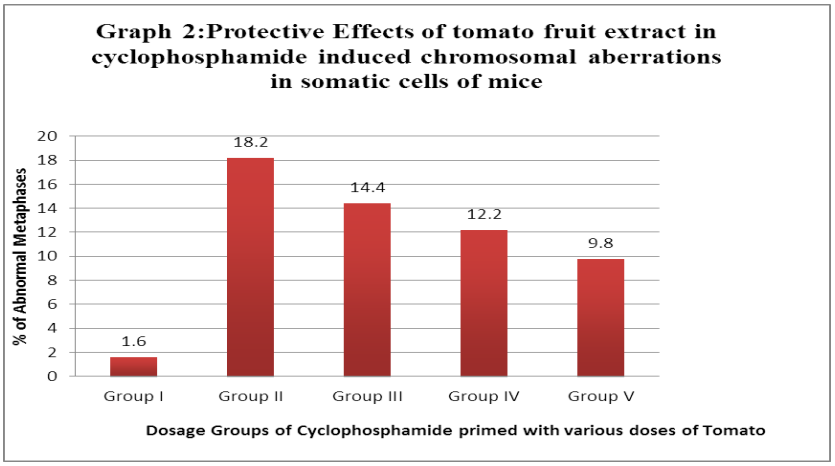
The values in parentheses are percentages

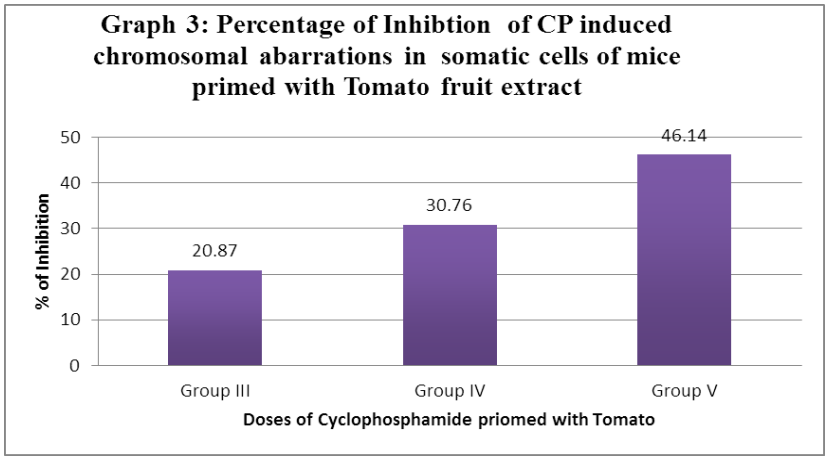
Gaps and polyploides are not included in total aberrations.

The frequency in abnormal metaphases in tomato fruit extract primed mice were 14.40, 12.20& 9.80 respectively for 250+50, 500+50 & 1000+50 mg/kg body wt. tomato primed mice when compared with cyclophosmide alone administered mice were 18.20%. This

clearly indicates that the priming of tomato fruit extract to mice has shown a significant antimutagenic effect against the drug (Table-2 & 3, Graphs 1-3). The frequency of breaks were 6.40, 5.40 & 4.40 and the percentages of fragments were 6.40, 5.20 & 4.00 in 250+50, 500+50 & 1000+50 mg/kg body wt. tomato primed mice when compared with 9.00 (breaks), 5.00 (fragments) in cyclophosphamide alone treated animals and frequency of chromatid separations were 0.60,0.40,0.40 in 250+50,500+50, 1000+50mg/kg tomato fruit treated groups. The frequency of exchanges were 0.80 in cyclophosphamide treated animals and decreased to 1.20, 1.00 & 1.00 in 250+50, 500+50 & 1000+50 mg/kg body wt. tomato primed mice. The differences in the frequencies of chromosomal aberrations between controls and treated mice with 50mg/kg body wt. of cyclophosphamide on priming with 250+50, 500+50 & 1000+50 mg/kg body wt. were analyzed using X^2 test and the results were found to be significant ($P<0.05$). The Inhibitory effects of ginger extract against cyclophosphamide induced chromosomal aberrations in somatic cells of male mice were and in 48hrs20.87 30.76 ,46.14 in which were grouped as III, IV & V and depicted in Table 2 & 3.The results clearly indicate the protective effects of tomato fruit extract treated groups in CP induced cytotoxicity in mice.







DISCUSSION

The actively proliferating cells from bone marrow provide maximum information on the effect of any test compound.[21] Although chromosome damage in somatic cells is not transmitted to the offspring, such damage in its own right is of cardinal importance to the individual and its presence is an evidence of exposure of the individual to mutagens [22]. Further, they are also considered as stable anomalies and continue to next generations; furthermore, the studies on somatic cells are of significant importance, since severe genetic damage in somatic cells lead to cancer and perhaps other diseases.

In this study, the protective effect of solanum lycopersicum fruit extract is reported against cyclophosphamide (cp)-induced cyto genetic damage formation in mouse bone marrow cells. The three test doses, namely 500, 1000 and1000 1500 mg/kg body weight of s. Lycopersicum fruit extract provided protection when given 24 hr prior to the single ip administration of cyclophosphamide (50 mg/kg body weight). A dosefdependent inhibition of CA's formation was observed which was statistically significant ($p<0.05$) as compared to the cyclophosphamide group. It was observed that s. Lycopersicum (tomato) extract alone couldnot induced micronuclei formation at the test dose 500 mg/kg body weight. Its seem to have a preventivepotential against cp-induced micronuclei formation in swiss mouse bone marrow cells.

