ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Cytotoxic Effects of Some Medical Plant Extract Against Cancer Cell Line Using Tissue Culture Technique.

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ABSTRACT

Anethumgraveolens L., commonly known as dill belonging to the family of Umbelliferae, is one of the most useful essential oil bearing spices as well as medicinal herb. The current study aimed studying the effect of Iraqi traditional medical plant extracts using tissue culture technique to examine their cytotoxicity in human hepatocarcinoma HepG2, mouse cell and L20B cell lines. Anethumgraveolens leaves were extracted using aqueous and 99% alcoholic extractions. Eight crude concentrations were made by serial dilution, with concentrations of 3.9, 7.81, 15.62, 31.25, 62.5, 125,250 and 500mg /ml, respectively. These concentrations were added in triplicate to the micro titter plate containing 1x105 cells/well and 200 µl of the medium. The eight concentrations were used in triplicate to investigate their cytotoxic and anti-proliferative effects.Alcoholic extracts of Anethumgraveolens showed the highest potent cytotoxicity in the HepG2 and L20B cell lines; while aqueous extracts showed the lowest cytotoxicity. All concentrations of crude extracts showed different cytotoxicity activity in vitro.

Keywords: Anethumgraveolens; cytotoxicity; cancerous cell line.

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ISSN: 0975-8585

INTRODUCTION

Cancer chemoprevention refers to the use of natural, artificial or biologic compounds to reverse, suppress or avoid the development of invasive cancer. Photochemical are increasingly attractive and significant sources of chemo preventive agents, chiefly as they can reveal their useful potential at all stages of tumor configuration [1,2].

Among plant secondary metabolites are phenols which are of persistent interest for their anticancer activity. Phenols have reduced risk of incidence of several cancers in individuals for their richness of active compound work as anticancer [3,4]. Concerning the pharmaceutical production, it is essential to improve the early detection of drug-induced hepatotoxicity for being among the most important reasons for attrition of candidate drugs during advanced stages of drug development [5].The disease-prevention properties of fruits and vegetables are attributed to the biological activities of vitamins, dietary fiber, photochemical and minerals in plants. However, several studies suggested that the protective effects of leaves against chronic diseases are due mainly to the photochemical content of plants [6,7]. In the traditional system of medicine, the leaves have been in clinical use for centuries. Studies have shown that the extract of *Anethumgraveolens* L. has a growth inhibitory effect on cell line in vitro, especially breast cancer [1]. Aqueous and alcoholic extract of *Anethumgraveolens* L. have shown good anti-cancer and antioxidant activity [9]. Anethumi acid found in *Anethumgraveolens* is a strong modulator of nontoxic oxidative mutagens and a potent scavenger of free radicals [10]. Also, Anethumgraveolens L. leaves and oil represent a source of secondary metabolites like phenols and anthocyanin which are considered as an excellent anticancer activity and antioxidant [11]. Beside, leaves extracts of *Anethumgraveolens* L. contain appreciable levels of polyphenols that have anticancer action and radical scavengers [12,13].The *Anethumgraveolens* L. extract has a therapeutic action in different studies; preserving liver cirrhosis and fibrosis, protecting them against oxidative stress [14] and have anti-carcinogenic effects [15,16]. Therefore, this study aims evaluating the cytotoxic effects of *Anethumgraveolens* L. extracts against HepG2 and $L_{20}B$ cell lines *in vitro.*

MATERIALS AND METHODS

Anethumgraveolensis materials

Anethumgraveolensis materials were collected from various parts of Baghdad, Iraq. Authentication of plant materials was conducted at the herbarium of Bio Technology Department, College of Sciences, University of Baghdad, Iraq. *Anethumgraveolensis* were rinsed thoroughly with tap water and 90% of ethanol separately to remove extraneous contaminants and cut into small pieces, oven-dried at 50°C until stability of dry weight was observed, and then grounded in the powder with an electric-grinder to prepare it for extraction [13].

Preparation of crude extracts of *Anethumgraveolensis:*

Extraction was carried out by Macerating (100 g) in 500 ml of 95% ethanol and distal water in (25-30°C) for 3 days in flasks. The extracted solvent was filtered and separated through using filter paper Whatman No. 1. The extracts (ethanol and aqueous)were evaporated using rotary evaporation. Finally, the crude extracts powder was weighed and stored at 4°C to be used in cytotoxic activity [14].

