



## Anticancer Activity of Sunflower (*Helianthus annuus* L.) Seeds oil against cell lines

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### Abstract:

This study investigated the role of sunflower (*Helianthus annuus* L.) seed extracts as a cytotoxic agent against two cell lines (RD and L20B) using different concentrations of sunflower seeds oil 1.3 , 2.6 , 5.2 , 10.4 , 20.8, 41.6 , 83.2 and 166.4 µg / ml for different exposure time (24 ,48 and 72 hrs). The results revealed a clear cytotoxic activity of sunflower seeds oil on growth of RD cancer cell line, and the effect was concentration-dependent.

The signification inhibition ( $P<0.05$ ) was obtained at a concentration of 83.5 and 166.4 µg / ml as compared to control. In addition, the growth of RD cell line was more sensitive to sunflower seeds oil in comparison with the growth of L20B transform cell line. In conclusion, the extract showed inhibitory effect on cancer cell line and it is promising to use as anticancer drug.

**Kay word:** Sunflower oil, Cytotoxic effects, Anticancer activity

## فعالية زيت بذور زهرة الشمس (*Helianthus annuus* L.) ضد الخطوط الخلوية السرطانية

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### الخلاصة:

تهدف هذه الدراسة إلى التحري عن التأثيرات السمية الخلوية لزيت بذور زهرة الشمس (*Helianthus annuus* L.) في اثنتين من الخطوط الخلوية RD و L20B باستخدام ثمانية تراكيز مختلفة 1.3 ، 2.6 ، 5.2 ، 10.4 ، 20.8 ، 41.6 ، 83.2 و 166.4 مايكرو غرام /ملنتر وضمن فترة تعريض مختلفة ( 23 ، 48 و 72 ساعة ) . أظهرت النتائج وجود تأثير سمي واضح وبمعنوية عالية لمستخلص زيت بذور زهرة الشمس في نمو الخط الخلوي السرطاني RD، علما ان نسبة السمية زادت بزيادة التركيز وسجل أعلى تأثير تنبيطي في نمو الخط الخلوي السرطاني RD لتركيز 83.2 و 166.4 خلال فترة تعريض 24 و 72 ساعة مقارنة بالسيطرة. في حين لم يكن هناك تأثير واضح للمستخلص بذور زيت زهر الشمس في نمو الخلايا المتحولة L20B.

### Introduction:

The practice of herbal medicine dates backs to very early periods of known human history. There is evidence of herbs having been used in treatment of disease and for revitalizing body

systems in almost all ancient civilization such as the Egyptian, India, Chinese, Greek and Roman civilization, [1]. In the last years, the research on bioactive compounds of natural origin has gained much interest. According to a

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recent estimate of the World Health Organization (WHO), 70-80 % of the world population especially in developing countries, relies on traditional medicine, mostly plant drugs for their primary health care needs [2, 3]. However, plant secondary products have complementary and overlapping actions, including antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system, reduction of inflammation, modulation of steroid metabolism, and antibacterial and antiviral effects [4, 5].

The sunflower, *Helianthus annuus* major is agricultural crop and its seeds are widely used for the production of edible oil and food. In addition, research has also suggested that this seed is a good source of phytochemical, including tocopherols (Vitamin E), choline, betaine, lignan, arginine and phenolic acids, [6, 7]. Such constituents may justify the folkloric medicinal applications of the plant in treating rheumatism, sedative in cough, antispasmodic, antimalaria, urinary antiseptic, liver disorders and peptic ulcers, furthermore, the plant has also used as anti-inflammatory, anti-oxidant, anti-tumor and antimicrobial agent [8, 9].

Cancer is one of the leading causes of death in the world despite newly developed and miracle drugs for treatment and diagnosis [10]. Although several anticancer drugs are already commercially available and a number of adverse effects sometimes occur during chemotherapy [11]. Therefore, researches are necessary to find alternative effective, safe and inexpensive therapies [12].

According to Cardellinall *et al.* [13] cytotoxicity screening models provide important preliminary data to help selecting plant extract with potential antineoplastic properties. Also, cytotoxicity test is a qualitative and quantitative tests to determine how cell death [14]. For this purpose this study was designed to evaluate the anticancer activity of the sunflower (*Helianthus annuus* L.) seed oil on the growth of cancer cell lines *in vitro*.

