Contents lists available at www.innovativejournal.in

INNOVATIVE JOURNAL OF MEDICAL AND HEALTH SCIENCE

Journal homepage: http://www.innovativejournal.in/index.php/ijmhs

ISSN No. 2277-4939

PROTECTIVE EFFECTS OF *SOLANUM LYCOPERSICUM* FRUIT EXTRACT ON CYCLOPHOSPHAMIDE INDUCED MICRONUCLEI IN BONE MARROW CELLS OF MICE.

K. Rudrama devi*, Sri Vani, Minny Jael .P

Human genetics & toxicology lab, Department of Zoology, Osmania University, Hyderabad.

ARTICLE INFO

Corresponding Author:
K. Rudrama devi Human genetics & toxicology lab, Department of Zoology, Osmania University, Hyderabad.
rudramadevi_k@yahoo.com

Key words: Micronuclei, Cyclophosphamide, Bone Marrow Cells and *Solanum Lycopersicum*.

ABSTRACT

In the present investigations the antimutagenic effect of *solanum lycopersicum* fruit extract has been evaluated against cyclophosphamide (CP) induced micronuclei in bone marrow cells of mice. Single i.p administration of *solanum lycopersicum* fruit extract at various test doses namely 250, 500 and 1000 mg/kg b.w have given protection when 24hr prior administration of single doses of cyclophosphamide (50mg/kg b.w). A dose dependent inhibition of micronuclei was observed when animals were primed with *solanum lycopersicum* fruit extract. Thus the results indicate preventive effects of *solanum lycopersicum* fruit extract against cyclophosphamide induced genotoxicity in bone marrow cells of mice. Therefore the data showed *SL* fruit extract is a safer dietary component in cancer chemo preventive strategy.

INTRODUCTION

Cyclophosphamide is an anti cancerous alkylating agent. The metabolites of this compound can alkylate nucleophilic sites in DNA, RNA and protein [1,2]. It induces DNA single strand breaks at molecular level in rat embryos [3,4] in testicular cells [5]. Further cyclophosphamide is capable of inducing structural chromosomal aberrations in Chinese Hamster cells, in human chorionic villae and various stages of spermatogenesis in germ cells. Further cyclophosphamide exposure produced adverse effects on progeny outcome by altering sperm nuclear components. The morphometric analysis of head region of spermatozoa with chronic doses of cyclophosphamide showed significant increase over controls [6]. Further cyclophosphamide induced chromosomal aberrations in somatic and germ cells of mice [7].

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 [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 casecontrol 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 antimutagenic effect of *Solanum lycopersicum* fruit extract using the micronucleus test in mouse bone marrow cells.

MATERIALS AND METHODS

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 Prof. Prathiba devi (Voucher Specimen No: WR/101/LGOB/2006), Department of Botany, Osmania university, Hyderabad, Andhra 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 micronucleus test, three dose of *S. lycopersicum* i.e. 250, 5000 and 1000 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.

Swiss albino mice weighing about 22-24 g aged 8-10 weeks old were utilized in the present study. The drug was supplied by Reddy Labs Hyderabad. For each dose group of five animals were used. The animals were fed with 50 mg/kg cyclophosphamide intraperitonally in two installments within 24 hr interval. The control group of mice received 0.5 physiological saline simultaneously. The animals were scarified 6 hr after the last administration, bone marrow preparations were made by an air drying technique and stained with May Grunwald and Giemsa stains according to the method described by smith (1975). For each animal 2000 polychromatic erythrocytes (RBC) and corresponding normochromatic RBC were scored for the presence of micronuclei the appearance of micronuclei in polychromatic erythrocytes was used as an indicator of genetic damage. The ratio of polychromatic to normochromatic RBC was utilized to estimate the effect on the proliferative activity of bone marrow. The data obtained from these studies were analyzed using t-test adopted form Gold stein (1965).

RESULTS

Table 1: Results on the frequencies of micronuclei in bone marrow erythrocytes of mice treated with various doses of so *Solanum Lycopersicum* fruit extract.

	Micronuclei in polychromatic erythrocytes	Micronuclei in normochromatic erythrocytes	Micronuclei in total P+N cells	P/N ratio
Control -I	44/16000 (0.27)	14/16020 (0.08)	58/32020 (0.18)	0.99
Solanum Lycopersicum fruit extract 250 mg/kg	52/16000 (0.32)	32/16740 (0.19)	84/32740 (0.25)	0.95
500mg/kg	56/16000 (0.35)	40/17100 (0.23)	96/33100 (0.29)	0.93
1000mg/kg	60/16000 (0.37)	52/19960 (0.26)	112/35960 (0.31)	0.80

P>0.05

Table2: Protective effects of Solanum Lycopersicum fruit extract on cyclophosphamide induced micronuclei in bone marrow cells of mice.

