The Effect of *Artemisia Dracunculus* L. on Mitotic Index in Bone Marrow and Spleen Cells of Mice: *In Vivo* Study

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Abstract

Artemisia dracunculus L. (tarragon) is a rich source of herbal remedies with antioxidant and anti-inflammatory properties. In the present study, the proliferation of bone marrow and spleen cells of mice was evaluated after extraction of *Artemisia dracunculus* with 80% of ethanol. Two doses of the extract (500mg/kg body weight and 1000mg/kg body weight) were given to the mice for seven successive days. On day eight, mice were sacrificed and cells from bone marrow and spleen were collected; mitotic index was calculated and results were compared with that of methotrexate at a dose 20mg/kg body weight (positive control) and distilled water (negative control). The results showed that the dose 500mg/kg body weight of *Artemisia dracunculus* extract caused significant increase in mitotic index in both bone marrow and spleen cells of mice when compared with negative control. While, the dose 1000mg/kg body weight of *Artemisia dracunculus* extract caused significant decrease in mitotic index in both bone marrow and spleen cells of mice when compared with negative control.

Keyword: *Artemisia dracunculus, bone marrow cells, methotrexate, mitotic index spleen cells*

Introduction

Artemisia dracunculus (*A. dracunculus*) L., commonly as tarragon, is a perennial herb in the Asteraceae (daisy) family that has a long history of use in culinary traditions. *A. dracunculus* possesses a wide range of health benefits and has therefore been widely used as herbal medicine[1]. The botany and chemical constituents are well described in the literature, the latter mainly focusing on its essential oil composition that determines its distinct flavor. Additionally, a wide range of secondary metabolites (flavonoids, phenylpropanoids, coumarins, etc.) are reported, deter-mining *A. dracunculus* biological activities and its potential useas a source for plant-derived pharmaceutical chemical entities and complex extracts[2].

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In traditional medicine, *A. dracunculus* is commonly used to improve a malfunctioning digestive system by increasing appetite, to flush toxins from the body, and as a digestive stimulant, especially in cultures with a high consumption of red meat[3]. Arabic cultures have used *A. dracunculus* to treat insomnia and to dull the taste of medicines. Additionally, *A. dracunculus* has also been used as an anesthetic for aching teeth, sores, and cuts. It has been used widely in central Asia and Russia for the treatment of skin wounds, irritations, allergic rashes, and dermatitis[4]. In the traditional medicine of Azerbaijan, tarragon was used as an antiepileptic, laxative, antispasmodic, and carminative remedy[5].

A. dracunculus have been reported to have an important groups of biologically active secondary metabolites like essential oil, coumarins, flavonoids, and phenolic acids[6]. *A. dracunculus* usually contains >1.0% coumarins including, herniarin, coumarin, esculetin, esculin, capillarin, 8-hydroxycapillarin, artemidin, 8-hydroxyartemidin, artemidinol, and others[7]. Coumarins have long been recognized to possess anti-inflammatory, antioxidant, antiallergic,

hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities[8].

Different extracts from the aerial parts of *A. dracunculus* have revealed IC50 values ranging from 80 to 150 µg/mL against L5178Y lymphoma cells, and that 100 mg/kg of acetonitrile extract administered for 15 successive days has resulted in significant decrease in L5178Y lymphoma tumor size in mice[9].

Mitotic index is a measure for the proliferation status of a cell population, and defined as the ratio between the number of cells in mitosis and the total number of cells. The mitotic index can be worked out from a slide, even with light microscopy[10]. There is a direct relationship between cancer and value of mitotic index; cancer cells have high mitotic index and they grow uncontrollably and divide fast. In a normal lung tissue, the percent of dividing cells is 5% while in a cancerous lung the percent of dividing cells is 25%[11]. Low mitotic index indicates slower proliferation kinetics. Cytotoxic or genotoxic agents may interfere with mitosis rate by different mechanisms; including, interfering with tubulin polymerization or tubulin associated proteins[12].

The study aims to study the effect of the ethanolic extract of *A. dracunculus* on the mitotic index of cells obtained from the lymphoid organs bone marrow and spleen.

Materials and Methods

Plant Collection

The plant was brought from the Iraqi market. A voucher sample was kept at the department of pharmacognosy & medicinal plants, College of pharmacy/ University of Baghdad. The plant's aerial parts were air-dried at room temperature and crushed by mortar and pistol.

Preparation of an ethanol extract of *A. dracunculus*

One hundred fifty grams of dried aerial part were heated to 80°C with 1 liter of 80% ethanol for 2 hours. The extraction was continued for an additional 10 hours at 20°C. The extract was then filtered through filter paper and evaporated with a rotary evaporator; the yield weight was 23.2 gram[13].

