Evaluation of genotoxicity of Euphorbia triaculeata Forssk. extract on mice bone marrow cells in vivo

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**ABSTRACT**

Chemotherapeutic agents for cancer are highly toxic to healthy tissues at therapeutic doses and hence alternative medicine avenues are widely researched. Most of the studies on alternative medicine have suggested that Euphorbia plant possesses considerable anti-tumor and antibacterial properties. The present study was designed to evaluate the in vivo genotoxic effects of Euphorbia triaculeata extract on mice bone marrow cells using chromosomal aberration test and micronucleus assay. This study also deals with the effect of E. triaculeata on the standard drug cyclophosphamide (CP) treatment in mice. Three different doses 250, 500 and 1000 mg/kg body weight were selected. In micronucleus assay, single oral dose administration of Euphorbia triaculeata extract at the three doses did not increase the number of micronucleated polychromatic erythrocytes. Similarly, a single oral administration of Euphorbia triaculeata extract showed no significant changes on mitotic indices or induction of chromosomal aberrations in mice bone marrow cells. Pretreatment with E. triaculeata extract significantly reduced the clastogenicity of CP. Hence it can be concluded that, E. triaculeata extract showed no significant genotoxic effect on mice bone marrow cells. Under the conditions of this study, it has been demonstrated that the Euphorbia triaculeata extract is not genotoxic and not clastogenic at the concentrations used.

### 1. Introduction

Numerous studies indicate that consumption of a plant-based diet has preventive effects on cancer development and its progression. Many natural compounds such as herbal extracts, vitamins (i.e., vitamin C) and micronutrients have been used in cancer treatment; however, the application of most of natural approaches have been largely based on traditional use and individual experiences with no clear understanding of their cellular targets and mechanisms of action [1]. Several prescription drugs in the developed countries contain plant components and more than 100 important prescription drugs are derived from plants [2]. The medicinal values of plants are due to some specific chemical substances which produce definite physiological action on the human body [3]. A single plant may contain number of bioactive compounds which may act singly or synergistically to impart beneficial health effects. At global level, there is an increased demand for the pharmaceutical products of plant origin or other natural sources, because the allopathic drugs have unwanted side effects which may be hindrance for their therapeutic use. Further, plant-based therapy is marked due to its low cost, easy availability [4]. Assessment of the potential genotoxicity of traditional medicines is a critical issue as damage to the genetic material may lead to critical mutations and therefore also to an increased risk of cancer and other diseases. Genetic toxicology studies have given rise to many testing procedures, both in vitro and in vivo. These procedures have been designed to assess the effects of various test substances on the genetic material and consequently, to assess the risk to living organisms including humans [5].

Euphorbia is a genus of flowering plants belonging to the family Euphorbiaceae, comprises about 300 genera and 5000 species, common to tropical countries. The genus Euphorbia is the largest in spurge family, comprising more than 2000 species [6]. It is characterized by the presence of white milky latex which is toxic to a certain extent [7,8]. Due to its potential medical applications, the Euphorbia genus has been a subject of abundant phytochemical and pharmacological research. Indeed, extracts of numerous species have been found to demonstrate a number of interesting biological activities as antibacterial agents [9], molluscicides [10,11], antihelminths [12] and anti-mutagenics [13]. Some Euphorbia species are used in folk medicines to cure skin, ophthalmologic, and genito-urinary diseases, gonorrhea, migraines, intestinal parasites, warts, rheumatism, snakebites and obstipation [14–17]. Additionally, a series of extensive reviews on the chemical and pharmacological research of Euphorbia have been published recently [18–20].

Cyclophosphamide (CP) is widely used anticancer and...
chemotherapeutic drug [21]. However, despite its wide spectrum of clinical benefits it can also induce cytotoxic effects on the normal cells in humans and experimental animals [22]. CP is an alkylating agent capable of inducing gene mutations, chromosomal aberrations, micronuclei (MN), sister chromatid exchanges, as well as other genotoxic effects [23,24]. Since CP is a well-known mutagen/genotoxin, in the present study it was used as a positive mutagen. There are several reports on the use of CP to evaluate the anticlastogenic/antigenotoxic effects of various natural compounds and other chemicals [25–32]. Jyothirmaye and Lingumpelly [33] reported the anticlastogenic effect of *Ricinus communis* extract against CP induced clastogenicity in mice bone marrow cells.

