

## Research Article



## Evaluation of Genotoxicity and Effect of Petroleum Ether Extract of *Euphorbia lathyris* on Bone Marrow Cells: *In-Vivo* Study

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### ABSTRACT

Iraqi species of (*Euphorbia lathyris* F. Euphorbiaceae) have been used in the present study to evaluate genotoxicity of petroleum ether fraction in two doses (100mg/kg and 200mg/kg) on bone marrow cells in mice for seven successive days, comparing their effects with a positive control (methotrexate at a dose 20 mg/kg). Both doses of petroleum ether fraction of *Euphorbia lathyris* significantly decrease mitotic index ( $P < 0.05$ ) compared to dimethylsulfoxide (negative control) group. For total chromosomal aberration, the petroleum ether fraction of *Euphorbia lathyris* (in both doses) produced significant increase ( $P < 0.05$ ) compared to dimethyl sulfoxide (negative control) group. At the same time, 20mg/kg methotrexate significantly increases ( $P < 0.05$ ) in total chromosomal aberration compared to control.

**Keywords:** *Euphorbia lathyris*, genotoxicity, total chromosomal aberrations, mitotic index

### INTRODUCTION

**E**uphorbia *lathyris* is a species of spurge native to southern Europe (France, Italy, Greece, and possibly southern England), northwest Africa, and eastward through southwest Asia to western China. Other names occasionally used include Gopher Spurge, Gopher Plant or Mole Plant. Its belong to family (Euphorbiaceae)<sup>1</sup>.

*Euphorbia* is enormous genus belongs to the very diverse with at least 7,500 species. The variation within this genus is amazing; from low-growing garden weeds called spurges to giant, cactus-like succulents that rival in size. South African euphorbias have evolved succulent, spine-covered stems that greatly resemble North American cacti, a biological phenomenon known as convergent evolution<sup>2</sup>.

In folklore usages, people in Australia have used the milky sap of specific type of euphorbia to cure cancerous spots on their skin. The fresh sap is applied directly to the cancerous spot. After a few days the area develops into an enlarged, swollen sore, followed by a scab that eventually dries and falls off<sup>3</sup>. According to specific research, the sap penetrates the skin and destroys the malignant tissue. This is essentially what happens when a dermatologist applies liquid nitrogen directly to a superficial cancerous growth on the skin. A patent has already been applied for this remarkable discovery<sup>4</sup>.

Now a day several studies have been done to several types of euphorbia, they found that it had broad pharmacological effects, these effect includes; Antibacterial activity<sup>5,6</sup>, Antimalarial activity<sup>7</sup>, Anti-inflammatory activity<sup>8</sup>, Antidiarrheal activity<sup>9</sup>, Antioxidant activity<sup>10</sup>, Antiamoebic activity<sup>11</sup>, Antifungal activity<sup>12</sup>,

*Euphorbia lathyris* produce many types of active constituents. Its contain quercetin, kaempferol, diterpenoids, daphnetin, ingenol, b-sitosterol, p-coumaric acid, ferulic acid, lathyrol, esculetin, glycerides, isoprenoids, protein, rubber, oleic acid, and wax<sup>13-20</sup>.

Genotoxicity which is damage to cellular DNA is involved in mutagenesis and the development of cancer<sup>21</sup>. The DNA in a human cell undergoes several thousand to a million damaging events per day, generated by both external (exogenous) and internal metabolic (endogenous) processes<sup>22</sup>.

Changes to the cellular genome can generate errors in the transcription of DNA and ensuing translation into proteins necessary for signaling and cellular function<sup>22</sup>.

Genotoxicity tests can be defined as *in vitro* and *in vivo* tests designed to detect compounds, which induce genetic damage directly or indirectly by various mechanisms. These tests should enable hazard identification with respect to damage to DNA and its fixation<sup>23</sup>.

During normal course of life cycle, structural rearrangements in chromosomes do occur but less frequently but, under the effect of mutagenic agents such as radiations and conditions of stress like very high temperature, chromosomes are more prone to these changes<sup>24</sup>.

Structural changes in chromosomes were previously referred as chromosomal mutations. However, now, they are described as chromosomal aberrations not categorized as true mutations. In strict sense of the term, mutation is taken as architectural changes in gene (DNA) only<sup>25</sup>.



Despite a relatively large variety of different kinds of chromosome aberrations each aberration usually results in one of three possible outcomes:

1. Reduction of chromosomal segments within a chromosome or between chromosomes that leads to a recombination of genes.
2. Loss of genetic material from the entire chromosome or part of it.
3. Gain of a whole chromosome(s) or a chromosomal segment<sup>26</sup>.

