Effects of *Solanum lycopersicum* Fruit Extract on Cyclophosphamide-induced chromosome aberrations in mouse bone marrow cells

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Summary

In the present study, the clastogenic effect of *Solanum lycopersicum* fruit extract has been evaluated against cyclophosphamide (CP)-induced chromosomal aberrations in the bone marrow cells of the mice. Single i.p. administration of *Solanum lycopersicum* fruit extract at different test doses, namely 500, 1000 and 1000 mg/kg body weight have provided protection, when given 24 hr prior to the single i. p. administration of cyclophosphamide (50 mg/kg body weight). A dose dependent inhibition of chromosomal aberrations was observed which was statistically significant ($p<0.05$) as compared to the cyclophosphamide group. It's seems to have a preventive potential against CP-induced chromosomal aberrations in the bone marrow cells of the mice. Therefore, the results suggest a genotoxic potential of *Solanum lycopersicum* fruit extract.

Keywords: Chromosome, cyclophosphamide, bone marrow, *Solanum lycopersicum*.

Introduction

Recently a variety of compounds that possess antimutagenic properties has been detected in vegetables and spices, and evidence is accumulating that their dietary intake decreases the risk of cancer and other malignant diseases in human (1). *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 (2-9). 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.

Initial studies have suggested that cooked tomatoes (i.e., tomato sauce or paste) are a better source of available lycopene than raw tomato juice because the heating action allows the body to quickly absorb the lycopene. It has been suggested that lycopene is a powerful antioxidant (8). 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 (3). In animal studies the antitumour effect of Lycopene was reported in S180 tumor which inhibited the growth of S180 tumor (10). The antitumor effect may be related to its immune function and antioxidative effect. Pre-treatment with lycopene had significantly reduced the frequency of MNNG-induced bone marrow micronuclei and chromosomal aberrations, (11) plasma lycopene was significantly associated (inversely) with total mortality in the full study population. Smoking modifies associations between nutrients and mortality (12). Lycopene did not caused direct maternal or developmental toxicity in rats or rabbits at dosages as high as 2000 or 3000 mg/kg/day (13). Therefore, we have made to study the antimutagenic effect of *Solanum lycopersicum* fruit extract using the chromosomal aberrations assay in mouse bone marrow cells.

Materials and Methods

Chemical

Cyclophosphamide was purchased from Sigma Chemical Co. (St Louis, MO, USA). Other Reagent grades chemical were procured locally.

Extract Preparation

The identification of the plant *Solanum lycopersicum* (family*: Solanacae*) was done by botanist Dr. S. S. Khan (Voucher Specimen No: WR/101/LGOB/2006), Department of Botany, Safia Science College, Bhopal, Madhya Pradesh India. The *S. lycopersicum* fruit were collected. The pieces of fruits were taken and cut in to small pieces. After that paste was taken in a separating funnel and added double distilled water and extracted with double distilled water by refluxing for 36 hrs. at 60˚C. On the day of experimentation, the desired amount of powder was dissolved in double distilled water for the final administration.

Animal and Treatment

The study was conducted on random bred, 6-7 weeks old and 24- 28 gm body weight male *Swiss albino* mice. They were maintained under controlled conditions of temperature and light (light: dark, 12 hrs: 12 hrs.). They were provided standard mice feed and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC, Ref. No.- 2157/225/2006).

For Chromosomal aberration assay, three dose of *S. lycopersicum* i.e. 500, 1000 and 1500 mg/kg body weight were administered. *S. lycopersicum* extract were dissolved in double distilled water and administered as single dose in 0.2 ml per mouse 24 hours prior to cyclophosphamide (CP) administration.