	Micronuclei in polychromatic erythrocytes	Micronuclei in normochromatic erythrocytes	Micronuclei in total P+N cells	P/N ratio
Control-II	42/16000 (0.28)	15/16082 (0.09)	57/32082 (0.17)	0.99
Cyclophosphamide 50mg/kg	140/16000 (0.87)	32/16982 (0.18)	172/32982 (0.56)	0.94
250+50mg/kg	118/16000 (0.73)	42/17800 (0.23)	160/33800 (0.41)	0.89
500+50mg/kg	92/16000 (0.50)*	50/18000 (0.27)	142/34000 (0.41)	0.88
1000+50mg/kg	78/16000 (0.48)	58/20100 (0.48)	136/36100 (0.37)	0.79

*P<0.05**

The results on the induction of micronuclei in bone marrow erythrocytes of mice were depicted in Table 1. In Cyclophosphamide treated animals there was an increase in the polychromatic cells with micronuclei (Table 1). The frequency in control animal was 0.27% and the values were 0.32%, 0.35% and 0.37% after the administration of 250, 500 and 1000 mg/kg *Solanum lycopersicum* extract respectively (Table 1). The percentage of normochromatic cells with micronuclei was 0.18% in control mice, while the frequencies were 0.08%, 0.19% 0.23%, 0.26 SLE respectively (Table 1). The P/N ratio was 0.99 in control mice and it has decreased at all dose levels. The differences in the frequencies of micronuclei in polychromatic cells were in significant between the control and SLE treated groups (P>0.05).

DISCUSSION

In primed experienced, the frequency of micronuclei in bone marrow cells was 0.87% in CP treated animals. However the frequencies were reduced to 0.73%,0.50% and 0.48% after the administration of 250, 500 and 1000mg/kg of SLE 24hrs prior treatment. The significant protective effects of SLE was observed at 500 and 1000mg/kg SLE only (Table 2 P<0.05).

The invivo micronucleus test is one of best methods to screen the clastogenic effects of chemicals and drugs [20] using this procedure the mutagenicity of various alkylating agents [21-24] drugs [25] was also established. 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

and Lyco Vit 10% formulated products each contain approximately 10% synthetic lycopene. These products were evaluated for toxicological and behavioural effects during a 13-week oral dosing study with male and female Wistar rats. The no-observed-adverse-effect level (NOAEL) for this study was concluded to be 3000 mg/kg body weight per day for both Lycopene CWD and Lyco Vit [27]. The present data demonstrate that In *S. lycopersicum* fruit extract was dose dependent inhibition of micronuclei induced by CP in mouse bone marrow cells. *S. lycopersicum*, when tested for mutagenic effect at various test dose levels, failed to induce micronuclei. Pre-treatment with lycopene had significantly reduced the frequency of CP-induced bone marrow micronuclei [28]. The similar kinds of earliar studies have also been reported that several naturally occurring compounds exhibited antimutagenic activity. These include Indole-3-carbinol (I3C) [29].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 [30]. Further the protective role of lycopene on bisphenol a induced changes in sperm characteristics, testicular damage and stress in rats was reported [31].

In one study, lycopene inhibited human colon carcinoma, myeloid leukemia, and lymphoma cell lines in a dose-dependent manner [32]. Lycopene and eicosapentaenoic acid (EPA) also suppressed signal transduction pathways in human colon cancer cells, thus inhibiting cancer cell growth [33]. Another study documented activity against a liver adenocarcinoma cell line and noncancerous lung cell line [34]. Lycopene prevented chemically-induced DNA and chromosome damage and tumor-promoting activity in liver cells through antioxidant activity and inhibition of growth factors and signaling pathways [35]. 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 [36].

Carotenoids, as potential antioxidants, are well known as highly efficient scavengers of singlet oxygen ($^1O_2$) 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 [37]. Lycopene has been shown to have the highest antioxidant activity among the carotenoids in cell protection against hydrogen peroxide and nitrogen dioxide radical components. In addition, lycopene has been reported to attenuate oxidative stress and exert anticancer effects both in vitro and in vivo [38]. Previous studies reported that oral lycopene therapy in men with idiopathic infertility provided an improvement in male infertility, especially in sperm characteristics [39].Spermatozoa have been considered to be highly susceptible to the damage induced by ROS because of their high content of polyunsaturated fatty acids. To counteract the effects of ROS, spermatozoa are equipped with antioxidant defense systems, which prevent cellular damage [40]. In our study, significant decrease in abnormal sperm rates was observed in rats treated with CP alone, but normalization of these parameters was observed when animals were primed with lycopene. A rational mechanism for potential anticarcinogenic and antimutagenic effects of β-carotene and other carotenoids is their ability to scavenge free radicals that cause oxidative DNA damage [41]. These findings are in agreement with the data of the present study. The protective effects of lycopene against CP induced abnormal sperm rates may be attributed to the antioxidant properties of lycopene. These observations might also indicate that lycopene has protective role on CP induced genetic damage in bone marrow cells of mice. Naturally there is a dynamic balance between the amount of free radicals generated in the body and antioxidant defense system that scavenge them and protect the body against their deleterious effects [42].

ACKNOWLEDGEMENT

One of the authors Sri Vani, is thankful to Prof.L.Rajeshwari Anand, HOD, Department of Zoology, Osmania University for providing laboratory facilities and to Dr. Bhargavi, HOD, Department of Zoology, Vanitha college, for her encouragement for research work.

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