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 College of Pharmacy, University of Baghdad. 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 follow: the first group was treated with the vehicle (distilled water) and served as negative control; the second group was treated with methotrexate (20mg/kg) dissolved in water and administered intraperitoneally (i.p.) as a single dose; the third and the fourth groups were treated with 500 and 1000mg/kg of *A. dracunculus* extract dissolved in distilled water; respectively. Negative control group and test groups' treatments were administered as i.p. daily doses for seven consecutive days.

Preparation of Bone Marrow Cells

After seven days of treatment, all animals were injected i.p. with 1mg/kg colchicine (Sigma, USA); two hours later, they were scarified by cervical dislocation. Bone marrow samples was aspirated from the femur bone and processed using aseptic technique for evaluation of mitotic index as previously reported elsewhere[14].

Assessment of Mitotic Index: the number of cells in division expressed as a percentage of the total number of cells was calculated, according to the following formula[15] :

Mitotic index = (Number of cells in mitosis /Total number of cells) × 100

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.

Percent of the response is calculated as follow:

Response %=A-B/B×100

Where; A= values for tests groups or positive control B=values for negative control

Results

As shown in table 1 and 2, the dose 500mg/kg of *A. dracunculus* was associated with a significant increase in mitotic index of bone marrow and spleen cells (39.5% and 47.4%; respectively) ($P<0.05$); and the dose 1000mg/kg was associated with a significant decrease in this parameter of bone marrow and spleen cells (-51.5%, -46.8%; respectively) ($P<0.05$), both compared with the distilled water treated group. Methotrexate treated group showed (-74.3% and -84.5%) ($P<0.05$) decrease in mitotic index in bone marrow and spleen cells; respectively, compared with the distilled water treated group.

Table 1: Mitotic index and response percent in bone marrow of mice treated with distilled water, methotrexate and different doses of *A. dracunculus* extract in distilled water

Mitotic Index of Bone Marrow	Mean ±SD	Response %
Distilled water (negative control)	8.62±0.42	
Methotrexate (20mg/kg) (positive control)	2.21*a±0.14	-74.3%
A. dracunculus 500mg/kg	12.02*Ab±1.16	39.5%
A. dracunculus 1000mg/kg	4.18*Bb±0.73	-51.5%

Data for mitotic index are expressed as mean±SD; *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 ($P<0.05$).

The mitotic index levels in methotrexate treated group, in both bone marrow and spleen cells, were significantly lower than that of *A. dracunculus* treated groups; moreover, mitotic index in animals treated with the dose 1000mg/kg of *A. dracunculus* was significantly lower than those treated with the dose 500mg/kg.

Table 2: Mitotic index and response percent in spleen of mice treated with distilled water, methotrexate and different doses of *A. dracunculus* extract in distilled water

Mitotic Index of Spleen	Mean ±SD	Response %
Distilled water (negative control)	7.11±0.24	
Methotrexate (positive control) (20mg/kg)	1.1*a±0.25	-84.5%
A. dracunculus 500mg/kg	10.48*Ab±1.28	47.4%
A. dracunculus 1000mg/kg	3.76*Bb±0.45	-46.8%

Data for mitotic index are expressed as mean±SD; *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).

Discussion

Cancer is ranked second, just after the cardiovascular diseases, as most common cause of noncommunicable diseases death, with global socioeconomic burden[16,17]. Cancer chemoprevention has received a great deal of attention and variety of plants and herbs extracts have been shown to provide some protection against cancer[18-21]. Anti-proliferative effect of certain species of genus *Artemisia* have been described previously[22.23].

A. dracunculus have been reported to be rich with coumarins[7], which have been presented in several researches as promising anticancer agents[8, 24-27]. The anticancer activity of coumarins and their derivatives is generally mediated by caspase dependent induction of apoptosis[27]. The cytotoxic and antitumor effects of *Artemisia dracunculus* extracts has been reported in murine model of lymphoma[9]; the present study investigated the effect of ethanolic extract of the plant on the mitotic index as a marker of cellular proliferation in normal murine bone marrow and spleen cells.

In the present study, the ethanolic extract of *A. dracunculus* at dose (500mg/kg body weight) caused significant increase, and the dose (1000mg/kg body weight) caused significant decrease in mitotic index in both bone marrow and spleen cells when compared with distilled water treated animals. That refers to dose-dependent effect of *A. dracunculus* extract on cell division and proliferation in both bone marrow and spleen cells. The proliferative effect of low dose of *A. dracunculus* extract needs further study for confirmation and elucidation of the potential mechanism(s). The anti-proliferative effects of *A. dracunculus* extract might be attributed to coumarins that present in this plant.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Obtained from Institutional ethical committee

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