PROTECTIVE EFFECTS OF SOLANUM LYCOPERSICUM FRUIT EXTRACT ON LEAD INDUCED MICRONUCLEI IN BONE MARROW CELLS OF MICE

*K Rudrama Devi and K Dilip Reddy

Department of Zoology, University college of Science, Osmania university, Hyderabad.

ABSTRACT

In the present investigation the antimutagenic effect of Solanum lycopersicum fruit extract has been evaluated against lead nitrate induced genotoxicity. Single IP administration of Solanum lycopersicum fruit extract at various doses i.e. 250, 500 and 1000 mg/kg. When treated individually did not induce micronuclei in polychromatic erythrocytes of mice. A single IP of 40mg/kg, of lead nitrate induced significant increase in the percentage of micronuclei in bone marrow cells of mice. However after co administration of three doses of Solanum lycopersicum fruit extract there was a dose dependent decrease in the % of micronuclei was observed. Thus the results clearly indicate the preventive effects of Solanum lycopersicum fruit extract against lead 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.

KEY WORDS: Solanum Lycopersicum fruit extract (SLFE), Lead Nitrate, Micronuclei.

INTRODUCTION

Lead (Pb) is a dangerous heavy metal and harmful even in small amounts. Nevertheless, humans get exposed to Pb through their environment and diet (1). The manifestations of Pb poisoning in humans are nonspecific. They may include weight loss, anemia, (2, 3) memory loss, (4) nephropathy, infertility, liver, testis and heart damages’ (5, 6) , DNA damage (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 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 antimutagenic effect of Solanum lycopersicum fruit extract using the micronucleus test in mouse bone marrow cells.

MATERIALS AND METHODS

Materials and Methods

Chemical

Lead Nitrate was purchased from Merk Chemical Ltd. Other Reagent grades chemical were procured locally.

Extract Preparation

The identification of the plant Solanum lycopersicum (family: Solanaceae) was done by botanist Prof. Prathiba devi, 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 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, 500 and 1000 mg/kg body weight were administered. *S. lycopersicum* extract were dissolved in double distilled water and administered to mouse 24 hours prior to Lead Nitrate administration. 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 [19]. 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**

The results on the induction of micronuclei in bone marrow erythrocytes of mice are depicted in Table-1 and the photographs of micronuclei are shown in Fig-1 & 2. The frequency of micronuclei in control was 0.27% and the values were 0.38%, 0.40% and 0.42% after the administration of 250,500 and 1000mg/kg *Solanum Lycopersicum* extract respectively (Table-1). Hence, the results clearly indicate the non mutagenic nature of SL fruit extract. In lead nitrate treated group, there was a significant increase in the percentage of micronuclei(1.80) in bone marrow cells of mice when compared to control –II value (0.28) (Table-II). However the frequency of micronucleiis decreased to 0.68%, 0.56% and 0.45% after the co-administration of 250, 500 and 1000mg/kg of SLF extract. The P/N ratio in bone marrow cells showed a decrease when compared with control values and graphically represented in fig -3 and 4. The differences in the frequency of micronuclei in control and lead treated group were found to be significant (P< 0.05), Table-II. The percentage of micronuclei between lead treated group and SLF extract primed group were found to be significant.
Table-1: Incidence of micronuclei in bone marrow erythrocytes of mice treated with solanum.

<table>
<thead>
<tr>
<th>Dose group</th>
<th>Micronuclei, polychromatic erythrocytes</th>
<th>Micronuclei in normochromatic cells</th>
<th>Micronuclei in total P+N cells</th>
<th>P/N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>46/16000 (0.28)</td>
<td>16/16040 (0.09)</td>
<td>62/32040 (0.19)</td>
<td>0.99</td>
</tr>
<tr>
<td>Solanum lycopercicum</td>
<td>56/16000 (0.35)</td>
<td>34/16760 (0.20)</td>
<td>90/32760 (0.27)</td>
<td>0.95</td>
</tr>
<tr>
<td>fruit extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250mg/kg</td>
<td>60/16000 (0.38)</td>
<td>42/17400 (0.27)</td>
<td>102/33400 (0.30)</td>
<td>0.91</td>
</tr>
<tr>
<td>500mg/kg</td>
<td>64/16000 (0.04)</td>
<td>48/17200 (0.27)</td>
<td>112/33200 (0.33)</td>
<td>0.93</td>
</tr>
<tr>
<td>1000mg/kg</td>
<td>68/16000 (0.42)</td>
<td>54/18800 (0.28)</td>
<td>122/34800 (0.35)</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Fig -3: Incidence of micronuclei in bone marrow erythrocytes of mice treated with Solanum Lycopersicum
Table-2: Protective effect of *Solanum lycopersicum* fruit extract in lead induced genetic damage in mice.