Studying Cytotoxic Activity of *Anethumgraveolensis Extracts in Vitro*

The anticancer efficacy of aqueous and ethanol extracts from anticancer activity against L20B cell line was evaluated. The colorimetric cell viability MTT assay was used as described by [15,16]. In the beginning, 100 µL/well of L20B cells (10^6 cell/ mL) were cultured in 96-well tissue culture plate. Different concentrations 3.9, 7.81, 15.62, 31.25, 62.5, 125,250 and 500 mg /ml of test solution were prepared by dissolving (3 mg/ ml) in water. After that, each well was filled with 100 µL of different concentrations and incubated at 37°C for 24h. Then, 10µL of MTT solution (5 mg/ mL) was added to each well and incubated at 37°C for 4 h. Finally, each well was filled with 50 µL of DMSO (dimethyl sulfoxide) and incubated for 10 min. L20B cells were cultured in complete medium without xxx solution as a control. An ELISA reader was used to measure the absorbance for each well at 620 nm. The calculation of live cells, percentage of viability and inhibition ratio was performed using the following formula:

ISSN: 0975-8585

$$GI\% = \frac{(OD\ of\ control\ wells - OD\ of\ test\ wells)}{OD\ of\ control\ wells} \times 100.$$

Hepatocarcinoma and $L_{20}B$ (a mouse cell line that expresses the genes for human cellular receptor for *polio viruses*) Cells were cultured in DMEM medium supplemented with 10% foetal bovine serum, L-glutamine. Cells were grown as a monolayer at 37 °C with 5% CO_2. The experiments were performed when cells were in the logarithmic phase of growth [17]. Cell line was incubated with different concentrations of each extract. The nine concentrations were used in triplicate to investigate their cytotoxic and anti-proliferative effects. A complete medium was used as negative control [18,19].

Statistical Analysis:

The results obtained were statistically analysed using SAS software (version 17; SAS Inc., Chicago, IL, USA) [20].

RESULTS AND DISCUSSION

The assay of 3-(dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) was achieved to determine the cytotoxic effect and anticancer activity. The tests of aqueous and ethanol extracts show clear inhibitory action against the proliferation of the hepatocellular carcinoma cell line after 48 h. The *Anethumgraveolensis* extracts to HepG2 cells were treated with increasing concentrations (3.9, 7.81, 15.62, 31.25, 62.5, 125,250 and 500 mg /ml). The aqueous and ethanolic extract resulted in a dose dependent decrease in cell viability. The sensitivity of ethanol and aqueous extract was more in higher dosage specially with aqueous extracts concentrations 3.9, 7.81, 15.62, 31.25, 62.5, 125,250 and 500 mg /ml(Table 1). Treatment with the Anethum*graveolensis* aqueous extract (250 μg/mL) decreased the cell viability of hepatocellular carcinoma HepG2 with value of 92.1 %IR after 48 h and further decreased to 88.00 %IR for aqueous extract after 48 h compared to the control cells, respectively (Table 1,2,3,4).

Table 1: "The cytotoxic effect expressed as the inhibition rate percentage (%IR) for different concentrations of *Aqueous extracts of Anethumgraveolensis* after 48 hours exposure on HepG2 cell lines"

Extract Conce.	Mean	S.D.	GI%
1(500)	0.404	0.049	**0**
2(250)	0.205	0.080	**42.4**
3(125)	0.141	0.053	**60.3**
4(62.5)	0.138	0.021	**61.2**
5	0.119	0.029	**66.5**
6	0.211	0.016	**40.7**
7	0.268	0.011	**24.7**
8	0.378	0.034	**0**
Control	0.356	0.080	

Table 2: "The cytotoxic effect expressed as the inhibition rate percentage (%IR) for different concentrations of *Aqueous extracts of Anethumgraveolensis* after 48 hours exposure on L20B cell lines"

Extract Conce.	Mean	S.D.	GI%
1(500)	0.060	0.001	**83.1**
2(250)	0.083	0.036	**76.6**
3(125)	0.079	0.026	**77.8**
4(62.5)	0.082	0.004	**76.9**
5	0.078	0.013	**77.9**
6	0.090	0.002	**74.7**
7	0.096	0.012	**73**
8	0.110	0.008	**69.1**
Control	0.356	0.080	

