In Vivo Study the Cytogenetic Effect of Ammi majus Methanolic Extract on Mitotic Index, Micronucleus Formation and DNA Damage on Mitoxantrone Treated Albino Male Mice

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Abstract

Medicinal plants or their secondary metabolites have shown different biological effects with a wide range of pharmacological properties; for instance, immune stimulator, anti-bacterial, anti-viral, anti-inflammatory, anti-oxidant, anti-mutagenic, anticancer, hepatoprotective, and many other properties. One of these medicinal plants is Ammi majus. The purpose of this research was to evaluate the cytogenetic impact of a methanolic extract of the Ammi majus plant on micronucleus formation, mitotic index and DNA damage in mice that had been given intraperitoneally mitoxantrone medicine (anticancer drug). Mice were divided into four groups, each group consisting of four mice: group I (positive control), mice treated with 0.008 mg/mouse mitoxantrone, group II (negative control), mice not given any treatment, group III, and group IV (interaction groups), mice treated with 50 mg/kg and 100 mg/kg of plant extract three through seven, respectively, after receiving injections of the mitoxantrone drug on days one and two. The findings showed that, in comparison to positive and negative controls, the plant has the ability to induce the mitotic index to 10.67±2.33% and 12.65±3.01% for 50 and 100 mg/kg respectively, compared to 5.31±1.33% for mitoxantrone treated mice. In addition, the results of micronucleus formation indicated the capacity of the plant to reduce its formation to 2.63 ±0.011 and 2.47±0.012 micronucleus/cell after treating the mice with Ammi majus two doses (50 and 100 mg/kg), in comparison to the drug that enhances micronucleus formation to (4.28± 0.00 micronucleus/cell). DNA damage results explained the ability of the plant to reduce DNA tail damage to 134 ±22.3 and 117 ±16.1% for 50 and 100 mg/kg of Ammi majus extract, respectively which increased to (176 ±11.23%) after mice were treated with drugs. All these findings may be attributed to plant-active compounds such as flavonoids and other constituents.

Keywords: Anticancer, Male mouse, Medicinal plant, Metaphase index, Methanolic extract.

Introduction

Herbal medicine is the oldest form of health care known to mankind, and medicinal plants have been used by all cultures throughout history. It represents an integral part of modern civilization development.
Herbal medicine is a major component of all indigenous people's traditional medicine and a common element in Ayurvedic, Homoeopathic, Naturopathic, Traditional Arabic, Oriental and Native American Indian medicine, and the WHO documented further that out of 119 plants derived pharmaceutical medicines, approximately 74% are used in modern medicine. Research on these healing plants, including toxicological and pharmacological analyses, is essential for the study of medicine and for public health initiatives. Based on pharmacological activity, researchers are constantly working on various plant components as well as various polarities of extracts to identify and isolate certain pharmacologically active phytochemicals that may be utilized as a treatment for illnesses.

From antiquity, researchers have continuously worked to understand the many aspects of the chosen plant species' pharmacological medicinal uses. Ammi majus is a natural vegetable plant that is often used to cure various illnesses and possesses different names, such as: the Arabic name Khillah, Khillah shytani, the English name Bishops weed, Latin and German name Ammi, and the French name Ammi commun. The fruits, stems, leaves, and roots are often used to treat illnesses. Ammi majus has a larvicidal impact on mosquito larvae and is utilized as an antibiotic, antifungal, and antioxidant. These pharmacological effects are a result of the valuable chemical components of the product, which mostly include flavonoids, polyphenolic chemicals, and essential oils including khellin and visnagin. According to reports, the plant's essential oil has antiviral and antibacterial properties, but the flavonoid concentration is what gives it its antioxidant properties. Moreover, the plant contains a chemical called -pyrone that acts on the kidney by minimizing renal colic and smoothing up stone passages in addition to having a relaxant impact on smooth muscle, particularly that of the coronary arteries. The plant is considered as field of winter crops in the Nile Delta and Valley, as well as in the Oases and the Mediterranean region. It is also distributed in North Africa, the Middle East, as well as western Asia. Currently, the selected plant is widely distributed in most of the European countries, India and tropical countries due to its significant biological activity and traditional use.

