

Cytotoxic activity of ethanol extract of *Sesamum indicum* seeds to cancer cell lines *in vitro*

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Abstract :

The present study was conducted to investigate the active constituents found in ethanolic extract of *Sesamum indicum* defatted seeds and its cytotoxic activity on three types of cancer cell lines (Hep-2, AMN-3, and RD) and one normal embryo rat cell line (Ref).

Results of general chemical detection showed that ethanolic extract of sesame seeds contain phenols, tannins, saponines, glycosides, alkaloids, coumarines, and flavonoids. The qualitative and quantitative determination of sesamin bioactive compound using High Performance Liquid Chromatography (HPLC) analysis was carried out and compared with standard sesamin. It was found that the concentration of sesamin was 79.9% of ethanolic extract according to total peak area.

Results of *in vitro* growth inhibition of sesame extract against cell lines demonstrated that the growth of these cells significantly decreased when compared with control cell lines (untreated), and the effects were dose and time- dependent for Hep-2, AMN-3, RD and Ref cells. A clear cytotoxic activity was observed after 72 hr at concentration (1000 µg/ml) which reached (85.83%, 40.06%, and 20.20%) in the cell lines respectively, while the percentage of inhibition during exposure time of 48 hrs recorded 31.03% at a concentration of 1000µg/ml for RD cells.

Key words: *Sesum indicum*, *sesamin*, *cell line*, *HPLC*, *cytotoxic activity*.

Introduction:

According to the World Health Organization, more than 11 million people are diagnosed with cancer every year and it is estimated that by 2020 there will be 16 million new cases per year, and moreover approximately 7 million people are died from cancer every year worldwide, which it is coming in the second level after cardiovascular diseases (1, 2).

In Iraq, it has been estimated that approximately 15000 people have been died of cancer in 2005, and such number represents 22.8% of the total deaths, moreover, it is projected that such percentage can be increased up to 35.4% in 2030 (3).

Chemotherapy is one of the conventional cancer treatments in addition to surgery and radiotherapy. All these types of treatments are costly and carry a high risk of side effects and resistance, besides of their unavailability, re-

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sulting in high morbidity and mortality rates especially in poor countries (4).

The researchers have developed other anti-cancer strategies to overcome such fatal disease, and accordingly novel pharmacological paradigms have been developed which quickly and efficiently moves prospective anti-cancer drugs from the discovery phase through pharmacology testing and into therapeutic trial assessment. Some of these developments are based on natural products (5).

Sesamum indicum Linn. (Pedaliaceae) has long been used extensively as a traditional food in the orient for various purposes and has varieties of medicinal properties. The seed powder is useful in amenorrhea, dysmenorrhea, ulcers and bleeding piles(6).

Sesamin is a major lignan constituent of sesame and sesame oil, many studies have revealed that sesamin is effective in preventing hypertension, thrombogenesis (7), and hypercholesteremia by increasing hepatic fatty acid oxidation (8). Additionally, sesamin exhibits antioxidative properties, by reducing peroxidation products in the plasma and liver of rats(9) and exerts an inhibitory effect on chemically induced cancers (10). In Iraq many studies have been carried out on assessing the cytotoxic activity of many local Iraqi plants on cancer cells *In Vitro* and *In*

Vivo(11,12,13). These studies showed promising results for cancer treatments.

This study aimed to detect the active compounds in crude and defatted ethanol extract in *Sesamum indicum* seeds and to assess their cytotoxic activity on cancer and normal cell lines.

Material and Methods:

Seeds collection and preparation of crude extract

Sesamum indicum seeds were collected and separated from undesirable materials or plant parts, then grinded into coarse powder by electrical grinder and kept in clean plastic cans until use.

Ground sesame seeds (100 g) were defatted by mixing with n-hexane (500 ml) with magnetic stirrer at room temperature. The resulting slurry was filtered in Buckner funnel through whatman No.1 filter paper and then left to dry for 12 hour. The dried defatted residue (63 g) was mixed with 80% ethanol for 6 hours, the resulting ethanolic mixture were filtered and evaporated under vacuum by rotary evaporator at 40°C, the final residue powder weighted 2.8 g and stored at 4°C until use (14).

Detection of chemical compounds in ethanolic extract

Chemical detection was carried out using different reagents as mentioned in (15) to determine the quality of active compounds exists in crude extract.

Detection of sesamin using (HPLC)

Quality and quantity analysis was done by HPLC technique analysis using C-18 column, 50 × 4.6 mm I.D column, the mobile phase used was 1% phosphate buffer (pH =4.5): acetonitrile:water (60:40), and the flow rate was 1ml/min at 264 nm. The volume of injected extract and standard sesamin were 20µl. The peak area was calculated and compared with standard (16).