#### Materials & Methods:

**Plant collection:** The dried seed of *Helianthus annuus* were obtained from a Department of Field Crops Science, College of Agriculture in Abu-Ghraib University of Baghdad. The seeds were powdered using an electric blender, and 50 grams of the powder was infused in hexane overnight, and then oil by soxhelt apparatus using hexane for 20 hours. Solvent was then distilled off under reduced

pressure below 4 °C using rotary evaporated and stored at 4°C until use [15].

#### Cell Culture and Cytotoxicity:

All the experiments of this study were performed in the laboratories of the tissue culture unit of Biotechnology Research Center / Al-Nahrin University. Two types of cell lines; rhabdomyosarcoma (RD) and murine L20B cells, were used. Cells were grown in RPMI-1640 medium containing 10% inactivated fetal calf serum (FCS) and penicillin (100U/ml)-streptomycin (100mg/ml) antibiotic. The cytotoxicity of hexane extract was tested using the method of [16]. The extract was dissolved in 0.1% dimethyl sulfoxide (DMSO) (Stock solution concentration 1mg/ml) and diluted with RPMI-1640 medium to give concentrations ranging from 1.3 – 166.4 µg / ml, the cells were grown in tissue culture flasks containing growth medium at 37°C in an atmosphere of 5% Co<sub>2</sub> and 95% relative humidity in a Co<sub>2</sub> incubator.

The cells at subconfluent stage were harvested from the flask by treatment with trypsin-versine solution (20 ml trypsin in 370 ml PBS containing 10 ml versine) and suspended in the medium. Cells with more than 97% viability (trypan blue exclusion) were used for determination of cytotoxicity. Cells were plated in 96 multi well plate for 24 hours in a Co<sub>2</sub> incubator at 37°C. Different concentration of the tested substance (1.3, 2.6, 5.2, 10.4, 20.8, 41.6, 83.2 and 166.4 µg /ml) were added to the cells (three replicate wells were prepared for each individual concentration) and reincubated for the selected exposure time (24, 48 and 72 hrs). Control cultures containing RPMI-1640 alone were tested for back ground cytotoxicity. After that, 50 µl of crystal violate stain were added to the wells, and the plates were incubated in a CO<sub>2</sub> incubator for 30 minutes at 37 °C. The stain was washed gently with tap water for three times reader at 492 nm. The inhibitory rate of cell growth was calculated as following formula [17]:

Inhibition (%)= [(Optical density of control wells - Optical density of test wells) / Optical density of control wells] \* 100.

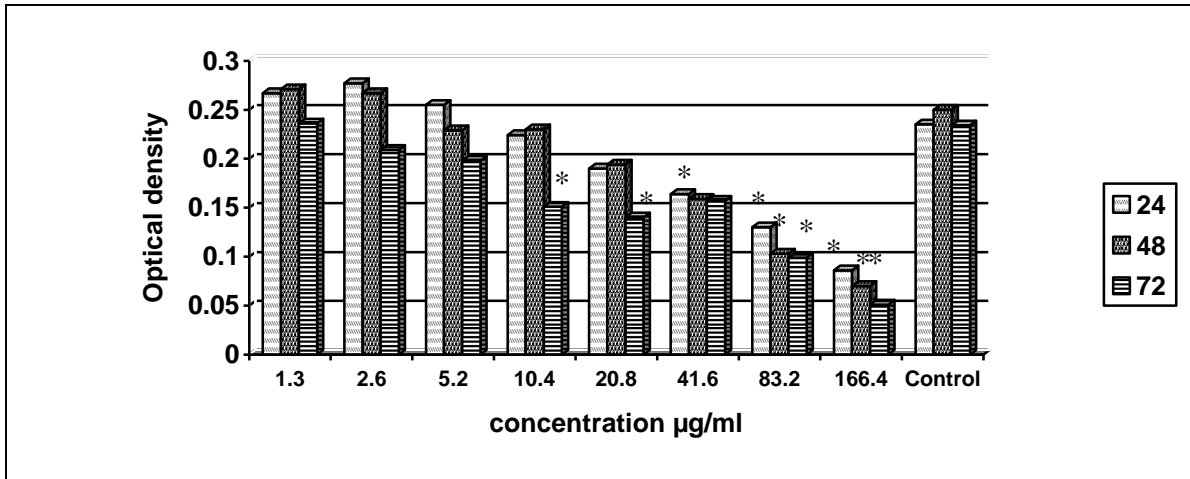
#### Statistical analysis:

The experimental data were analyzed using statistical software SPSS (SPSS 16.0 for windows, SPSS Ins. III., USA). Significant difference between control and sample means was assessed using student's t test and P values < 0.05 were considered significant.