Mitoxantrone (a synthetic anthraquinone) is an anticancer drug that has an asymmetrical structure; a planar heterocyclic ring (tricycles planar) and two basic side chains. A Pharmacacological effect of mitoxantrone is suggested to be closely related to its ability to bind DNA, and formation of the complex mitoxantron-DNA. Such a complex is able to trap and stabilize the enzyme Topoisomerase-II, which is an essential part of human cell replication and transcription machinery. The mitoxantrone-DNA-topoisomerase-II complex is able to induce apoptosis in cell. Therefore this research aimed to evaluate the ability of Ammi majus leaves methanolic extract to counteract any damage caused by chemotherapeutic drug mitoxantrone via in vivo study.

Materials and Methods

Plant gathering, naming, and extraction

The period of plant gathering is from April to June 2021, seeds were gathered from Baghdad gardens in Iraq. The Iraqi national herbarium verified their authenticity. Fresh Ammi majus leaves were rinsed with running water, and then allowed to dry naturally before being ground. The extract was created using the technique described by . A little under 50 g of plant powder was steeped in 250 ml of 80% methanol for an hour, subjected to sonication for 24 hours, and then filtered through a Buchner funnel twice. A rotary evaporator was used to dry the filtered solution, and after dry freezing, it was kept chilled at -20°C until usage.

Amounts of plant (doses of plant in vivo)

Two doses of plant extract (50, 100 mg/kg) were administered to mice in a model. The dried methanolic extract was dissolved in DMSO and distilled water to make these dosages. Whereas just one dosage of the mitoxantrone medication (0.008 mg/mouse; after calculated dose according to company leaflet) was utilized.
Animals used in research

Twenty albino mice, each weighing between 23 and 25 g, were employed for this investigation. The animals were acquired and cared for at the Biotechnology Research Center's animal home at Al-Nahrain University. The animals had unrestricted access to food and fresh water for drinking. They were split into four groups, each with four mice: group I (positive control), mice treated with 0.008 mg/mouse mitoxantrone, group II (negative control), mice not given any treatment, group III, and group IV, mice given injections of mitoxantrone drug for days one and two, followed by doses of plant extract (50 and 100 mg/kg) for days three through seven, respectively. All material was intraperitoneally administered in a single dosage on day 8 and sacrificed for testing in the lab.

Cytogenetics tests

Metaphase Index test

To estimate the metaphase index, cells collected from bone marrow after colchicine solution 0.25 ml were intraperitoneally given into a mouse, and after 1.5 to 2 hours, the animal was killed by cervical dislocation and dissected to extract femur cells. 5 ml of physiological saline was used to collect the cells, and tubes were centrifuged at 2000 rpm for 10 minutes. After discarding the supernatant, 5-8 ml of KCl (0.075 M) were added for 30 minutes. The supernatant was then discarded after being centrifuged for 10 minutes at 2000 rpm. After 30 minutes in the refrigerator (4°C), 5 ml of the fixative was added, and tubes were centrifuged for 10 minutes. The cell deposit was well mixed with 2 ml of fixative, deposited onto a spotless slide, stained for 15 minutes with Giemsa stain, and then thoroughly washed with distilled water. By dividing the total number of cells by the number of split cells*100; these were used to calculate the percentage of cells in metaphase (metaphase index).

Micronucleus formation assay

The method described in [13] was used to examine the formation of micronuclei: a mouse was slaughtered, and cells from the femur were collected with 2 ml of heat-inactivated human AB plasma. The tube was then centrifuged at 1000 rpm for 10 minutes, and the supernatant was discarded. A tiny smear of the cellular deposit was produced on a slide, carefully mixed, and stained for 15 minutes with Giemsa stain before being washed with distilled water. The number of micronucleus cells was used to calculate the micronucleus index; at least 1000 polychromatic erythrocytes (PCE) were examined for the presence of micronucleus formation.