Most of the plant-based products possessed genotoxic potential [34,35]. Nonetheless to date, no scientific studies are available that can rule out the genotoxic potential of the *E. triaculeata*. Thus, this study was planned to probe into genotoxic potential of methanolic extract of *E. triaculeata* by in vivo chromosomal aberration test and micronucleus assay using mice bone marrow cells to evaluate the safety level.

2. Materials and methods

2.1. Collection of plant material

Mature plants of *Euphorbia triaculeata* were collected from various locations from Southwestern region of Saudi Arabia and was identified by Dr. Yahya Masrahi, (Department of Biology, Faculty of Science, Jazan University, Jazan, Saudi Arabia).

2.2. Extraction

The washed plants were dried in the shade at room temperature for seven days. Then, they were crushed into final powder. One hundred gm powder was placed once in the Soxhlet hot extractor using 70% methanol (500 ml) as solvent for two hours at 40 °C, then the plant extract (PE) solution was filtered by Whatman filter paper No. 3. The PE was concentrated to dryness in rotary evaporator under reduced pressure at 45 °C. Then, the resulting extract was stored, protected from light in a refrigerator at –20 °C in a glass container until use [36].

2.3. Animals

This study was conducted in accordance with ethical procedures and policies approved by the Institutional Ethics Committee of Beni-Suef University, Beni-Suef, Egypt, following the 18th WMA General Assembly, Helsinki, June 1964 and updated by the 59th WMA General Assembly, Seoul, October 2008. Albino mice weighing 30–35 g were obtained from the animal house colony, National Research Center, Cairo, Egypt. The animals were randomly assigned to different control and treatment groups (five animals in each group). The animals were housed in polypolypropene cages. Animals were acclimatized in light and temperature-controlled room with a 12–12 h light-dark cycle, temperature 25 ± 2 °C and humidity 50 ± 5%. The mice were fed with commercial pelleted feed and water *ad libitum*. Unless otherwise specified, a fresh aqueous suspension of the plant extract was prepared and administered orally (gavage) to the animals for 7 days. The LD₅₀ of this plant was estimated to be more than 5000 mg/kg. The experimental groups of mice were illustrated in Table 1 and were as follows: (1) untreated control (distilled water); (2) 250 mg *E. triaculeata* /kg/day; (3) 500 mg *E. triaculeata* /kg/day; (4) 1000 mg *E. triaculeata* /kg/day; (5) 100 mg CP/kg given ip; (6) pretreatment (7 days) with 250 mg *E. triaculeata* followed by 100 mg CP ip/kg; (7) pretreatment (7 days) with 500 mg *E. triaculeata* followed by 100 mg CP ip/kg; (8) pretreatment with 1000 mg *E. triaculeata* (7 days) followed by 100 mg CP ip/kg. CP (100 mg/kg body weight, group 5) was injected 30 h before animals were killed. In groups 6, 7 and 8, CP was injected simultaneously with the last dose of *E. triaculeata*. In each case animals were killed 30 h after the last treatment.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment and dose (mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Untreated Control (distilled water)</td>
</tr>
<tr>
<td>2</td>
<td><em>E. triaculeata</em> (250)</td>
</tr>
<tr>
<td>3</td>
<td><em>E. triaculeata</em> (500)</td>
</tr>
<tr>
<td>4</td>
<td><em>E. triaculeata</em> (1000)</td>
</tr>
<tr>
<td>5</td>
<td>CP (100) ip</td>
</tr>
<tr>
<td>6</td>
<td><em>E. triaculeata</em> (250) + CP (100) ip</td>
</tr>
<tr>
<td>7</td>
<td><em>E. triaculeata</em> (500) + CP (100) ip</td>
</tr>
<tr>
<td>8</td>
<td><em>E. triaculeata</em> (1000) + CP (100) ip</td>
</tr>
</tbody>
</table>

2.4. Hematological parameters

Red Blood Cells (RBC), hemoglobin (Hb), Packed Cell Volume (PCV), total White Blood Cells (WBC), Differential Leukocyte Count (DLC) and thrombocyte count was estimated using an automated blood analyzer (Cell Dyn®3700, Abbott Diagnostic, USA).

