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Cytotoxicity of aflatoxin B1 on HepG2 cell line and study the protection role of *Adhatoda vasica* and *Panax ginseng extracts* (in vitro study)

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Abstract

Anethum graveolens L. commonly known as dill belonging to the family Umbelliferae, is one of the most useful essential oil bearing spices as well as medicinal herb. The present study aimed to study the effect of Iraqi traditional medical plants extracts using tissue culture teqnique stud their cytotoxicity in human hepatocarcinoma HepG2 and mouse cell $L_{20}B$ cell lines. *Anethum graveolensis* leaves were extracted using Aqueous and 99% and alcoholic extraction. eight crude concentration were made by serial dilution, with concentrations of 3.9, 7.81, 15.62, 31.25, 62.5, 125,250 and 500 mg /ml, respectively. These were added in triplicate to the microtiter plate containing $1x10^5$ cells/well and 200 µl of the medium. The eight concentrations were used in triplicate to investigate their cytotoxic and anti-proliferative effects. The alcoholic extracts of *Anethum graveolensis* showed the highest potent cytotoxicity in the HepG2 and $L_{20}B$ cell lines.while aqueas extracts showed the lowest cytotoxicity. All concentrations of crude extracts showed different cytotoxic activity *in vitro*.

Keywords: *Anethum graveolensis*; Cytotoxicity; cancerous cell line.

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1. Introduction

Cancer chemoprevention is the use of natural, artificial or biologic compounds to reverse, suppress or avoid the development of invasive cancer. Phytochemicals are attractive increasingly 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 phenols are of persistent interest as anticancer activity also reduced risk of incidence of several cancers in individuals with the rich of active compound work as anticancer [3,4]. In the pharmaceutical industry, improving the early detection of drug-induced hepatotoxicity is essential as it is one of the most important reasons for attrition of candidate drugs during the later stages of drug development [5]. The disease-prevention properties of fruits and vegetables are attributed to the biological activities of the dietary fiber, vitamins, minerals and phytochemicals in the plants, however many studies suggest the protective effects of leaves against chronic diseases are due in large part to the phytochemical content of the 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 *Anethum graveolens* L. has a growth inhibitory effect on cell line in vitro specially breast cancer [1]. Aqueous and alcoholic extract of *Anethum graveolens* L. have shown good anti-cancer and antioxidant activity [9]. Anethumi acid found in *Anethum graveolensis*is a strong modulator of nontoxic oxidative mutagens and a potent scavenger of free radicals [10]. Also, *Anethum graveolens* L. leaves and oil source of secondary metabolites like phenols and anthocyanin which considered as an excellent anticancer activity and antioxidant [11]. Beside , Leaves extracts of *Anethum graveolens* L. contain appreciable levels of polyphenols that have anticancer action and radical scavengers [12,13].The *Anethum graveolens* L. extract had a therapeutic action in different studies ; preserving liver cirrhosis and fibrosis protect them against oxidative stress [14] and have anti-carcinogenic effects [15,16]. The aim of this study was to evaluated of the cytotoxic effects of. *Anethum graveolens* L. extracts against HepG2 and L_{20}B cell lines *in vitro.*

2.Materials and Methods

Anethum graveolensis materials

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

Preparation of crude extracts of *Anethum graveolensis:*

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

Study Cytotoxic activity of *Anethum graveolensis 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]. At first, 100 µL/well of L20B cells (10^6 cell/ mL) were cultured in 96-well tissue culture plate. Different concentrations of of 3.9, 7.81, 15.62, 31.25, 62.5, 125,250 and 500 mg /ml, test solution were prepared by dissolving (3 mg/ mL) in water. Then, 100 µL of various concentrations was added to each well and incubated at 37◦C for 24h. After the incubation, 10µL of MTT solution (5 mg/ mL) was added to each well and incubated at 37◦C for 4 h. Finally, 50 µL of DMSO (dimethyl sulfoxide) was added to each well and incubated for 10 min. L20B cells were cultured in complete medium without xxx solution as a control. The absorbance was measured for each well at 620 nm using an ELISA reader. The live cells, percentage of viability and inhibition ratio were calculated according to the formula

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

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].

3.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 the aqueous and ethanol extracts shows clear inhibitory action against the proliferation of the hepatocellular carcinoma cell line after 48 h . The *Anethum graveolensis* ***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 the ethanol and aquius extract was more in higher dosage specially with aqueous extracts concentration's 3.9, 7.81, 15.62, 31.25, 62.5, 125,250 and 500 mg /ml (Table 1). Treatment with the *Anethum graveolensis* aquius 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 Anethum graveolensis* 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 Anethum graveolensis* after 48 hours exposureon 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	

Table 3: The cytotoxic effect expressed as the inhibition rate percentage (%IR) for different concentrations of *Alcoholic extracts of Anethum graveolensis* after 48 hours exposure on 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	

Table 3: The cytotoxic effect expressed as the inhibition rate percentage (%IR)for different concentrations of *Alcoholic extracts of Anethum graveolensis* after 48 hours exposure on HepG2 cell lines.

Extract Conce.	Mean	S.D.	GI%

1(500)	**0.206**	**0.033**	**41.9**
2(250)	**0.180**	**0.033**	**49.2**
3(125)	**0.112**	**0.004**	**68.5**
4(62.5)	**0.089**	**0.006**	**74.8**
5	**0.082**	**0.004**	**76.9**
6	**0.083**	**0.005**	**76.6**
7	**0.099**	**0.031**	**72.1**
8	**0.159**	**0.006**	**55.1**
Control	**0.356**	**0.080**	

Also , all these extracts have a same cytotoxic effects result against $L_{20}B$ cells line. Treatment with *Anethum graveolensis* extracts (500 μg/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. Further decreased to 41.8% IR after 48h for *Anethum graveolensis* extract compared to the control cells (Table 3).these results may be attributed to their contents of polyphenols, flavonoids, anthocyanin's, ellagitannins, and vitamin C. It is the phytochemicals that are responsible for many of the biological activities of theirs crude extracts, including antioxidant, reduce inflammato ry and anticancer properties [11,12,13,15].

Anethum graveolensis is often used as medicinal herbs in different areas of the world due to its biological activities such as bactericidal, antifungal, and antiviral as well as antioxidant activity [19], however, very few researches were reported the antitumor activity of *Anethum graveolensis* extracts. The cytotoxic effect mainly resulted by the Ascorbic acid extract extract on HepG2 and $L_{20}B$ cells may be attributed to the presence of polyphenol which considered to be the major constituent that possess a wide variety of biological activity. Such indication is in agreement with [20], which reported the extracts of of *Anethum graveolensis* showed a potent cytotoxic effect against $L_{20}B$. In addition, a significant inhibition (60% -90%) tumor cells was reached with polyphenol, one of the major constituents of $L_{20}B$ [20].

4.Conclusions

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

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