Cell Lines Culture

Human larynx epidermoid carcinoma (Hep-2) the passage number was 230, murin Mammary adenocarcinoma (AMN3) the passage number was 180, human Rhabdomyosarcoma (RD) its passage number was 41 and Rat embryo fibroblast (REF) with passage number 89 were obtained from Iraqi Center for Cancer and Medical Genetic Research (ICCMGR), AL-Mustansiriyah University. Cells were grown in RPMI-1640 media that had been supplemented with fetal calf serum (5% v/v). The stock cultures were maintained at 37°C in humidified atmosphere with 5% CO₂ in 95% air in T-25.

Cytotoxicity assays

Sesamum indicum defatted extract was tested for cytotoxicity against three cancer cell lines. Two hundred micro liter of cells suspension were seeded in 96-well micro-titration plates at the density 1×10⁵ cells/ml and incubated for 24hr in humidified atmosphere incubator at 37°C. Then cells were treated with various concentrations (1000, 500, 250, 125) µg/ml of samples in total volume (200 µl/well) for 48 and 72 hr. Five replicates wells were used for each concentration of extract and 200µl of maintenance medium (RPMI-1640) added to 6 wells represented as negative control.

At the end of the exposure period, cells were stained by 100 µl of 0.5% crystal violet solution and incubated at

37°C for 20 min; the stain was washed gently with tap water until the dye was removed. The plate was left at room temperature to dry. Then 100 µl of 33% glacial acetic acid was added to each well and mixed to extract the dye. The optical density of each well was read by using a micro-ELISA reader at 492nm transmitting wavelength (17).

The percentage of inhibition was calculated according to the following equation:

$$R \% = \frac{A - B}{A} \times 100$$

IR=inhibition rate, A= the optical density of control, B= Optical density of test sample (17).

Statistical analysis

A completely randomized design (CRD) was used with different factors. The statistical analysis system –SAS (18) program was used to calculate the effect of different concentrations of sesame extract on inhibition rate. Least significant differences (LSD) test was used to compare between means at p≤0.05 level.

Results:

Detection of some active compounds in sesame seed extract

Results obtained from general chemical detection revealed the presence of phenols, tannins, saponens, glycosides, alkaloids, coumarins, and flavonoids, in crude ethanol extract and the yield of partially purified sesamin obtained from seeds extract was about 2.8 g per 100 g.

Qualitative and quantitative analysis by HPLC technique

Determination of the most important bioactive phenolic compound (sesamin) in seeds was accomplished by HPLC technique using sesamin standard as a reference. Results showed that the retention time of standard sesamin was 2.905 min, while it was 2.848 min for partially purified sesamin. The percentage of isolated sesamin was 79.93% of ethanolic extract according to total peak area (TPA) and it was approximately close to standard peak. Fig (1)

1. Hep-2 cell line

The results demonstrated that a treatment with sesame extract decreased the growth of Hep-2 cells significantly as compared to control cells (untreated), and the effect was concentration dependent, as well as, time dependent. Results showed a significant increase in inhibition rate for the concentrations 125, 250, and 500 µg/ml which were 12.06%, 14.63%, and 17.33% respectively and reached to highest inhibition rate 73.36% when the concentration increased to 1000 µg/ml at 48 hours of exposure time. The pattern of inhibitory increased in all concentrations by increasing exposure time to 72 hours 12.8, 16.2, 20.56 and 85.83% at concentrations 125, 250, 500, and 1000 µg/ml, respectively. Table 2.