<table>
<thead>
<tr>
<th>Dose group</th>
<th>Micronuclei in polychromatic cells in mice</th>
<th>Micronuclei in normochromatic cells in mice</th>
<th>Micronuclei in total P + N cells</th>
<th>P/N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-II</td>
<td>42/16000 (0.28)</td>
<td>14/16080 (0.08)</td>
<td>56/32080 (0.17)</td>
<td>0.99</td>
</tr>
<tr>
<td>Lead Nitrate</td>
<td>162/16000 (1.8)*</td>
<td>30/16900 (0.17)</td>
<td>190/32900 (0.59)</td>
<td>0.94</td>
</tr>
<tr>
<td>250 + LA</td>
<td>110/16000 (0.68)</td>
<td>44/17680 (0.24)</td>
<td>154/33680 (0.45)</td>
<td>0.90</td>
</tr>
<tr>
<td>500 + LA</td>
<td>90/16000 (0.56)</td>
<td>52/18200 (0.28)</td>
<td>142/34200 (0.41)</td>
<td>0.87</td>
</tr>
<tr>
<td>1000 + LA</td>
<td>72/16000 (0.45)</td>
<td>56/20200 (0.27)</td>
<td>128/36200 (0.35)</td>
<td>0.79</td>
</tr>
</tbody>
</table>

P> 0.05

Fig-4:- Protective effect of *Solanum lycopersicum* fruit extract in lead induced genetic damage in mice.

**DISCUSSION**

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 agent drugs [21- 24] 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 cause direct maternal or developmental toxicity in rats or rabbits at dosages as high as 2000 or 3000 mg/kg/day[25] 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 [26]. 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. Pretreatment with lycopene had significantly reduced the frequency of CP-induced bone marrow micronuclei [27]. The similar kinds of earil studies have also been reported that several naturally occurring compounds exhibited antimutagenic activity. These include Indole-3-carbinol (I3C) [28]. 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 [29]. Further the protective role of lycopene on bisphenol a induced changes in sperm characteristics, testicular damage and stress in rats was reported [30]. 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 [31]. 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 [32].

Carotenoids, as potential antioxidants, are well known as highly efficient scavengers of singlet oxygen (1O$_2$) and other excited species. During 1O$_2$ quenching, energy is transferred from 1O$_2$ 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 [33]. 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 [34]. Previous studies reported that oral lycopene therapy in men with idiopathic infertility provided an improvement in male infertility, especially in sperm characteristics [35]. 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 [36]. 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 Lead Nitrate
induced genetic damage in bone marrow cells of mice. The protective effect of lycopine is because of pigment principally responsible for the characteristic deep red colour of ripe tomato fruits and tomato products.

Lycopene has been shown to have the highest antioxidant activity among the carotenoids in cell protection against free radicals [37]. The present study showed that lycopene supplementation reduced the thyroid cellular changes induced by deltamethrin indicating that lycopene contributes to the protection against thyroid damage. Of all carotenoids, lycopene has been shown to exhibit the highest physical quenching rate constant with ROS [38-40]. [41] suggested that the administration of lycopene might alleviate deltamethrin-induced oxidative stress. It may also contribute to the prevention or amelioration of oxidative damage to cells and tissues both in vivo and in vitro [42;]. Moreover, studies have suggested that the anticancer effects of lycopene are related to their effectiveness as antioxidants [43; 44]. Antioxidant activity of tomato and tomato products was related to lipophilic constituents such as b-carotene, lycopene, and organic phenolic compounds [45]. Tomatoes and related tomato products are the major sources of lycopene, and are also considered an important source of carotenoids in the human diet [46]. Our laboratory has been published on the protective effects of ascorbic acid [47]. and garlic extract [ 48] against lead induced genotoxicity in invivo and invitro test system.

CONCLUSION
In conclusion our results demonstrated that lead nitrate is known mutagenic, lycopene could be a suitable agent for preventing the lead induced genotoxicity.

REFERENCES