ISSN: 0975-8585

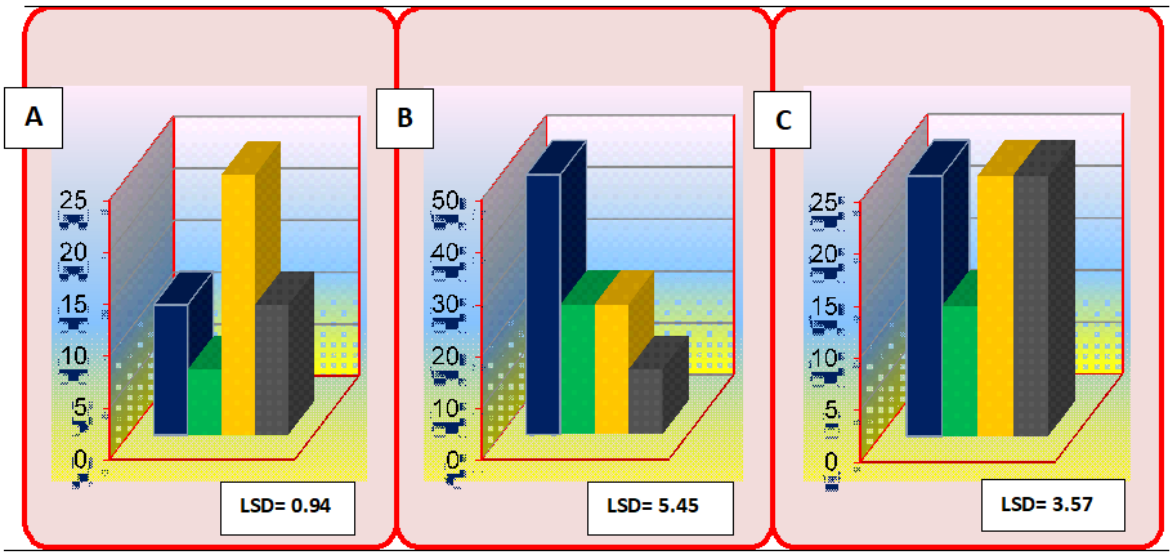
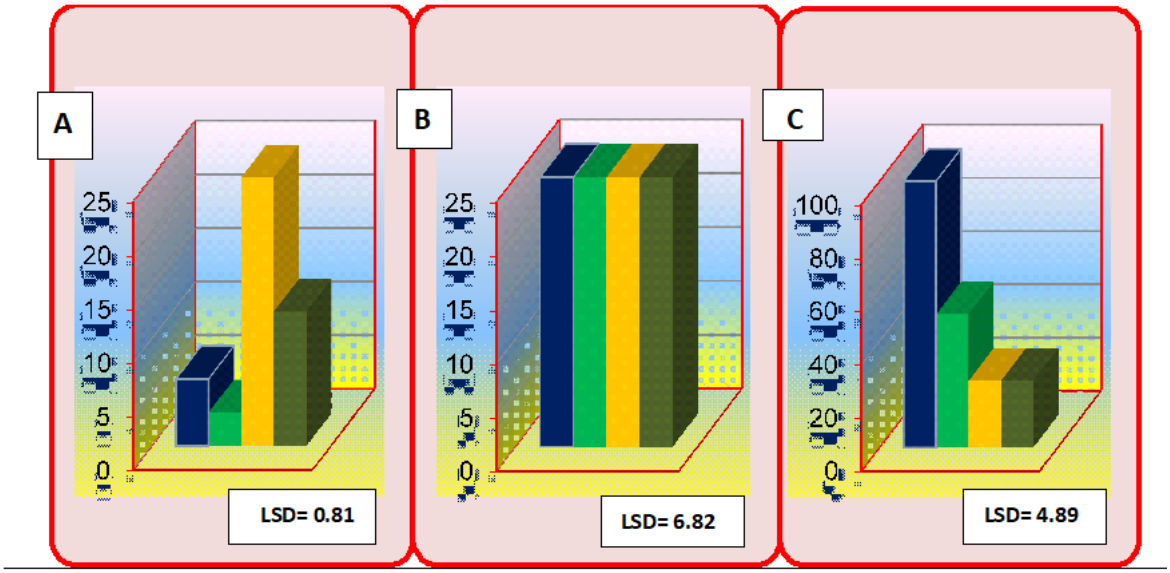
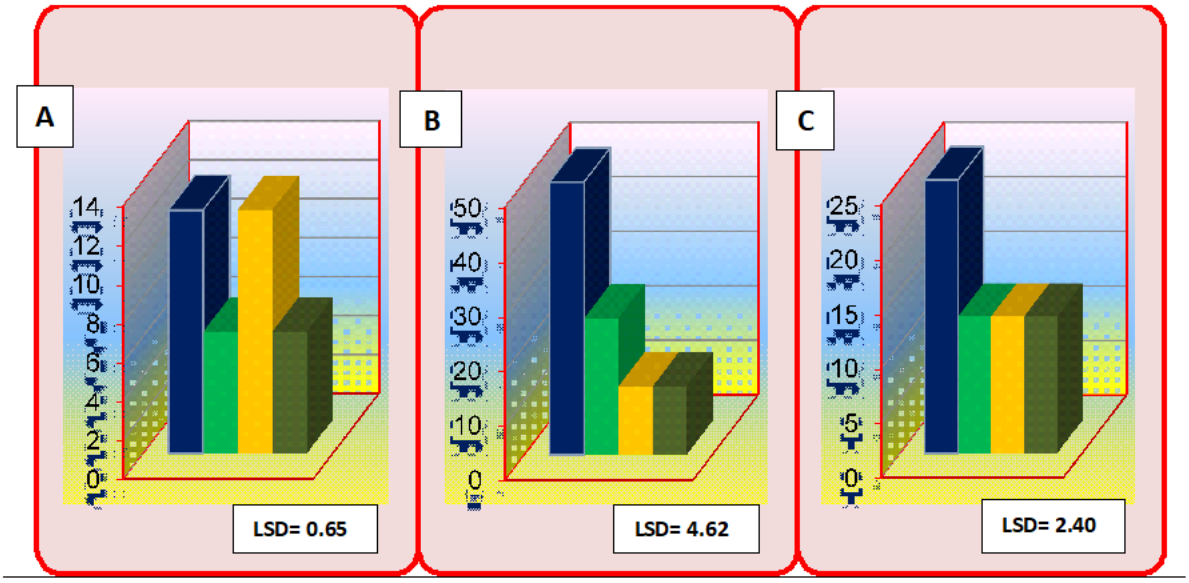