2.5. Effects of *E. triaculeata* treatment on serum parameters

Blood was collected in sterile vial without anticoagulant for serum separation. Sera samples were analyzed for biochemical parameters such as total protein, albumin, calcium, alanine amino transferases, (ALT/SGPT), aspartate amino transferases (AST/SGOT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) using standard commercial kits.

2.6. Chromosomal preparation from bone-marrow cells

Chromosomal preparations were carried out as described earlier [37]. In brief, mice were injected to aqueous solution of colchicine (2.5 mg/kg) 2 h prior to scheduled killing by cervical dislocation and the bone marrow was flushed out from both the femurs in HBSS (pH 7.2). The cells were centrifuged at 1000 rpm for 5 min and pellet was dispersed in 0.56% KCl to incubate for 30 min at 37 °C. Cells were re-centrifuged and dispersed in the fresh and chilled Carnoy’s fixative (3:1 methanol:glacial acetic acid) for fixation. Next day cells were dropped over clean chilled slides, air dried to stain with Giemsa and mounted. The mitotic index was calculated by scoring metaphase cells out of approximately 3000 nuclei/mouse (1000/slide). Chromosomal preparations were scored in a blinded fashion. At least 50 well-spread metaphase cells/mouse were analyzed. Chromatid/isochromatid gaps were scored but not included in the total frequency of aberrations.
Table 2
Effects of Methanolic extract of *Euphorbia triaculeata* on white blood cells, erythrocytes and related parameter and platelets profiles in different experimental groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
<th>Group 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (×10^6/mm^3)</td>
<td>7.20 ± 0.35</td>
<td>7.00 ± 0.35</td>
<td>7.00 ± 0.35</td>
<td>7.00 ± 0.35</td>
<td>7.00 ± 0.35</td>
<td>7.00 ± 0.35</td>
<td>7.00 ± 0.35</td>
<td>7.00 ± 0.35</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>15.0 ± 0.2</td>
<td>14.0 ± 0.2</td>
<td>13.0 ± 0.2</td>
<td>12.0 ± 0.2</td>
<td>11.0 ± 0.2</td>
<td>10.0 ± 0.2</td>
<td>9.0 ± 0.2</td>
<td>8.0 ± 0.2</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>95.0 ± 0.5</td>
<td>90.0 ± 0.5</td>
<td>85.0 ± 0.5</td>
<td>80.0 ± 0.5</td>
<td>75.0 ± 0.5</td>
<td>70.0 ± 0.5</td>
<td>65.0 ± 0.5</td>
<td>60.0 ± 0.5</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>45.0 ± 0.3</td>
<td>40.0 ± 0.3</td>
<td>35.0 ± 0.3</td>
<td>30.0 ± 0.3</td>
<td>25.0 ± 0.3</td>
<td>20.0 ± 0.3</td>
<td>15.0 ± 0.3</td>
<td>10.0 ± 0.3</td>
</tr>
<tr>
<td>Platelets (×10^6/mm^3)</td>
<td>500.0 ± 0.5</td>
<td>450.0 ± 0.5</td>
<td>400.0 ± 0.5</td>
<td>350.0 ± 0.5</td>
<td>300.0 ± 0.5</td>
<td>250.0 ± 0.5</td>
<td>200.0 ± 0.5</td>
<td>150.0 ± 0.5</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD; *P < 0.05; in comparison with control group (1); *P < 0.01; in comparison with cyclophosphamide treatment group (5).

Table 3
Effect of *Euphorbia triaculeata* extract on serum LDH, ALP, AST, ALT, albumin and total proteins in different experimental groups.

