

Cytotoxic effects of *Ammi visnaga* volatile oil on some cancer cell lines

التأثير السمي للزيوت الطيارة لعشبة الخلة البلدي على بعض خطوط الخلايا السرطانية

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Abstract:

Ammi visnaga a herbal plant is rich with important active constituents that make the plant to be described in traditional medicine and among them is the volatile oil. The oil extracted by distillation and then study it's cytotoxic effect on two cell line: The human pelvic rhabdomyosarcoma (RD) and The mouse cell line(L₂₀B) which expresses the genes for human cellular receptor for *Polio* viruses. Results showed that there were potent toxic effects on both cell lines RD&L₂₀B specially at the concentration (100,50 and 25)µl/ml of the essential oil ,then decreased as the oil concentrations decreased.

Key words: Cytotoxic, *Ammi visnaga*, volatile oil, cell lines

المستخلص

ان النبات العشبي الخلة البلدي (*Ammi visnaga*) غني بمكوناته الفعالة المهمة والتي جعلت النبات يدخل في وصفات طبية تقليدية ومن ضمن هذه المركبات الفعالة التي يحويها النبات الزيت الطيار . تم استخلاص الزيت بالتقطير و أجريت دراسة تأثير سميته الخلوية في خطين خلويين هما : RD ، L₂₀B . أظهرت النتائج ان زيت الخلة الطيار له تأثير سمي ملحوظ في كلا الخطين الخلويين خاصة عند التراكيز (25 ، 50 ، 100)مايكروليتر/مللتر من الزيت الطيار ويقل تأثيره كلما قل التركيز .

الكلمات المفتاحية : التسمم الخلوي ، *Ammi visnaga* ، الزيوت الطيارة ، خطوط الخلايا

Introduction:

Ammi visnaga Family(*Apiaceae*) grows in the Mediterranean region has been described in traditional medicine for angina pectoris, cardiac insufficiency, paroxysmal tachycardia, extra systoles, hypertonic, asthma, whooping cough and cramp-like complaints of the abdomen ,while the pharmacopeia described the plant as an antispasmodic, muscle relaxant and vasodilator[1]. The plant active constituents include :furochromones particularly khellin ,visnagin,khellol and kello glycoside. Also contains flavonoids , furanocumarins fatty oils and volatile oil [1,2]. The plant content of the essential oil represented by camphor α -terpineol and linalool[2,3]. Since the middle ages, essential oils have been widely used for bactericidal, virucidal, fungicidal, anti parasitic, insecticidal, medicinal and cosmetic applications, especially nowadays in pharmaceutical, sanitary, cosmetic, agricultural and food industries. Because of the mode of extraction, mostly by distillation from aromatic plants, they contain a variety of volatile molecules such as terpenes and terpenoids, phenol-derived aromatic components and aliphatic components [1,4].

The aim of this study is to investigate the effect of *Ammi visnaga* volatile oil on viability of two cancer cell lines: The human pelvic rhabdomyosarcoma (RD) and The mouse cell line(L₂₀B) (which expresses the genes for human cellular receptor for *Polio* viruses) might get the plant volatile oil an attention for being promise anticancer products.

Materials and method:

Sample preparation:

The volatile oil was supplied by Ministry of agriculture through extraction by Clevenger apparatus. Oil stock solution was made by mixing 400µl *Ammi visnaga* volatile oil with 10 µl Dimethylsulfoxide (DMSO) and complete the volume up to one ml using serum free medium to get the concentration of 400µl volatile oil/1ml medium. Then seven oil concentration starting with 200 µl /ml till 3.125µl/ml in a twofold dilution manner were applied to the microtiter plate containing 200 µl/weel of the mono confluent layer. The first weel concentration became 200 µl/ml for the volatile oil and the last concentration treated weel was 3.125 µl/ml .

Methodology:

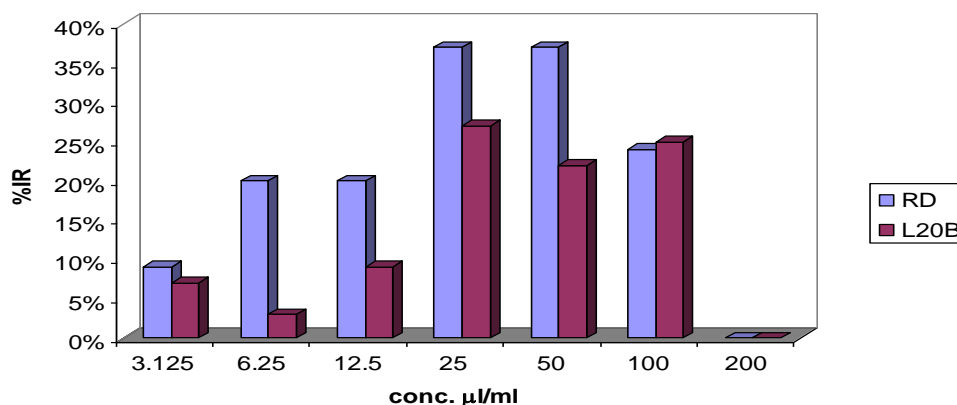
The cell lines were supplied by the center Laboratory for pathological analysis at Ministry of health. The RD cell line is a human cell line that derived from a biopsy specimen obtained from a pelvic rhabdomyosarcoma of a 7-year old Caucasian girl [5]. Passages [6] of cell line were used throughout this study, the cells were propagated and maintained in Minimum Essential Medium Eagle MEME medium. The L₂₀B cell line is a mouse cell line that expresses the genes for human cellular receptor for *polio viruses*. The passage number was (20) for this cell line used in this study.