Alkaline comet assay

Warm PBS (37°C) was eroding bone marrow from the femoral bone. Thereafter, 50 l of molten LM Agarose 37°C was pipetted at a ratio of 1:10 (v/v) onto a comet slide, which was then left at 4°C in the dark for 10 minutes. Slides were submerged for 30 to 60 minutes in 4°C lyses solution and for 20 minutes in room temperature alkaline unwinding solution. Slides were introduced to the Alkaline Electrophoresis Solution, set on an electrophoresis slide tray, and covered with a slide. Samples were dried for 10 to 15 minutes at 37°C. Each circle of dried agarose on the slide received 100 l of diluted SYBR Green, which was stained for 30 minutes followed by an examination by a fluorescence microscope. At least 50 randomly selected cells were analyzed per sample and the quantification was done by using the image analysis software comet score [14].

Statistical Analysis

The values of the investigated parameters were given in terms of mean ± standard deviation (SD), and differences between means were assessed by analysis of variance (ANOVA) using the computer programme SPSS version 13.0.

Results and Discussion

Micronucleus formation

The results of micronucleus formation in bone marrow cells declared that *Ammi majus* extract was able to prevent the development of micronuclei by mitoxantrone drug to be (2.63 ±0.011 and 2.47±0.012 micronucleus/cell) after mice were treated with *Ammi majus* extract at doses of (50 and
100 mg/kg), respectively, in comparison to drug (4.28± 0.00 micronucleus\cell) as shown in Figs. 1

**Figure 1.** Shows the development of micronucleus in several mouse groups.

**Bone marrow mitotic index of male albino mice**

In the control negative group mice, the percentage of the mitotic index was 15.32±3.11% compared to the medication's (mitoxantrone treated mice) in which the percentage of mitotic index was 5.31±1.33%, mice treated with plant extract following drug administration had mitotic indices of 10.67±2.33% and 12.65± 3.01% for 50 and 100 mg/kg, respectively as explained in Figs. 3 and 4.

**Figure 2.** Bone marrow cells of mice treated with mitoxantrone showing micronucleus formation (100X; Giemsa stain)

**Figure 3.** Shows the percentage of the mitotic index in several mouse groups.
In order for plants to generate therapeutic molecules and ultimately be employed by the medical industry to treat life-threatening ailments including heart disease, cancer, HIV, and diabetes, sophisticated biotechnology was used to create pharmaceutical plants. One of the most significant medicinal plant essential oils and their antioxidant properties is *Ammi majus* 15. This study’s species demonstrated a basic oil product and its anticancer properties. Bergapten, iso imperatorin, and imperatorin are important components of *ammi majus* and have antioxidant and cancer-inhibitory properties 16. Xanthotoxin (methoxsalen, 8-methoxypsoralen (8-MOP) ammoidin), marmesin, and heraclenin are a few more well-known dynamic combinations in *Ammi majus*. All three have antioxidant and anti-carcinogenic properties. There are more substances as well, and some of them, like flavonoids, may function in different routes to protect the genetic makeup 17. The flavonoid compounds possessed in addition to radical scavenging activity (antioxidant activity) serve other bio-functions like antimutagenic and anti-tumor activities 18. Flavonoids may have preservative consequences to the endogenous scavenging compounds by stimulating the activity of the most important detoxifying enzymes such as glutathione transferase (GST) and superoxide dismutase (SOD). Flavonoids have also been shown to inhibit nitric oxide in a dose-dependently manner. Additionally, it has been reported that flavonoids are the most potent inhibitor of xanthine oxidase. Another possible mechanism by which flavonoids act on reactive oxygen species is through interaction with various enzyme systems, and some effects may be a result of a combination of radical scavenging and interaction with enzyme functions 19. These findings have also been further augmented to be involved in increasing the mitotic index of bone marrow, these actions have been attributed to the chemical constituents of the plant with regard to the forthcoming repair mechanisms 20. It has been found that flavonoids enhance post-replication repair and stimulate the mechanism of error-free repair 21. In addition to the anti-oxidants and electrophile scavengers activity of flavonoids, other biological functions can be achieved; a stimulation of the immune system, inhibition of DNA adducts with carcinogens, inhibition of hormonal actions
and metabolic pathway associated with the development of cancer, and inducing phase I or II detoxification enzymes. Significant reduced frequency of MN formation can be consequenced in the light of these functions and the results of this study suggest that the administration of *ammi majus* is safe and with beneficial anti-mutagenic potential and has a protective effect on the DNA within the bone marrow cells of treated animals, as suggested by the reduced frequency of MN-induction. In addition to that, *Ammi majus* may exhibit anticancer potential against breast cancer cell lines due to coumarin xanthotoxin that possessed cytotoxic effects on a variety of cell lines, according to earlier reports.