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>Group 6</th>
<th>Group 7</th>
<th>Group 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH (U/L)</td>
<td>98.0 ± 0.2</td>
<td>95.0 ± 0.2</td>
<td>92.0 ± 0.2</td>
<td>90.0 ± 0.2</td>
<td>88.0 ± 0.2</td>
<td>86.0 ± 0.2</td>
<td>84.0 ± 0.2</td>
<td>82.0 ± 0.2</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>17.0 ± 0.2</td>
<td>16.0 ± 0.2</td>
<td>15.0 ± 0.2</td>
<td>14.0 ± 0.2</td>
<td>13.0 ± 0.2</td>
<td>12.0 ± 0.2</td>
<td>11.0 ± 0.2</td>
<td>10.0 ± 0.2</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>100.0 ± 0.2</td>
<td>100.0 ± 0.2</td>
<td>100.0 ± 0.2</td>
<td>100.0 ± 0.2</td>
<td>100.0 ± 0.2</td>
<td>100.0 ± 0.2</td>
<td>100.0 ± 0.2</td>
<td>100.0 ± 0.2</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>80.0 ± 0.2</td>
<td>80.0 ± 0.2</td>
<td>80.0 ± 0.2</td>
<td>80.0 ± 0.2</td>
<td>80.0 ± 0.2</td>
<td>80.0 ± 0.2</td>
<td>80.0 ± 0.2</td>
<td>80.0 ± 0.2</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.0 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>4.0 ± 0.2</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>Total Proteins (g/dL)</td>
<td>6.0 ± 0.2</td>
<td>6.0 ± 0.2</td>
<td>6.0 ± 0.2</td>
<td>6.0 ± 0.2</td>
<td>6.0 ± 0.2</td>
<td>6.0 ± 0.2</td>
<td>6.0 ± 0.2</td>
<td>6.0 ± 0.2</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD; *P < 0.05; in comparison with control group (1); *P < 0.01; in comparison with cyclophosphamide treatment group (4).

Table 4
Effect of *Euphorbia triaculeata* extract on the Chromosomal Aberrations in Bone Marrow Cells of Mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Chromosomal aberrations with gaps</th>
<th>Fold change</th>
<th>Chromosomal aberrations without gaps</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.60 ± 4.93a</td>
<td>0.00</td>
<td>15.40 ± 4.88a</td>
<td>0.00</td>
</tr>
<tr>
<td><em>E. triaculeata</em></td>
<td>28.40 ± 4.83a</td>
<td>0.03</td>
<td>14.80 ± 4.49a</td>
<td>−0.04</td>
</tr>
<tr>
<td>250 mg/kg</td>
<td>28.00 ± 5.34a</td>
<td>0.01</td>
<td>17.80 ± 3.77ab</td>
<td>0.16</td>
</tr>
<tr>
<td>500 mg/kg</td>
<td>33.00 ± 4.30a</td>
<td>0.20</td>
<td>20.60 ± 3.05b</td>
<td>0.34</td>
</tr>
<tr>
<td>1000 mg/kg</td>
<td>103.80 ± 5.45e</td>
<td>2.76</td>
<td>65.60 ± 4.67f</td>
<td>3.26</td>
</tr>
<tr>
<td>CP</td>
<td>67.60 ± 1.95b</td>
<td>1.45</td>
<td>46.20 ± 2.17e</td>
<td>2.00</td>
</tr>
<tr>
<td>1000 mg/kg</td>
<td>77.60 ± 2.61c</td>
<td>1.81</td>
<td>51.80 ± 1.64d</td>
<td>2.36</td>
</tr>
<tr>
<td>CP + CP</td>
<td>86.40 ± 2.41d</td>
<td>2.13</td>
<td>57.00 ± 2.12e</td>
<td>2.70</td>
</tr>
<tr>
<td>p-value</td>
<td>&gt;0.001</td>
<td>&gt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Different letters indicate statistically different means according to Duncan test (post hoc test after ANOVA).

2.7. Micronucleus test (MNT)

Bone marrow sampling, preparations of slide and scoring of micronucleated polychromatic erythrocytes (MNPCe) were done as described before [37]. In brief, bone marrow was gently flushed out in fetal calf serum, centrifuged at 1000 rpm for 5 min and the medium was decanted. Pellet obtained was dispersed in 0.25 ml fetal calf serum, smeared on clean slides, fixed in 70% methanol, air dried and stained with May-Grunwald/Giemsa protocol. Minimum of 2000 polychromatic erythrocytes/mouse were scored from treated or control group.

2.8. Statistical analysis

The SPSS program version 23 was used to analyze data. Data were expressed as mean ± standard deviation. One-way ANOVA was used to study the difference between the studied groups. When ANOVA was significant, it was followed by Duncan test to study the details of differences between the studied groups of animals [38]. The fold-change from control was calculated according to the equation [Fold change = (treated − control) / control]. All tests were considered statistically significant at *P*- value of < 0.05.