Table 3:"The cytotoxic effect expressed as the inhibition rate percentage (%IR) for different concentrations of *Alcoholic extracts of Anethumgraveolensis* after 48 hours exposure eon L20B cell lines"

Extract Conce.	Mean	S.D.	GI%
1(500)	0.218	0.009	**38.6**
2(250)	0.097	0.0007	**72.6**
3(125)	0.085	0.012	**75.9**
4(62.5)	0.081	0.011	**77.2**
5	0.078	0.018	**78**
6	0.099	0.011	**72.1**
7	0.085	0.009	**76**
8	0.160	0.000	**55**
Control	0.356	0.080	



ISSN: 0975-8585



Thymus vulgaris

Also, all these extracts have the same cytotoxic effects result against $L_{20}B$ cells line. Treatment with *Anethumgraveolensis* extracts (500μg/mL) decreased the cell viability of hepatocellular carcinoma $L_{20}B$ cells line value of 43.00 and 72.00 % IR after 48 h, respectively. It is further decreased to 41.8% IR after 48h for *Anethumgraveolensis* extract compared to the control cells (Table 3). These results may be attributed to their contents of polyphenols, flavonoids, anthocyanin, ellagitannins and vitamin C. It is the phytochemicals that are responsible for many of the biological activities of their crude extracts, including antioxidant, reducing inflammatory and having anticancer properties [11,12,13,15].

Anethumgraveolensis are often used as medicinal herbs in different regions of the world because of their biological activities such as bactericidal, antifungal, antiviral as well as antioxidant activity. [19] However, very few antitumor activity researcheshave been reported on Anethumgraveolensis extract. The cytotoxic effect of the extract of ascorbic acid on HepG2 and L20Bcells can be attributed to the presence of polyphenols, which is the main component that possesses a wide range of biological activity. This indicator is consistent with [20], which reported extracts of Anethumgraveolensis potent cytotoxic effect against L20B. In addition, significant inhibition (60% -90%) of cancer cells with polyphenols is one of the major components of $L_{20}B$ [20].

CONCLUSIONS

The crude extract of the *Anethumgraveolensis extracts* has the ability to inhibit the growth activity and reduce the proliferation of cell lines used in the study.

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