

## Prevention of Cyclophosphamide-Induced Micronucleus Formation in Mouse Bone Marrow by *Solanum lycopersicum* Extract

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**Abstract:** In this study, the protective effect of *Solanum lycopersicum* fruit extract is reported against cyclophosphamide (CP)-induced micronuclei formation in mouse bone marrow cells. The three test doses, namely 500, 1000 and 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 dose dependent inhibition of micronuclei 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 could not induced micronuclei formation at the test dose 500 mg/kg body weight. Its seem to have a preventive potential against CP-induced micronuclei formation in Swiss mouse bone marrow cells. Therefore, the results suggest a antimutagenic potential of *Solanum lycopersicum* fruit extract.

**Key words:** Micronucleus • Bone marrow • Chemopreventive • *Solanum lycopersicum* • Cyclophosphamide

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### INTRODUCTION

Tomato (*Solanum lycopersicum*) is the second most produced and consumed vegetable nationwide and it is a rich source of lycopene, beta-carotene, folate, potassium, vitamin C, flavonoids and vitamin E [1, 2]. Some epidemiological and experimental data suggest an inverse relation between intake of tomato and risk of cancer at various anatomical sites, especially prostate and colon [3-5]. It is believed that lycopene is a powerful antioxidant, a compound that blocks the action of activated oxygen molecules, known as free radicals that can damage cells [6, 7]. There are a few experimental studies on the role of lycopene in preventing or treating cancer is reported. One animal study found that lycopene treatment reduced the growth of brain tumors, suppressed breast tumor growth [8, 9].

A population-based case-control study found that *Solanum lycopersicum* based foods was associated with a small reduction in risk for prostate cancer [10]. High concentration of lycopene in prostate tissues resulted in a nearly three-fold increase in programmed cell damage among cancer cells. In animal studies the antitumour effect of lycopene was reported in S180 tumors [11]. 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 [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]. We have therefore undertaken antimutagenic effect of *Solanum lycopersicum* extract in Swiss mouse bone marrow cells of mice.

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## MATERIALS AND METHODS

**Animal:** 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).

**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: *Solanaceae*) 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.

**Micronucleus Assay:** For the micronucleus assay, the extract at the volume of 0.2 ml at different doses level such as 500, 1000 and 1500 mg/kg body weight was injected 24 hours before the treatment of cyclophosphamide, to six animals. The positive control group received single i. p. injection of 50 mg/kg cyclophosphamide in 0.9% saline. The animals were sacrificed by cervical dislocation and bone marrow cells were harvested. The slides were prepared essentially as described by Schmid [14] and modified by Aron *et al.* [15]. After staining with May-Gruenwald and Giemsa, a total 1000 cells were scored at the magnification of x1000 (100 x 10x) for each group. The data are expressed as the average number of micronucleated cells/thousand polychromatid erythrocytes cells (PCE) cells/animals ( $\pm$ SE) for a group of six animals. The results were compared with the vehicle control group using Student's 't' test with significance determined at  $p < 0.05$ .

### Groups:

Group 1 (Positive Control): The cyclophosphamide 50 mg/kg was given.

Group 2 (*S. lycopersicum* 500 mg/kg + Cyclophosphamide)

Group 3 (*S. lycopersicum* 1000 mg/kg + Cyclophosphamide)

Group 4 (*S. lycopersicum* 1500 mg/kg + Cyclophosphamide)

Group 5 Solvent alone: Solvent alone was given in this group.

**Animals:** The experiment was performed on the Swiss albino mice. The assay was divided into five different sub groups. Each group consisted of 10 animals.

**Rout:** The dose of the plant extract was given by i. p.

**Study Parameters:** Different types of micronucleus formation and PCE/NCE ratio.

## RESULTS AND DISCUSSION

In our study, a single administration of *S. lycopersicum* fruit extract resulted in a dose dependent inhibition of micronuclei formation induced by CP in mouse bone marrow cells. *S. lycopersicum*, when tested for mutagenic effect at various test dose levels, failed to induce micronucleus formation. 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 [12]. We have also found an anticarcinogenic effect of *S. lycopersicum* extract using skin papilloma and melanoma model [16].