**Conclusion**

The conducted study detailed the ability of the *Ammi majus* plant to counteract the damage caused by mitoxantrone chemotherapeutic drug through evaluation of micronucleus formation, percentage of mitotic index in addition to decreased damaged DNA. As described by results, the plant is able to decrease micronucleus formation, enhances mitotic index and decrease DNA damage after interaction with medical drug. All these activities may be attributed to secondary metabolite present in the plant.

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**Authors’ Declaration**

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Al-Nahrain.

**Authors’ Contribution Statement**

-R. M. Al.: Collected the experimental data and arranged it as a graph.
-M. H. A.: Wrote the conclusion section and arranged references according to the journal author guidelines.

-H. M. Kh.: Wrote the introduction section and the goal of the study.

**References**


دراسة التأثير الوراثي الخلوي للمستخلص الميثانولي لنبات الخلة داخل جسم الكائن الحي على معامل الانقسام الخلوي، تكوين النوى الصغيرة وتحطم الدنا الوراثي (DNA) على الفئران البيض المعاملة بعقار الميتوزانترون

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الخلاصة
تتمثل النباتات الطبية أو مركبات الأيض الثانوي فيها فوائد بيولوجية مختلفة وهامة صيدلانية على سبيل المثال: محفزات مناعية، مثبطات للكبد، مضادات البكتريا، مضادات السرطان، حماية الجلد، وفعالية بيولوجية أخرى. هذه النباتات الطبية في نبات الخلة. وقد صممت هذه الدراسة للكشف عن التأثير الوراثي الخلوي للمستخلص الميثانولي لنبات الخلة على معامل الانقسام الخلوي. نصيب الفرد بالأدوية والتركيز (8.880 ملغ/كغم) ونسبة انقسام العينة إلى معامل الانقسام في الفئران. استخدمت في هذه الدراسة أربعة جماعات: جماعة الفئران (4 فئران لكل مجموعة). المجموعة الأولى (الفئران المعالجة بعقار الميتوزانترون) 100 ملغ/كغم، المجموعة الثانية (الفئران المعالجة بعقار الميتوزانترون) بتراكيز 8.880 ملغ/كغم. الفئران الغير معالجة. أما المجموعة الثالثة والرابعة (مجاميع التفاعل) كانت الفئران المعالجة بالعقار لليومين الأول والثاني ومن اليوم 3 إلى 7 عوامات الفئران بالمستخلص النباتي بتراكيز (50 و100 ملغ/كغم) على التوالي. أظهرت النتائج قابلية النبات على زيادة معامل الانقسام الخلوي إلى 10.67±2.33% و12.65±3.01% للتركيزات 50 و100 ملغ/كغم على التوالي بالمقارنة مع الفئران المعالجة بالعقار الذي اقتسمه الفئران إلى 5.31±1.33%. أظهرت نتائج قتص النوى الصغيرة قدرة المستخلص النباتي على خفض معدل تكوين النوى إلى 1.01±0.012 و2.63±0.47 تواجـًا. مشكلة نتائج الحمض النووي النباتي وذ كالتراكيز على زيادة النوى إلى 2.48±0.28 و0.00±0.00 تواجـًا. كما أظهرت نتائج الحمض النووي النباتي على تقليل ذلك إلى 11.23% و22.3±134% للجرعتين المستخدمتين بالمقارنة مع المعاملات النباتية. ممكن أن تؤدي النتائج التي تم الحصول عليها إلى المركبات الفعالة الموجودة بالنبات كالفلافونويد وغيرها.

الكلمات المفتاحية: مضادات السرطان، المستخلص الميثانولي، النباتات الطبية، ذكر الفأر، معامل الانقسام الاستوائي.