

Evaluation of the Genotoxicity of the Aerial Parts of Iraqi *Euphorbia cyathophora* on Bone Marrow and Spleen Cells in Mice

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Abstract

Euphorbia cyathophora Family: Euphorbiaceae, is an ornamental annual herb that has a poisonous latex. Traditionally it was used for fever, and mouth sores. Various phytochemicals were identified in this plant; flavonoids, tannins, glycosides, and others that resulted in its proven pharmacological effects.

Evaluation of the Genotoxic effect of methanolic fraction of aerial part of *Euphorbia cyathophora* on bone marrow cells and spleen cells in mice. 200 gm of *E. cyathophora* fine powder extracted by cold maceration 80% ethanol for seven days. The extract was filtered and dried in a rotary evaporator then the dried extract was suspended with water and consecutively extracted using chloroform, ethyl acetate for each. The aqueous layer was then mixed with 100ml methanol. Methanol fraction was dried under reduced pressure to obtain the dry extract. Twenty-four Albino mice were divided into four groups: Group 1: Mice were treated with distilled water daily for seven successive days. Group 2: Mice were treated with a single dose (20mg/kg) of methotrexate (positive control). Group 3: Mice were treated with (100mg/kg) of menthol fraction for seven successive days. Group 4: Mice were treated with (200mg/kg) of methanol fraction for seven successive days. Mice were sacrificed by (spinal dislocation). Samples of bone marrow cells and spleen cells were taken and genotoxic analyses.

Methanol fraction of *Euphorbia cyathophora* at a dose of 100mg/kg demonstrated a significant decrease in mitotic index and a significant increase in total chromosomal aberrations as compared to distilled water in both bone marrow cells and spleen cells ($p < 0.05$). 100 mg/kg and 200 mg/kg of methanol fraction of *Euphorbia cyathophora* that showed to be significantly higher in mitotic index and significantly lower in total chromosomal aberration as compared to methotrexate ($p < 0.05$).

The present study revealed that the methanol fraction of the aerial parts of *Euphorbia cyathophora* is genotoxic but its genotoxicity is less than that of methotrexate.

Keywords: *Euphorbia cyathophora*, genotoxicity, methotrexate

تقييم السمية الجينية للأجزاء الهوائية من الفربيون العراقي السياتوفورا على نخاع العظام وخلايا الطحال في الفئران

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الخلاصة

ان الهدف من الدراسة هو استخلاص المركبات الموجودة في الاجزاء الهوائية من *Euphorbia cyathophora* مع الميثانول وتقييم تأثيره على مؤشر الانقسام والانحراف الكروموسومي الكلي خلية نخاع العظام و خلية الطحال في الفئران. تم نزع 200 جرام من مسحوق *E. cyathophora* الناعم ثم استخلاصه بالنقع البارد 80٪ إيثانول لمدة سبعة أيام. يرشح المستخلص ويجفف في مبخر دوار ثم يمزج المستخلص المجفف مع الماء ويستخلص على التوالي باستخدام الكلوروفورم، أسيتات الإيثيل لكل منهما. ثم بعد ذلك خلط الطبقة المائية مع 100 مل ميثانول. يتم تجفيف هذه المستخلصات تحت ضغط منخفض للحصول على المستخلص الجاف. تم استخدام أربعة وعشرين فأر أبيض للتجربة. تم تقسيم الحيوانات إلى أربع مجموعات: المجموعة الأولى: عولجت الفئران بالماء المقطر. تعطى الجرعة يومياً لمدة سبعة أيام متتالية. المجموعة الثانية: عولجت الفئران بجرعة وحيدة (200 مغم / كغم) من الميثوتريكسات (السيطرة الإيجابية). المجموعة الثالثة: عولجت الفئران ب (100 مغم / كغم) من جزء المنقول لمدة سبعة أيام متتالية. المجموعة الرابعة: عولجت الفئران بجرعة (200 مغم / كغم) من الميثانول لمدة سبعة أيام متتالية. تم التضحية بالفئران عن طريق (خلع العمود الفقري). تم أخذ عينات من خلايا نخاع العظام وخلايا الطحال وتحليل السمية الجينية. لقد تسبب مستخلص الميثانول من *Euphorbia cyathophora* بجرعة 100 مغم/كغم في انخفاض كبير في مؤشر الانقسام وزيادة كبيرة في إجمالي الانحرافات الصغوية مقارنة بالماء المقطر في كل من خلايا نخاع العظام وخلايا الطحال ($p < 0.05$). 100 مغم / كغم و 200 مغم / كغم من جزء الميثانول من *Euphorbia cyathophora* الذي أظهر أنه أعلى بشكل ملحوظ في مؤشر الانقسام وأقل بكثير في إجمالي انحراف الكروموسومات مقارنة بالميثوتريكسات. $P < 0.05$. أظهرت الدراسة الحالية أن مستخلص الميثانول من الأجزاء الهوائية من الفربيون سام وراثياً ولكن سمية مستخلص الميثانول أقل من تلك الموجودة في الميثوتريكسات.