The cytotoxic assay was applied according to Iraqi center for cancer and medical genetic researches (ICCMGR) protocol modified from Fresheny method [6] and as follow :

When the cells are in the exponential phase ,the cells be in full activity so these cells were collected after dissociation the monolayer with 2-3 ml trypsin/versine for 5-15 min., then reseeding the cells in 96 well microtiter plate to be incubated in CO₂ incubator at 37°C for 24 hour and get adhering monolayer that treated later with the serial concentrations of the volatile oil sterile solution in three replicates, leaving about 12 wells as control (cells with serum free medium only). After the exposure time was finished, the medium was removed from the plate and washed with Phosphate buffer saline (PBS) and 0.2 ml of neutral red solution was added to each well, incubated for 4 hours . Discarded the excess of the dye and resolved the amount of the dye taken up by the living cells with methanol/acetic acid extraction solution 1/1 after incubation for further half an hour. Finally the plate was read by ELISA at 490nm. The cytotoxic effect was measured as The percent rate of cell inhibition (%IR) = [absorption at 490nm for control- absorption at 490 nm for volatile oil/ absorption at 490nm for control] x 100 [6].

Results and Discussion:

The cytotoxic effect of different concentrations for the *Ammi visnaga* volatile oil after treating both cell lines (RD and L₂₀B) for 48 hours intervals is shown in figure-1.



Fig(1): The cytotoxic effect as percent inhibition rate(%IR) of different concentration of *Ammi visnaga* volatile oil after 48 hours exposure on RD and L₂₀B cell lines .

As the figure showed there was a potent toxic effect on both cell lines RD&L20B specially at the concentration (100,50 and 25)µl/ml of the essential oil that gave an inhibition rate on RD cell line(%IR) of 24%,37% and 37% and 25%,22% and 27% on L20B cell line respectively ,then decreased as the oil concentrations decreased. In general, the cytotoxic activity of essential oils is mostly due to the presence of phenols, aldehydes and alcohols in their composition [4,7] and because of the great number of essential oils constituents they seemed to have no specific cellular target and in vitro physicochemical assays characterised most of them as antioxidants[7]. As they considered typical lipophiles, they passed through the cell wall and cytoplasmic membrane and disrupted the structure of the membrane different layers lead to permeablized them, so the cytotoxicity effect appeared to include such membrane damage. However, a work showed that in eukaryotic cells, essential oils can act as prooxidants affecting inner cell membranes and organelles such as mitochondria[8]. Depending on type and concentration, they exhibit cytotoxic effects on living cells but are usually non-genotoxic. In some cases, changes in intracellular redox potential and mitochondrial dysfunction induced by essential oils can be associated with their capacity to exert antigenotoxic effects[8]. A big advantage of essential oils

is the fact that they are usually devoid of long-term genotoxic risks [8,9]. Moreover, some of them show a very clear antimutagenic capacity which could well be linked to an anticarcinogenic activity. Plants of the Apiaceae family possess a range of compounds with many biological activities. Some of the main properties were ability to induce apoptosis, antibacterial, hepatoprotective and vaso-relaxant activities, cyclooxygenase inhibitory effect and antitumor action[9], and as Ammi visnaga is among Apiaceae family that showed to be rich in volatile oil and in a recent study on chemical composition of the oil revealed that about forty one constituents mainly linalool, isoamyl 2-methyl butyrate and isopentyl isovalerate with another non-terpene esters were the major components represented Ammi visnaga volatile oil [9].

A study has demonstrated that the prooxidant activity of essential oils or some of their constituents, as of some polyphenols, is very efficient in reducing local tumor volume or tumor cell proliferation by apoptotic and/or necrotic effects. These findings suggest that, at least in part, the encountered beneficial effects of essential oils are due to prooxidant effects on the cellular level [10]. The present study on the Iraqi cultivated Ammi visnaga volatile oil exhibit cytotoxic effects on both cancer cell lines need to further investigation to know mechanism by which the oil act in comparison to traditional anticancer drug that might get the plant volatile oil an attention for being promise anticancer product.

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