*Solanum lycopersicum* fruit extract ip at 500, 1000 and 1500 mg/kg body weight was found to inhibit the micronuclei formation induced by CP given ip at 50 mg/kg body weight. A dose dependent response was remarkable and statistically significant (Table 1 and Fig. 1). In the positive control group CP induced micronucleus formation at the dose of 50 mg/kg body weight. It was noteworthy that different doses of *S. lycopersicum* and CP used in the present experiments were not cytotoxic for PCE/NCE (normochromatid erythrocytes) ratio in *S. lycopersicum* fruit extract treated and positive control as compared to the solvent control group, which remained unchanged (Table 1 and Fig. 2).

Table 1: Effect of *S. lycopersicum* (tomato) extract on micronucleus formation in mouse bone marrow cells.

S.N.	GROUPS	MN PCE $\pm$ SE	PCE/NCE $\pm$ SE
1.	Positive control Cyclophosphamide alone ( 50mg/kg)	3.24 $\pm$ 0.476	0.451 $\pm$ 0.006
2.	<i>S. lycopersicum</i> ext. (500mg/kg) + cyclophosphamide (50mg/kg)	2.00 $\pm$ 0.408*	0.536 $\pm$ 0.022
3.	<i>S. lycopersicum</i> ext. (1000mg/kg) + cyclophosphamide (50mg/kg)	1.5 $\pm$ 0.289*	0.667 $\pm$ 0.016
4.	<i>S. lycopersicum</i> ext. (1500mg/kg) + cyclophosphamide (50mg/kg)	1.00 $\pm$ 0.577*	0.730 $\pm$ 0.012
5.	<i>S. lycopersicum</i> ext. (500mg/kg) alone	0.50 $\pm$ 0.288	0.874 $\pm$ 0.028
6.	Solvent (water)	0.25 $\pm$ 0.249	0.947 $\pm$ 0.006

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

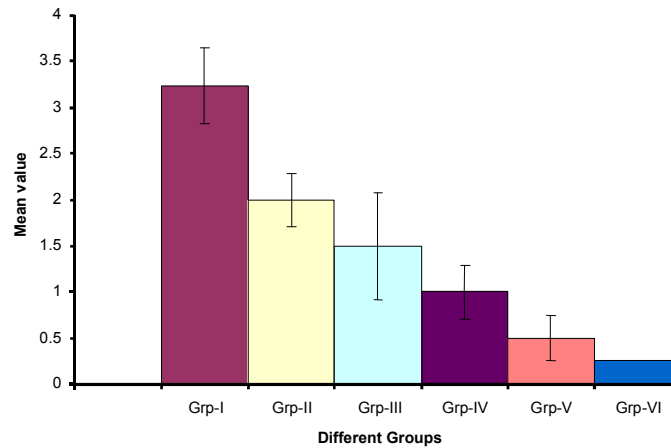


Fig. 1: Effect of *S. lycopersicum* fruit extract on micronuclei formation in Bone Marrow cells of the mice

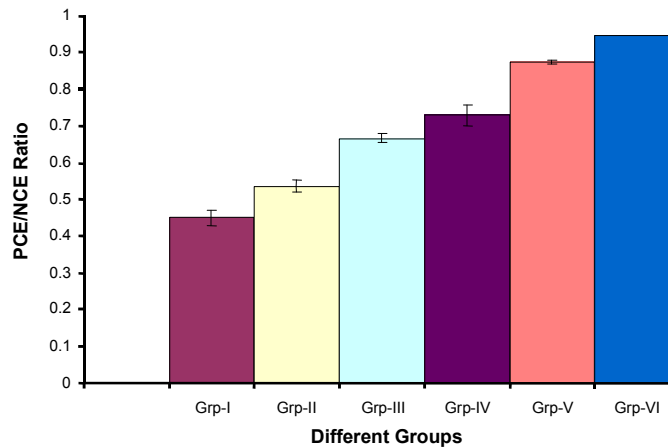


Fig. 2: Effect of *S. lycopersicum* fruit extract on PCE/NCE ratio in bone marrow cells of the mice

### CONCLUSIONS

The present study demonstrates that different doses of *Solanum lycopersicum* fruit extract was found to inhibit the micronuclei formation induced by CP given a dose dependent response was remarkable and statistically significant. 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 neutrophills 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 [17]. The *S. lycopersicum* fruit extract was also reported on dose dependent inhibition of chromosomal aberration induced by CP in mouse bone marrow cells [18]. These results are important with respect to

preventive aspects of diet and nutrition in chemical carcinogenesis. This result is important because the tomato is an important vegetable in Indian diet and considerable importance has been given for the role of tomato and lycopene in prevention of different types of diseases.

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