**Results:**

The effect of treating RD cells of *H. annuus* seed oil are shown in Figure 1. Growth inhibition in a dose – dependent manner was observed in RD cell line. *H. annuus* seed oil has significant effect ( $p<0.05$ ) on RD cell line at the concentrations (83.2 and 166.4  $\mu\text{g/ml}$ ) at 24, 48

and 72 hrs. Also, there is significant effect ( $p<0.05$ ) for the concentration (10.4 and 20.8  $\mu\text{g/ml}$ ) at 72 hrs, and 41.6  $\mu\text{g/ml}$  at 24 hrs. Where the other concentrations showed non-significant effect during all the period of exposures, figure 1.

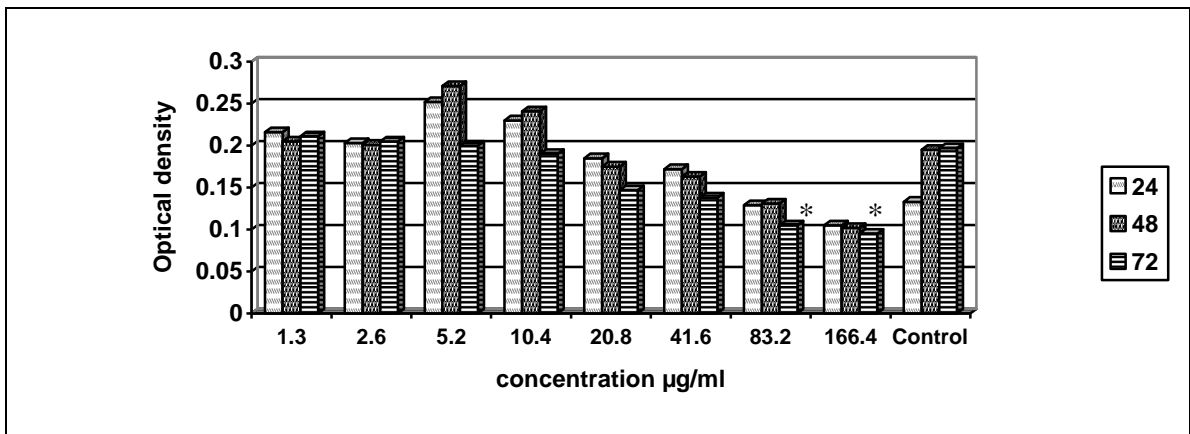


**Figure 1-** The effect *H. annuus* seed oil at different concentration on optical density of RD cell line during 24, 48 and 72 hours of exposure.

\* Significant  $P<0.05$

The effect of hexane extract of *H. annuus* seed oil on L20B cell line was demonstrated in figure 2, most concentration of sunflower seed oil had no significant effects on the growth of

L20B cell line ( $p>0.05$ ). Only the treatment with concentration (83.2 and 166.2  $\mu\text{g/ml}$ ) caused significant effect ( $p<0.05$ ) on L20B cell line at 72 hrs.



**Figure 2-** The effect *H. annuus* seed oil at different concentration on optical density of L20B cell line during 24, 48 and 72 hours of exposure.

\* Significant  $P<0.05$

Based on the results of optical density the growth inhibiting (G1) effect was calculated, the results in table 1- revealed that the RD cell line was more sensitive to the sunflower seed oil after period of exposure. Also, the results in table 1- showed that best exposure period was 72 hrs than the other periods (24 and 48 hrs).

The G1 percentage for sunflower seed oil on RD cell line at 166.4  $\mu\text{g/ml}$  concentration was 63.40%, 72% and 78.21% after 24, 48 and 72 hrs., respectively. The results also showed the G1 percentage for sunflower extract on L20B cell line by the same concentration was 20.4,

47.69% and 51.78% after 24, 48 and 72 hrs, respectively.

The results also showed that the G1 percentage for the *H. annuus* seed oil on RD cell line at 83.2 µg/ml was 44.68%, 58.8% and 57.69% after 24, 48 and 72 hrs., respectively, while the G1 percentage for *H. annuus* seed oil on L20B cell line at the same concentration was

2.27%, 32.82% and 46.70% after 24, 48 and 72 hrs., respectively. on the other hand, the results showed that the G1 for RD cell line was decreased at the lower concentrations (1.3, 2.6, 5.2 and 10.4 µg/ml) during all exposure time. The results also showed there was less G1 for L20B at lower concentrations during all exposure time.