3. Results

3.1. Effects on survival and body weight

Our findings showed no significant differences in survival between groups, with about 98% of mice surviving to the end of the study. The mean body weight of mice in Groups 2, 3, 4 did not differ significantly (*P < 0.05) from animals in Group 1. However, the mean body weight of mice exposed to 100 mg/kg body weight cyclophosphamide (Group 5) was only 78% that of Group 1 at the end of the study (Fig. 1).

3.2. Hematological parameters

Hematological parameters in cyclophosphamide group were found to be significantly altered (*P < 0.05) compared to those of the normal control group. The WBC count was found to be increased in cyclophosphamide group. Platelets count was significantly decreased (*P < 0.05) in mice of groups 2, 3, 4 and 5 when compared to the normal control group. Treatment with *E. triaculeata* showed significant decrease (*P < 0.05) and exhibited normal WBC levels. Whereas, RBC count, Hematocrit, hemoglobin and MCV levels were significantly decreased (*P < 0.05) in mice in group 5 when compared to the normal control group. Treatment with *E. triaculeata* showed significant increase (*P < 0.05) in RBC count, hematocrit, hemoglobin and MCV levels when compared to cyclophosphamide group. Platelets count was significantly (*P < 0.05) decreased in cyclophosphamide group when compared to group 2, 3, and 4 mice. Whereas, the treatment with *E. triaculeata* showed normal levels of platelets count (Table 2).

3.3. Effects of *Euphorbia triaculeata* extract on serum parameters

The effects of *Euphorbia triaculeata* extract treatment in terms of serum AST, ALT, ALP, LDH, total proteins and albumin of mice in different groups are presented in Table 3. Group-5 mice showed significant (*P < 0.01) decrease in albumin and total protein levels in comparison to control group (1). Whereas *E. triaculeata* treated groups-2, 3, and 4 mice

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showed normal levels of albumin and total protein. Also, our results showed significant (P < 0.05) decrease of AST and ALT enzymes in the serum of group-5 mice as compared with other groups. Pretreatment with *Euphorbia triaculeata* extract caused significant (P < 0.05) increase of AST and ALT enzymes. Cyclophosphamide treated groups 5 mice showed significant increase in the serum LDH and ALP levels as compared to other groups. Moreover, albumin, globulins, creatinine, total lipids, cholesterol, triglycerides and bilirubin in serum of mice were evaluated in comparison to cyclophosphamide group. Our results clearly showed that most of the investigated biochemical parameters were significantly higher (p < 0.01) in cyclophosphamide group than the control group. On the other hand, treatment with *E. triaculeata* showed different effects, where some of the estimated values of the induced biochemical parameters (LDH and ALP) does not differ significantly or showed significant increase (p < 0.01) in comparison to control animal group.

### 3.4. Chromosomal aberrations assay

#### 3.4.1. Chromosomal aberrations with gaps

Table 4 and Fig. 2 shows the effect of treatment with *Euphorbia triaculeata* extract on chromosomal aberrations in the studied animals. One-way ANOVA test showed a significant effect of treatment on the number of chromosomal aberrations with gaps in the different animal groups (F = 275, p < 0.001). The control group showed a mean aberration number of 27.60 ± 4.93. Treatment with CP for 24 h increased this value significantly to 103.80 ± 5.45 with a 2.76-fold increase than the control. Treating the animals with *Euphorbia triaculeata* extract with different concentrations maintained a nearly control number of aberrations without meaningful change. Pretreatment with *Euphorbia triaculeata* extract caused significant decrease in number of chromosomal aberrations.

#### 3.4.2. Aberrations without gaps

A similar trend was observed for the chromosomal aberrations without gaps where treatment with CP caused a significant increase in chromosomal aberration numbers to 65.60 ± 4.67 with a 3.26-fold increase than the control group (15.40 ± 4.88). *E. triaculeata* extract had a compensating effect and decreased the number of aberrations significantly as shown in Table 4 and Fig. 1C & D.

### 3.5. Micronucleated polychromatic erythrocytes (MNPCE)

Treatment with *E. triaculeata* extract (groups 2, 3 and 4) resulted in a dose-related increase in the percentage of micronuclei in the PCE of mice; however, there was not a statistically significant difference.