الكلمات المفتاحية: *Euphorbia cyathophora*، السمية الجينية، انحراف الكروموسومات. الفلافونويدات

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Introduction

For thousands of years, the majority of the world's population used traditional medicine, and about 80% of developing countries people rely on traditionally used medicinal plants for disease management⁽¹⁾. The discovery of the medicinal or toxic potential of the plant was assessed during their use for therapeutic purposes⁽²⁾. Plants hold certain therapeutic components with remarkable physiological effects which can be extracted and used in drug preparation⁽³⁾. Lots of the medicinal plants possessed cytotoxic, genotoxic, and mutagenic potential, therefore their uses might be associated with a higher incidence of tumor formation⁽⁴⁾. Further investigations are required before the consumption of these plants, particularly in the terms of their mutagenic potential⁽²⁾. *Euphorbia cyathophora* is also called *Poinsettia cyathophora* Murray⁽⁵⁾, it is family Euphorbiaceae (Spurge family). In the wild world, about 300 genera and 8000 species are included in this family, but most members of this family have poisonous milky latex⁽⁶⁾. *Euphorbia cyathophora* is naturally grown or indigenous in tropical America and cultivated in tropical and subtropical areas as ornamental. This annual herb rises to one-meter height⁽⁷⁾. Traditionally the airy parts of the plant were used for fever, jaundice, mouth sores, and improved milk production⁽⁸⁾. The previous study for the aerial part shows that there are different types of active constituents which include flavonoids, tannins, glycosides, saponins, and cardiac glycosides are present in aerial parts of the plant, other studies show the anti-inflammatory, antioxidant, antidiabetic, antibacterial, hepatoprotective and significant anti-Parkinson activity^(9, 10). Silver nanoparticles of aqueous extract of *Euphorbia cyathophora* leaves exhibit an anticancer effect against HT-29 cell line⁽¹¹⁾.

Genotoxicity is the destruction of cellular genetic materials which may result in undesirable consequences as mutagenesis or tumor induction⁽¹²⁾. Genotoxicity was evaluated by different tests such as the bacterial Ames test, chromosomal aberration (cytogenetic parameter), and other tests. Chromosomal aberration can be performed through vivo and in vitro studies⁽¹³⁾.

Methotrexate (MTX) is an anti-metabolite drug widely used in the treatment of neoplastic disorders, rheumatoid arthritis, and psoriasis. Developed as an analog of folic acid, it inhibits purine and pyrimidine synthesis that accounts for its therapeutic efficacy as well as for its toxicities. MTX has a narrow therapeutic index and its toxicity has been reported in various organ systems including gastrointestinal, haematologic, and central nervous systems⁽¹⁴⁾.

The basis for its therapeutic efficacy is the inhibition of dihydrofolate reductase (DHFR), a key enzyme in the folic acid (FA) metabolism. FA is reported to

have protective effects on MTX-induced genotoxicity in the somatic cells⁽¹⁵⁾.

This study was intended to evaluate the genotoxic potential of *Euphorbia cyathophora* methanolic fraction by measuring mitotic index and the total chromosomal aberration in both bone marrow cells and spleen cells in mice.

Materials and Methods

Collection and authentication

Euphorbia cyathophora aerial parts were obtained from the medicinal plants garden in the college of pharmacy/ University of Baghdad during May/2021, authenticated by Assist. Prof. Dr. Sokaena Abass in the College of the Science / University of Baghdad. The desired aerial parts were rinsed with tap water and then dried at room temperature for fourteen days, grounded in a grinder to a fine powder.