The present data demonstrate that In *S. lycopersicum* fruit extract was dose dependent inhibition of chromosomal aberrations induced by CP in mouse bone marrow cells. *S. lycopersicum*, when tested for mutagenic effect at various test dose levels, failed to induce chromosomal aberrations in somatic cells of mice.

The present results are comparable with that of Asita el al[23] investigated the intraperitoneal injection of mice with a single dose of 40 mg/kg body weight of CP induced a significant increase in the frequency of MNPCE, 24 h after injection, when compared with animals that received water treatment. The present results are comparable to Santos Renato et al., 2005; reported that cyclophosphamide at 135mg/kg dose induced a significant increase in the frequency of micronuclei in plychromatic erythrocytes of male mice Further, the percentage of chromosomal aberrations was 59.33 in 50mg/kg body wt. cyclophosphamide treated mice.[24]

Naturally occurring antioxidants have been extensively studied for their capacity to protect organisms and cells from oxidative damage. Many plant constituents including *S. lycopersicum* and Lycopene appear to be potent antimutagens and antioxidants. Lycopene did not caused direct maternal or developmental toxicity in rats or rabbits at dosages as high as 2000 or 3000 mg/kg/day(26) and Synthetic crystalline lycopene provides an alternative to extracts of naturally occurring lycopene for use in dietary supplements and functional foods. BASF Lycopene 10 CWD.

The results are comparable with earlier studies such as the antigenotoxic effects of *solanum lycoperscium* fruit extract were studied in Cisplatin induced chromosomal aberrations in somatic cells of mice. Various doses of SL fruit 250, 500 and 1000 mg/kg extracted were tested using standard protocol in bone marrow chromosomes of mice. The results of SLF extract individually showed no cytoxic effects in somatic cells of mice. However when SLF extract administered for seven days for modulation studies prior to cisplatin treatment (10 mg/kg i.e.,) showed significant protection in the percentage of chromosomal aberration at 500 & 1000 mg/kg SLF treated groups.[25]

Pre-treatment with lycopene had significantly reduced the frequency of CP-induced bone marrow micronuclei.[26] The similar kinds of earliar studies have also been reported that several naturally occurring compounds exhibited antimutagenic activity. The non mutagenic effect of Lycopene active constituent of *S. lycopersicum* extract has been also observed also in MNNG-induced micronuclei formation hromosomal aberration test system.[27] In one study, lycopene inhibited human colon carcinoma, myeloid leukemia, and lymphoma cell lines in a dose-dependent manner.[28] In a clinical trial, lycopene supplementation (30 mg/day for 2 months) had beneficial effects in healthy women with a high risk of breast cancer but not in breast cancer survivors.[29] Carotenoids, as potential antioxidants, are well known as

highly efficient scavengers of singlet oxygen (1O2) and other excited species. During 1O2 quenching, energy is transferred from 1O2 to the lycopene molecule, converting it to the energy-rich triplet state. Trapping of other ROS, such as OH, NO-2 or peroxynitrite, in contrast, leads to oxidative breakdown of the lycopene molecule. Thus, lycopene may protect in vivo against oxidation of lipids, proteins, and DNA.[30]

The present results are comparable with that of Emerit et al.[31], who reported cyclophosphamide induced chromosomal breaks and rearrangements in different strains of mice (C3H, AKR and C57B1ZLII Orl.). However in 1978. Renault had shown a decrease in the frequency of SCE in AKR mice. Similarly six fold increase over the controls in the induction of SCE was observed in bone marrow cells of mice. Chinese hamsters one per cell, per pg DNA content and per chromosome basis.[32] Further, acute and chronic exposure of cyclophosphamide had showed significant increase in SCE in human, mouse, rat and rabbit.[33] Similar results were reported by cyclophosphamide in bone marrow cells in Swiss albino mice.[34]

The non mutagenic effect of Lycopene active constituent of *S. lycopersicum* extract has been also observed also in MNNG-induced micronuclei formation and chromosomal aberration test system.[27] Tomato fruit is a reservoir of diverse antioxidant molecules, such as ascorbic acid, vitamin E, carotenoids, flavonoids and phenolic acids. Lycopene, the carotenoid of interest (in tomato), has the highest antioxidant activity among all dietary antioxidants and has also been shown to induce cell-to-cell communications and modulation of hormones, immune systems and other metabolic pathways[35] Dietary intake of lycopene is epidemiologically correlated with diminished risk of prostate cancer and it has been found to be superior to α- and β-carotene in inhibiting cell proliferation in various human epithelial cancer cell lines[36] Tomatoes also contain moderate amounts of α- and β-carotene and lutein. β-Carotene is known for itsprovitamin A activity and lutein for reduced risk of lung cancer.[37]

Earlier from our laboratory we have reported a significant reduction in the frequency of chromosomal aberrations by phyllanthus emblica and Curcumin in somatic cells of mice.[38] cisplatin.[39]

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