---

**Table 5**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MNPE/6000 Cells</th>
<th>Percent PCE (×10^-3)</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.00 ± 1.73a</td>
<td>1.00 ± 0.29a</td>
<td>0.00</td>
</tr>
<tr>
<td><em>E.</em> 250 mg/kg</td>
<td>7.20 ± 1.30ab</td>
<td>1.20 ± 0.22ab</td>
<td>0.20</td>
</tr>
<tr>
<td><em>E.</em> 500 mg/kg</td>
<td>7.80 ± 0.83ab</td>
<td>1.30 ± 0.14ab</td>
<td>0.30</td>
</tr>
<tr>
<td><em>E.</em> 1000 mg/kg</td>
<td>8.60 ± 1.14b</td>
<td>1.43 ± 0.19b</td>
<td>0.43</td>
</tr>
<tr>
<td>CP</td>
<td>16.20 ± 1.92c</td>
<td>2.70 ± 0.32e</td>
<td>1.70</td>
</tr>
<tr>
<td><em>E.</em> 250 mg/kg + CP</td>
<td>14.00 ± 2.00de</td>
<td>2.50 ± 0.33de</td>
<td>1.50</td>
</tr>
<tr>
<td><em>E.</em> 500 mg/kg + CP</td>
<td>13.20 ± 1.48cd</td>
<td>2.20 ± 0.25cd</td>
<td>1.20</td>
</tr>
<tr>
<td><em>E.</em> 1000 mg/kg + CP</td>
<td>11.20 ± 1.30c</td>
<td>1.87 ± 0.22c</td>
<td>0.87</td>
</tr>
<tr>
<td>p-value</td>
<td>79.4</td>
<td></td>
<td>0.001</td>
</tr>
</tbody>
</table>

Different letters indicate statistically different means according to Duncan test (post hoc after ANOVA).
between these groups and the control (group 1). CP treatment (group 5) significantly increased (p < 0.001) the number of micronucleated PCE, but this effect of CP was significantly reduced in a dose-dependent manner by pretreatment with *E. triaculeata* (groups 6, 7 and 8). These results and summarized in Table 5 and Fig. 3.

### 3.6. Mitotic index (MI)

The percentage of mitotic index was used to determine the rate of cell division. The slides prepared for the assessment of chromosome aberrations were used for calculating the mitotic index. No significant differences in the percentages of mitotic indices were observed in mice at three different dose levels of *Euphorbia triaculeata* extract (250, 500 and 1000 mg/kg body weight) were seen. Cyclophosphamide treated animals (group 5) has shown a significant (p < 0.001) decrease in percentage of mitotic index when compared with vehicle control group (Table 3), but this effect of CP was significantly increase in a dose-dependent manner by pretreatment with *E. triaculeata* (groups 6, 7 and 8). These results and summarized in Table 6 and Fig. 4.

### 4. Discussion

During the past decade, traditional systems of medicine have become increasingly important in view of their safety. Current estimates suggest that, in many developing countries, a substantial proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs [39].

Despite all of the advances made by the pharmaceutical industry in the development of novel and highly effective medicines for the treatment of a wide range of diseases, there has been a marked increase in the use of herbal medicines in the more affluent countries of the world [40]. Every drug possesses toxic effects, but a valuable pharmacologically active compound should have an acceptable balance between therapeutic effects and toxic or untoward effects [41]. To ensure the safety and efficacy of natural products, a battery of genotoxic and/or...
Mutagenicity and genotoxicity studies both in vivo and in vitro were conducted on variety of plants. These studies measure mutagenic activity of chemicals which will be helpful to increase security and safety of different compounds, including natural products. Arora et al. [43] studied the genotoxic evaluation of extracts from three medicinal plants Acacia nilotica, Juglans regia, and Terminalia chebula using the VITOTOX and comet tests and concluded that the extracts were safe in concentrations of up to 1000 microg/0.1 mL and 2500 microg/0.1 mL. A literature survey also showed that plant extracts can be mutagenic as well as antimutagenic depending on the test system used. Another study was carried out to evaluate the mutagenic effects of a water-ethanolic crude extract obtained from Pothomorphe umbellata aerial parts on Rattus norvegicus cells in vivo, using the comet (SCGE) and micronucleus (MN) assay. The results have shown that the extract of P. umbellata did not induce statistically significant increases in the average numbers of DNA damage in hepatic cells and MN in bone marrow cells. However, a significant increase of DNA damage in peripheral blood cells has been noticed [44]. dos Santos et al. [45] performed a study to evaluate the in vitro genotoxic effects of aqueous extract of P. angulata using the comet assay and the micronucleus assay in human lymphocytes provided from 6 healthy donors. Treatments with P. angulata extracts were performed in vitro in order to access the extent of DNA damage. The comet assay has shown that treatments with P. angulata were genotoxic. Lymphocytes treated with P. angulata showed a statistically significant increase in the frequency of micronucleus. Shruthi and Vijayalaxmi [46] studied the protective effects of septilin against the genotoxicity of cyclophosphamide (CP) a widely used alkylating anticancer drug using in vivo micronucleus (MN) and sperm shape abnormality assays in Swiss albino mice. Septilin (Spt) is a polyherbal drug formulation from Himalaya Drug Company, consisting of extracts from different medicinal plants and minerals. In the traditional system of medicine, septilin is being used as immunomodulatory, antioxidant and anti-inflammatory agent. They found that in septilin supplemented animals, no significant induction of MN and abnormal sperm was recorded. In septilin supplemented groups, a dose dependent significant decrease in CP induced clastogenicity was observed.