Mitotic Index is defined as the ratio between the numbers of cells in a population undergoing mitosis to the number of cells not undergoing mitosis. The purpose of the mitotic index is to measure cellular proliferation.<sup>27</sup>

Mitotic index is an important prognostic factor predicting both overall survival and response to chemotherapy in most types of cancer<sup>28</sup>. An elevated mitotic index indicates more cells are dividing, and thus obvious in cancer cells, the mitotic index may be elevated during necessary processes to life, such as the normal growth of plants or animals, as well as cellular repair the site of an injury<sup>29</sup>. The decrease in the MI considered a delay in the cell proliferation kinetics and it may be suspected to be due to the cytotoxicity or genotoxicity of the drug at specific dose on mitosis<sup>30</sup>.

## MATERIALS AND METHODS

### Plants Collection

The plant was brought from the Iraqi market and authenticated by, Department of Pharmacognosy & Medicinal plants, College of Pharmacy, and University of Baghdad. A voucher sample was kept at the Department of Pharmacognosy & Medicinal plants, College of pharmacy/University of Baghdad. The plant seeds crashed by mortar and pestle to be extracted by reflux apparatus.

### Preparation of Petroleum Ether Fraction

Seeds were smashed into powder by triturating in a mortar, and then 500 gram of the powder was introduced in a soxhlet apparatus and extracted with 1500 ml of petroleum ether (30°-60°). Extraction was conducted for 15 hours then cooled and filtered. The extract was concentrated under vacuum until all the petroleum ether was removed. The yielding values were 153 grams for petroleum ether fraction<sup>31</sup>.

### Phytochemical Investigation

Preliminary phytochemical investigation was carried out for petroleum ether fraction using, Dragendorff's spray reagent and 5% ethanolic KOH spray reagent, vaniline/H<sub>2</sub>SO<sub>4</sub> reagent.

### Animals and Treatment Protocols

Twenty-four albino Swiss mice, weighing 23-27 g, were used in this study in accordance with the guidelines of the Biochemical and Research Ethical Committee at College of Pharmacy, University of Baghdad (Canadian Council on

Animal Care guidelines). Animals were purchased from the animal house of National Center for Drug Control and Research. They were housed for 2 days under standard conditions (well ventilated, temperature 22±2°C, relative humidity 50–60% and 12 h day and night cycle). Food consisted of normal animal chow and water was provided *ad libitum*. Care was taken to avoid stressful conditions. All experimental procedures were performed from eight to ten a.m. All the experimental work with the animals was carried out after obtaining approval from the Institutional Animal Ethical Committee. The animals were allocated into 4 groups (6 mice in each) and treated as follows:

First group treated with the vehicle (dimethylsulfoxide) and served as negative control; second group treated with methotrexate (20mg/kg) dissolved in water as intra peritoneally single dose; third and fourth groups are treated with 100 and 200mg/kg of dried petroleum ether fraction of *euphorbia lathyris* dissolved in dimethylsulphoxide respectively. Negative control group and test groups' treatments administered as intra peritoneally daily doses for seven consecutive days.

### Evaluation of Genotoxicity in Bone marrow

After seven days of treatment, all animals were injected intraperitoneally with 1mg/kg colchicines, and then two hours later they are sacrificed by cervical dislocation. Bone marrow samples was aspirated from the femur bone and processed using aseptic technique for evaluation of mitotic index and total chromosomal aberration as previously reported elsewhere<sup>32</sup>.

### Statistical Analysis

Data are expressed as Mean ± SD; unless otherwise indicated, statistical analyses were performed using unpaired *t*-test.

If the overall *F* value was found statistically significant (*P*<0.05), further comparisons among groups were made according to post *hoc* Tukey's test. All statistical analyses were performed using SPSS GraphPad InStat 3 (GraphPad Software Inc., La Jolla, CA, USA) software.

## RESULTS AND DISCUSSION

### Phytochemical Investigations

Phytochemical investigations revealed the presence of terpenoids in the petroleum ether fraction.

### Gene toxicity of different concentrations of petroleum ether fraction of *Euphorbia lathyris*

In Table (1), both doses (100mg/kg and 200 mg/kg) of petroleum ether fraction of *Euphorbia lathyris* significantly decrease mitotic index (*P*<0.05) when compared to negative control (dimethylsulfoxide), meanwhile a none significant increase in this parameter compared to positive control group (20mg/kg methotrexate) was seen on the dose to 100mg/kg, but when the dose is increased to 200mg/kg, mitotic index,



showed not significant decrease when compared to positive control (20mg/kg methotrexate).

**Table 1:** Incidence of mitotic index in bone marrow of mice treated with methotrexate and different doses of petroleum ether fraction of *Euphorbia lathyris*

| Treatment Groups                                      |          | Mitotic Index              |
|---|----------|----------------------------|
| Negative control (dimethylsulphoxide)                 |          | 5.12 ± 0.44                |
| Positive control (methotrexate 20mg/kg)               |          | 2.1 ± 0.23 <sup>*a</sup>   |
| Petroleum ether fraction of <i>Euphorbia lathyris</i> | 100mg/kg | 2.52 ± 0.41 <sup>*aA</sup> |
|   | 200mg/kg | 1.62 ± 0.49 <sup>*aB</sup> |

Data for mitotic index are expressed as Mean ± S.D; \*significantly different compared to negative control (P<0.05); values with non-identical superscripts (a, b) among treatment groups are significantly different (P<0.05), values with non-identical superscripts (A, B) between test groups are significantly different (P<0.05).