200 gm of *E. cyathophora* fine powder was defatted twice in 800 ml hexane for seven days, the defatted plant material was then extracted twice by cold maceration using 800 ml 80% ethanol for seven days. The extract was filtered and dried in a rotary evaporator yielding dry extract. The dried extract was suspended in 100ml water and consecutively extracted using chloroform and ethyl acetate (100ml x 3) for each. The aqueous layer was then mixed with 100ml methanol. These fractions (chloroform, ethyl acetate, and methanol) are dried under reduced pressure to obtain dry extract⁽¹⁶⁾.

Preliminary chemical tests

Preliminary phytochemical analysis was carried out for methanolic fraction using Mayer's reagent (alkaloids), 5% ethanolic KOH (flavonoids), FeCl₃ (tannins), foam test for saponins, Benedict's test for sugars^(17, 18), and Ninhydrin test for proteins⁽¹⁹⁾.

Experimental model

Twenty-four Albino Swiss mice (*Mus musculus*) were used for each experiment. They were supplied by the animal house/Department of Pharmacology and Toxicology/ University of Baghdad. Their weights were 20-25 grams. They were divided into four groups; each was kept in a separate plastic cage. The animals were maintained at a temperature of 23 – 25°C, and they had free excess to food (standard pellets) and water (*ad libitum*).

The animals were divided into four groups (six mice of each) as follow:

Group 1: Mice were treated with distilled water. This group was served as normal control the dose was given (I.P.) daily for seven successive days.

Group 2: Mice were treated with a single dose (20mg/kg) of methotrexate. This group was served as a positive control⁽²⁰⁾.

Group 3: Mice were treated (oral) with (100mg/kg) of menthol fraction of *Euphorbia cyathophora* for seven successive days.

Group 4: Mice were treated (oral) with (200mg/kg) of methanol fraction of *Euphorbia cyathophora* for seven successive days.

After seven days of treatment, all animals were injected intraperitoneally with 1mg/kg colchicine, and then two hours later they are sacrificed by cervical dislocation. Bone marrow samples were aspirated from the femur bone and processed using an aseptic technique for evaluation of mitotic index and total chromosomal aberration as previously reported elsewhere⁽²¹⁾.

Statistical analysis

Data are expressed as Mean \pm SD; unless otherwise indicated, statistical analyses were performed using ANOVA test. If the overall P value was found statistically significant ($P < 0.05$), further comparisons among groups were made according to post hoc Tukey's test.

Results and Discussion

The phytochemical analysis of the methanol fraction of *Euphorbia cyathophora* (Table 1) revealed the existence of flavonoids, tannins, sugar, proteins, and saponins, while the alkaloids are absent⁽¹⁰⁾.

Table 1. Phytochemical analysis of methanol fraction of *Euphorbia cyathophora*

Compound	Flavonoids	tannins	saponins	alkaloids	sugar	Proteins
Result	+	+	+	-	+	+

In the Table (2), the methanol fraction of *Euphorbia cyathophora* at a dose of 100mg/kg caused a significant decrease in the mitotic index as compared to distilled water in both bone marrow cells and spleen cells ($p < 0.05$). 100 mg/kg and 200 mg/kg of methanol fraction of *Euphorbia cyathophora* showed to be significantly higher in the mitotic index as compared to methotrexate ($p < 0.05$), also there were significant differences between methanol fraction doses ($p < 0.05$).

Table 2. Incidence of mitotic index in the bone marrow and spleen cells of albino mice treated with different doses of the methanol extract of *Euphorbia cyathophora* compared to methotrexate and distilled water.

Treatment Groups	Mitotic Index	
	Bone Marrow Cells	Spleen Cells
Distilled water (Normal control)	5.12 \pm 0.44	3.38 \pm 0.2
Methotrexate (MTX) (positive control) 20mg/kg	2.1 \pm 0.23*	1.31 \pm 0.27*
Methanol Fraction 100mg/kg	3.1 \pm 0.21* ^{#A}	1.98 \pm 0.4 ^{#A}
Methanol Fraction 200mg/kg	4.62 \pm 0.11 ^{#B}	2.22 \pm 0.17 ^{#B}

Data are expressed as mean \pm S.D; n=6 animals in each group; *significantly different compared to distilled water (negative control) ($P < 0.05$); #significantly different compared to methotrexate group (positive control) ($P < 0.05$); Values with non-identical capital letters superscripts (A, B) consider significantly different when compared between tests doses ($P < 0.05$).