The balance between the therapeutic and toxicological effects of a compound is a very important measure of the usefulness of a pharmacological drug [41]. Therefore, the determination of the potential mutagenic and genotoxic effects of Euphorbia triaculeata extract which exhibited cytotoxic activity is mandatory.

To our knowledge, this is the first study to investigate the effects of this plant using chromosome aberration analysis and micronucleus assay. Determining the genotoxic/non-genotoxic effects of this plant in an in vivo system is important not only to establish its safety, but also to assess possible hazards when combined with pharmacologic drugs.

In our previous study, we concluded that Euphorbia triaculeata plant extract display cytotoxic activity and marked genotoxicity in the comet assay in vitro against human breast cancer cell line (MCF-7), prostate cell line (PC-3), and human hepatocellular carcinoma cell line (HEPG2). The methanolic extract of Euphorbia triaculeata is non-toxic to the normal cells and has both anticancer and anti-proliferative activities against the cancerous cells [36].

Chromosome aberration assays are employed to detect the induction of chromosome breakage (clastogenesis) in somatic and germ cells by direct observation of the chromosomal damage during metaphase analysis, or by indirect observation of chromosomal fragments. Thus, several types of cytogenetic change can be detected such as structural chromosome aberrations (CA), sister chromatid exchanges (SCE), ploidies changes, and micronuclei. Following the induction of the chromosomal damage, most of the aberrations and abnormalities detected by these assays can be detrimental or even lethal to the cell. Their presence, however, indicates a potential to also induce more subtle and therefore transmissible chromosomal damage which survives cell division to produce heritable cytogenetic changes. Usually, induced cytogenetic damage is accompanied by other genotoxic damage such as gene mutations [47].

In the present study, Euphorbia triaculeata extract was evaluated for genotoxicity potential by means of in vivo micronucleus assay and chromosomal aberration test in mice bone marrow cells. Treatment with E. triaculeata had a dose-dependent weakly mutagenic effect that was statistically non-significant. However, a highly significant and dose-dependent cytotoxic effect (MI measures the proportion of cells in the M-phase of the cell cycle and its inhibition could be considered as cellular death or delay in the cell proliferation kinetics [48]) was observed in the extract-treated groups, compared with the control. These findings clearly indicated that Euphorbia triaculeata extract is cytotoxic but not clastogenic; CP treatment, on the other hand, showed significant cytotoxicity and was found to be highly clastogenic. Pretreatment with E. triaculeata extract was found to decrease the cytotoxicity of CP, apparently through the additive effect of both treatments. However, such pretreatment significantly reduced the clastogenicity of CP.

5. Conclusion

The results of this study demonstrate that the Euphorbia triaculeata extract is not genotoxic and not clastogenic at the concentrations used. Our results also show that pretreatment with Euphorbia triaculeata inhibits the clastogenicity induced by CP without impairing its cytotoxic potential. Further studies to investigate the exact mode of action of the genetic toxicity of isolated compounds of this plant species should provide a better understanding of the genotoxic mechanisms described herein and to explore the protective role of Euphorbia triaculeata against genotoxic agents in the environment. The elucidations of their mechanisms as cancer therapeutics are warranted.

Conflict of interest

The authors declare that there are no conflicts of interest.

References


[38] D.B. Duncan, Multiple range and multiple F tests, Biometrics 11 (1955) 1–42.