In Table (2), which concerning the effect on chromosomal aberration, the petroleum ether fraction of *Euphorbia lathyris* (in both doses 100mg/kg and 200 mg/kg) produced significant increase in this marker compared to negative control (P<0.05), (Figures 1, 2, 3). Meanwhile, this fraction at a dose 100 mg/kg showed significant decrease in this parameter when compared to methotrexate but a dose 200mg/kg showed significant increase in aberration when compared to positive group.

**Table 2:** Incidence of total chromosomal aberrations in bone marrow of mice treated with methotrexate and different doses of petroleum ether fraction of *Euphorbia lathyris*

| Treatment Groups                                      |          | Total Chromosomal Aberrations |
|---|----------|-------------------------------|
| Negative control (dimethylsulphoxide)                 |          | 0.121 ± 0.08                  |
| Positive control (methotrexate 20mg/kg)               |          | 0.322 ± 0.009 <sup>*a</sup>   |
| Petroleum ether fraction of <i>Euphorbia lathyris</i> | 100mg/kg | 0.289 ± 0.008 <sup>*bA</sup>  |
|   | 200mg/kg | 0.51 ± 0.01 <sup>*bB</sup>    |

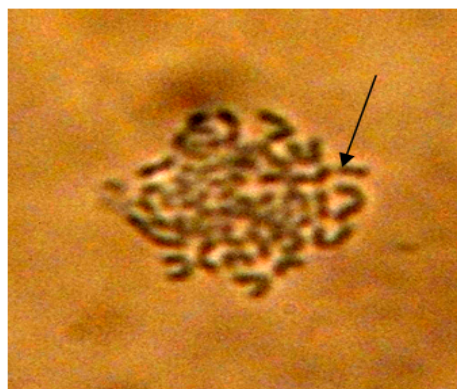
Data for total chromosomal aberration are expressed as Mean ± S.D; \*significantly different compared to negative control (P<0.05); values with non-identical superscripts (a,b) among treatment groups are significantly different (P<0.05), values with non-identical superscripts (A,B) between test groups are significantly different (P<0.05)



**Figure 1:** Chromosomal aberration (ring chromosome) in bone marrow of mice treated with *Euphorbia lathyris* petroleum ether fraction



**Figure 2:** Chromosomal aberration (chromosomal break) in bone marrow of mice treated with *Euphorbia lathyris* petroleum ether fraction



**Figure 3:** Chromosomal aberration (chromosomal gap) in bone marrow of mice treated with *Euphorbia lathyris* petroleum ether fraction

Phytochemical investigation showed that *Euphorbia lathyris* contain terpenoid compounds besides several types of fixed oils especially croton oil as reported in previous study<sup>33</sup>. Croton oil which is very toxic oil, Small doses taken internally cause diarrhea. Externally, the oil can cause irritation and swelling. Croton oil is the source of the organic compound phorbol<sup>34</sup>. This oil reported to induce chromosomal aberrations<sup>35</sup>. At the same time, phorbol which is derived from croton oil also reported to have clastogenic effects in many research<sup>36</sup>.

Terpenoid which is other active constituents that present in the petroleum ether fraction of *Euphorbia lathyris* have anticlastogenic effect which reported in many researches<sup>37,38</sup>.

According to the results of the present study, it have been seen that, at a dose 100 mg/kg of petroleum ether fraction of *Euphorbia lathyris* showed significant increase in total chromosomal aberrations of bone marrow cells when compared to negative control also when the dose increased to 200 mg/kg of this fraction showed significant increasing in the total chromosomal aberration of bone marrow cells when compared to negative control, even significantly elevated when compared to the methotrexate which is the positive control (high clastogenic effect).

This high tendency of clastogenic effect of this fraction is could be return to croton oil and its phorbol which overwhelm the other anticlastogenic compounds like terpenoid which is also present in the same fraction.

When there is a chromosome aberration, cell cycle is stop in order ether to repair the damage, or stop division at all or it may be undergoing apoptosis.<sup>39</sup>

According to this, the mitotic index which is reflected the tendency of cell to divide, have been decrease in both dose when compared to negative control.

## CONCLUSION

In the present study, the clastogenic effect of fixed oil like croton oil and phorbol dominated on the effect of anticlastogenic effect of terpenoid which is also present in the same fraction.

This causing raise in total chromosomal aberration and decreasing in mitotic index as a results. The domination is occur ether due to high quantity of croton oil and phorbol and/or low quantity of terpenoids.

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