In Table (3), the methanol fraction of *Euphorbia cyathophora* at both doses caused a significant increase in total chromosomal aberration as compared to distilled water in both bone marrow cells and spleen cells ($p < 0.05$). 100 mg/kg and 200 mg/kg of methanol fraction of *Euphorbia cyathophora* showed to be significantly lower in total chromosomal aberration as compared to methotrexate ($p < 0.05$) also there were significant differences between methanol fraction doses ($p < 0.05$).

Table 3 . Total chromosomal aberration in the bone marrow and spleen cells of albino mice treated with different doses of the methanol extract of *Euphorbia cyathophora* compared to methotrexate and distilled water.

Treatment Groups	Total Chromosome Aberration	
	Bone Marrow	Spleen Cells
Distilled water	0.121 \pm 0.08	0.108 \pm 0.08
Methotrexate (MTX) (Positive)	0.322 \pm 0.01*	0.272 \pm 0.23*
Methanol Fraction 100mg/kg	0.282 \pm 0.03* ^{#A}	0.142 \pm 0.06* ^{#A}
Methanol Fraction 200mg/kg	0.276 \pm 0.01* ^{#B}	0.139 \pm 0.05* ^{#B}

Data are expressed as mean \pm S.D; n=6 animals in each group; *significantly different compared to distilled water (negative control) ($P < 0.05$); #significantly different compared to methotrexate group (positive control) ($P < 0.05$); Values with non-identical capital letters superscripts (A, B) consider significantly different when compared between tests doses ($P < 0.05$).

In the Table (2) both doses of methanol fraction, mitotic index was significantly higher as compared to MTX in both bone marrow and spleen cells in a dose-dependent manner ($p < 0.05$), in the Table (3), there was a significant decrease in total chromosomal aberration as compared to MTX ($p < 0.05$).

The mitotic index is an indicator for cell genome health, a previous study showed that any damage or disruption in the genetic material (chromosomes) will activate a repair mechanism, this activation leads to prevent cell division and a decrease in the mitotic index as a result⁽²²⁾.

According to phytochemical investigation for *Euphorbia cyathophora*, tannins and flavonoids are major active constituents present in the plant. A previous study has been shown that tannin has a genotoxic activity which manifested by an increase in chromosomal aberration and a decrease in mitotic index⁽²³⁾, this explain the increase in chromosomal aberration in both bone marrow cells and spleen cells when compared with negative control at the same time it explains the decrease in the mitotic index in both bone marrow cells and spleen cells as compared to the negative control.

Other important active constituents that are present according to the phytochemical investigation are flavonoids. Flavonoids constitute a group of polyphenolic compounds characterized by a common gamma-benzo-pyrone structure considered in numerous biological systems to possess an antioxidant capacity. A previous study has been investigating the effect of flavonoids on chromosome integrity, and found that flavonoids had a protective effect on chromosomes and decrease in the chromosomal aberration⁽²⁴⁾.

In the present study, the presence of genoprotective flavonoids with genotoxic tannin explain the significant decrease in total chromosomal aberration in both methanol fraction as compared with methotrexate, besides the increase in the mitotic index in both doses as compared to methotrexate also related to the mixture of both compounds (flavonoids and tannin). Besides the increase in total chromosomal aberration for the methanolic fraction in both bone marrow cells and spleen cells as compared with the healthy group give a hint that either the quantity of tannic acid as higher than the quantity of flavonoids or the clastogenic effect of tannic was higher as compared to the anti-clastogenic effect of flavonoids.

Conclusion

The current study demonstrated that the methanol fraction of aerial parts of Iraqi *Euphorbia cyathophora* is genotoxic but its genotoxicity is less than that of methotrexate.

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Conflicts of Interest

The authors declare that there is no conflict of interest

Ethics statements

The study was approved by ethical Committee of the College of the Pharmacy/ University of Baghdad (acceptance number 2285 on 7/3/2022).

Author Contribution

Equally contributed

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