Control mice were administered an equal volume of vehicle alone. The positive control group also received a single i.p. injection of 50 mg /kg CP in 0.9 % saline. The animals were sacrificed by cervical dislocations and bone marrow cells harvested. Colchicine (4 mg/kg b. wt.) was administered intraperitoneally 2 hrs. before the harvest of the cells. The slides prepared essentially as per modified method of Preston, *et. al*., (1987) (14). Briefly, femur bones were excised and the bone marrow extracted in 0.56 % KCl. The harvested cells were incubated at 37 ^{0}C for 20 minutes and then centrifuged for 10 minuets at 1000 rpm. Cells were fixed in Carnoy's fixative (methanol:acetic acid = 3:1)and bursed opened on clean slides to release chromosome. The slide were stained with 5%Giemsa solution for 15 min and then put in xylene and mounted with DPX. A total of 100 well spread metaphase plates were scored for chromosomal aberrations at a magnification of 1000 X (100 x 10) for each groups. Different types of chromosomal aberrations such as chromatid breaks, gaps, pulverization, centromeric association etc. were scored and expressed as % chromosomal aberrations. The statistical significance was determined using Student's 't' test.

Results

Data summarized in Table 1 show that *S. lycopersicum* fruit extract at different dose level induced significantly different types of chromosomal aberrations in bone marrow cells of Swiss albino mice. The *S. lycopersicum* extract administered intraperitonial at the dose of 500, 1000 and 1500 mg/kg body weight 24 hours prior the cyclophosphamide (CP) administration provided protection against cyclophosphamide induced chromosomal aberrations in bone marrow cells in mice. The degree of protection was 28.65, 42.69 and 60.12% respectively. A statistically significant ($p<0.05$) protection was observed with all the dose levels tested. All kinds of observed aberrations like a breaks, gaps, Fragmentation's, Ring formation and Associations were found to be protected.
In the positive control group cyclophosphamide induced different types of the chromosomal aberrations at the dose level tested. *S. lycopersicum* extract alone did not induced the significant increase in frequency of any of these aberration at the test dose level and was found to be non-mutagenic. The test doses level of *S. lycopersicum* ext. protected against cyclophosphamide induced cell damage.

Table 1 Effects of *S. lycopersicum* (tomato) fruit extract on Chromosomal aberration in mouse bone marrow cells

S. N.	Groups	Mean± SE	Different aberration in %					% Inhib-ition
			Chro. Frag.	Chro. Break	Chro. Gap	Chro. Ring	Chro. Asso.	
1.	Cyclophosphamide alone 50mg/kg	59.33±2.36	18.00	15.66	12.33	6.67	6.67	-
2.	*S. lycopersicum* ext.500mg/kg+ Cyclophosphamide 50mg/kg	42.33±2.60*	12.33	10.33	9.00	6.00	4.67	28.65
3.	*S. lycopersicum* ext.1000mg/kg+ Cyclophosphamide 50mg/kg	34.00±2.88*	11.67	11.00	6.00	2.00	3.33	42.69
4.	*S. lycopersicum* ext.1500mg/kg+ Cyclophosphamide 50mg/kg	23.66±1.76*	11.00	7.67	1.67	2.00	1.33	60.12
5.	*S. lycopersicum* ext. (alone) 500 mg/kg	13.00±7.76	6.00	5.00	1.00	Nil	1.00	-
6.	Solvent (water)	11.33±1.20	4.33	5.00	2.00	Nil	Nil	-

* denotes statistically significant as compared to cyclophosphamide group at $p<0.05$.

Discussion

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. The present data demonstrate that In *S. lycopersicum* fruit extract was dose dependent inhibition of chromosomal aberration induced by CP in mouse bone marrow cells. *S. lycopersicum*, when tested for mutagenic effect at various test dose levels, failed to induce chromosomal aberration. The similar kinds of earliar studies have also been reported that several naturally occurring compounds exhibited antimutagenic activity. These include Indole-3-carbinol (I_3C) (15).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 (16). We have also found an anticarcinogenic effect of *S. lycopersicum* extract using skin papilloma and melanoma model (17).

The exact mechanism of protection is however unknown but *S. lycopersicum* (tomato) extract an active principal lycopene which have been shown to be able to participate in various mechanism of the chemoprevention virtue are acting as a neutrophillas an antioxidant. Several mechanisms may contribute to protection such as scavenging of potentially toxic electrophills and free radicals and modification of enzyme profile to inhibit that enhance the detoxification pathway. Antitumour potential of *S. lycopersicum* extract was also reported in S180 tumour (18). The present observation thus supports the mutagenic potential of *S. lycopersicum* extract in mammalian test system.

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