**Table 1-** Percentage of inhibition of RD and L20B cells by using the hexane extract of *H. annuus* seed oil three periods exposure.

Type of cell line	Concentration (µg/ml)	Growth Inhibition (%)		
		24 hrs.	48 hrs.	72 hrs.
RD	1.3	-	-	-
	2.6	-	-	10.68
	5.2	-	8.4	15.38
	10.4	4.68	8	35.47
	20.8	17.02	22.4	40.17
	41.6	30.21	36.4	32.91
	83.2	44.68	58.8	57.69
	166.4	63.40	72	78.21
L20B	1.3	-	-	-
	2.6	-	-	-
	5.2	-	-	-
	10.4	-	-	3.55
	20.8	-	10.23	25.38
	41.6	-	16.41	29.95
	83.2	2.27	32.82	46.70
	166.4	20.45	47.69	51.78

- No Inhibition

### Discussion:

In this study, sunflower seeds demonstrated different cytotoxicity *in vitro* toward RD cell line according to its concentration. A support of such results has been presented by Zhang *et al.*, [7] who demonstrated that the plant have antiproliferative action and growth inhibition on various cancer cell lines including Hela and Glioma cells.

According to [18], one of the most prominent natural product research efforts is in the field of antineoplastic drugs in an attempt to discover a product that more susceptibly affect the signaling pathways in tumorigenic cells than non- tumorigenic cells. 61% of new antitumor drugs were natural products or derived from natural products [19]. Sunflower polyphenols such as caffeic, chlorogenic and ferulic acids extract a high antioxidative potential and they have antimutagenic activity by blocking the metabolic activation of the mutagens and screening the free radicals produced from

mutagens metabolism [20, 21]. According to Maria [22], the high antioxidant capacity observed for the aqueous extract of the sunflower seed oil suggests that the intake of this seed may prevent *in vivo* oxidative reactions responsible for the development of several diseases, such as cancer.

In a recent study, sunflower trypsin inhibitor (SFT1), the protein ring, can be in its natural form to block breast cancer enzymes and in a modified form to block enzymes associated with other types of cancer [23]. It has also been reported that subcutaneous treatment with sunflower seed oil (SSO) inhibits the solid tumor growth and increase lymph node cell number in animals with solid tumor [24]. P-coumaric is generally considered to be the most important element of *H. annuus* seed oil, and recently it has been demonstrated that coumarins have antioxidant properties and exerted a reduced proliferative activity of cancer cell lines [25, 26].

Coumarins have also been found to induce glutathione-s-transferase (GST) activity in the for stomach, liver and intestine of mice [27]. Tannins is another phenolic phytochemicals, which can be extracted from sunflower seed. Tannins is a widely distributed plant phenolic that was recently reported as an anticancer agent, It induced cell death in cancer cells in a dose-depended manner but did not affect the growth of normal cells [28 , 29].

The numerous other active compounds in sunflower seeds oil identified include palmitic acid, linolenic acid,  $\alpha$ -Linoleic acid and Stearidonic acid all of them have antioxidant properties and also anti-carcinogenic effects [26 , 30].

Fractionation of a petroleum ether extract of *H. annuus* L. led to the isolation of three diterpene acid ; grandiflorolic, kaurenoic acid and trachylobanoic acid. These three compound reduced , in concentration dependent manner nitric acid (ON), prostaglandin E2 (PGE2) and tumor necrosis factor (TNF- $\alpha$ ) production[31]. Sunflower seeds oil are an excellent source of vitamin E. Clinical studies show the vitamin E has been associated with reduced risk of certain cancer such as colon, bladder and prostate [32 , 33].

Selenium is other compound of sunflower seeds works with vitamin E as an antioxidant and protects cells from damage that may lead to cancer [34]. Sunflower seeds are a great natural source of potassium and phosphorus, as well as, protein, iron and magnesium with is antioxidant activity and enhance immune function [35]. Also, the sunflower seeds contain high concentration of zinc, which plays an essential role in DNA replication, transcription and protein synthesis influencing cell division and apoptosis [36].

In conclusion, the results of this study demonstrated that the sunflower seeds oil had anti-cancer activity.

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