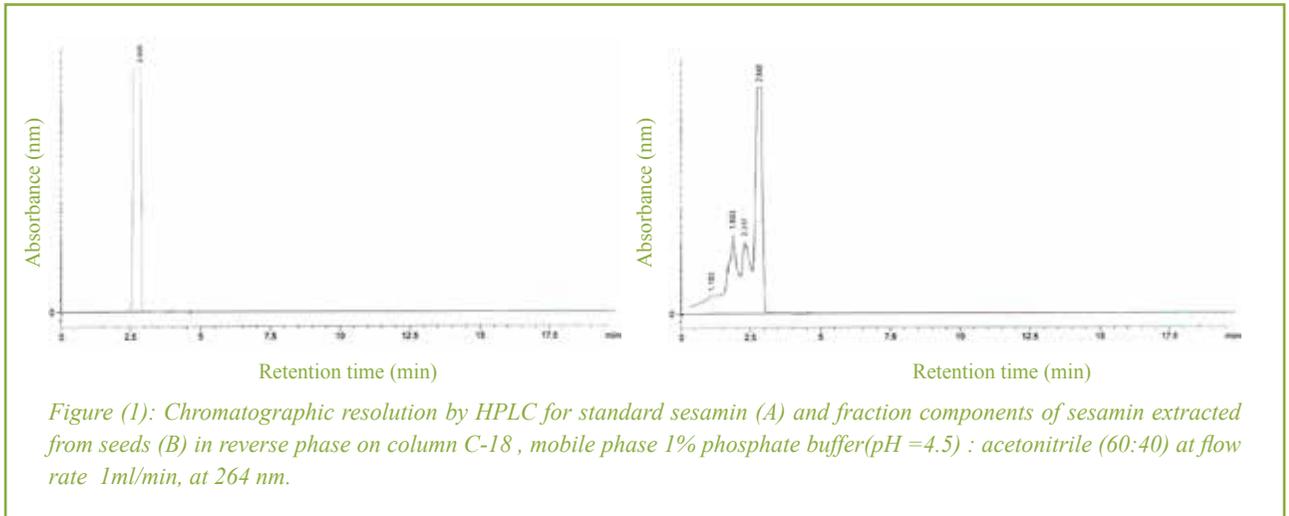


Figure (1): Chromatographic resolution by HPLC for standard sesamin (A) and fraction components of sesamin extracted from seeds (B) in reverse phase on column C-18 , mobile phase 1% phosphate buffer(pH =4.5) : acetonitrile (60:40) at flow rate 1ml/min, at 264 nm.

Table 2: Growth inhibitory rate of different concentrations of sesame extract on Hep-2 cell line after 48 and 72 hrs of exposure.

Concentration (µg/ml)	Inhibition % (mean ± SE)	
	48 hrs	72 hrs
125	12.06 ± 0.27 d	12.8 ± 0.34 d
250	14.63 ± 0.32 c	16.26 ± 0.24 c
500	17.33 ± 0.24 b	20.56 ± 0.47 b
1000	73.36 ± 0.24 a	85.83 ± 0.31 a

* ($p \leq 0.05$) . Different letters refer to a significant differences between treatments

2. AMN-3 cell line

As shown in table 3, the inhibition rate of sesame extract on AMN-3 cells increased after 48 hours of exposure time.

The concentrations of sesame extract 125 and 250µg/ml demonstrated no cytotoxic effect (-5.00% and -15.00%) respectively on the growth of AMN-3 cells but slight effects were shown at concentration 500µg/ml reached to 2.53% and moderate effect showed at concentration 1000 µg/ml

(25.06%).

The pattern of inhibition was differed when exposure time extended to 72 hour, the results showed lowest inhibitory rate (27.2%) at concentration 125 µg/ml and increased moderately and significantly (36.36%, 36.36%, and 40.06%) at concentrations 250, 500, and 1000 µg/ml respectively. Such results illustrate that inhibitory rate was time dependent.

Table 3: Growth inhibitory rate of different concentrations of sesame extract on AMN-3 cell line after 48 and 72 hrs of exposure.

Concentration (µg/ml)	Inhibition % (mean ± SE)	
	48 hrs	72 hrs
125	- 5.00 ± 0.21 c	27.20 ± 0.26 b
250	- 15.00 ± 0.26 d	36.36 ± 0.24 a
500	2.53 ± 0.18 b	36.36 ± 0.17 a
1000	25.06 ± 0.31 a	40.06 ± 2.32 a

* ($p \leq 0.05$) . Different letters refer to a significant differences between treatments

3. RD cell line

The results of inhibitory effects of sesame extract on RD cells shown in table 4 demonstrated that all concentrations under study decreased the growth of RD cells significantly as compared to control cultures (untreated). The lowest inhibitory rate 5.7% of sesame extract obtained at the concentration 250µg/ml while the highest inhibitory rate 31.03% reported at concentration 1000µg/ml of sesame extract after 48 hour exposure, and there were significant

increasing of inhibition rate dependent on sesame extract concentrations.

When the exposure time of RD cells was increased to 72 hrs, the inhibitory percentage was 4.03%, 6.76%, 17.33%, and 22.86% at concentrations 125, 250, 500, and 1000 µg/ml respectively. Increasing of inhibitory rate of RD cells depended on exposure time except the concentration 125 µg/ml.

Table 4: Growth inhibitory rate of different concentrations of sesame extract on RD cell line after 48 and 72 hrs of exposure.

Concentration (µg/ml)	Inhibition % (mean ± SE)	
	48 hrs	72 hrs
125	7.03 ± 0.29 c	4.03 ± 1.17 d
250	5.70 ± 0.15 d	6.76 ± 0.12 c
500	8.70 ± 0.34 b	17.33 ± 0.26 b
1000	31.03 ± 0.67 a	22.86 ± 0.20 a

* ($p \leq 0.05$). Different letters refer to a significant differences between treatments

4. REF cell line

The results obtained from treating REF-cell line with sesame extract are presented in table 5 which

showed decreasing in growth of REF-cells as compared to control cultures. The lower concentrations 125 and 250 µg/ml showed no cytotoxicity -12.06% and -12.6%, while

slight inhibition rate reported 4.00% and 15.03% at concentration 500 and 1000 µg/ml after 48 hour.

The inhibition percentage was increased significantly with increasing the concentrations and exposure time to 72 hour, which were 7.8%, 8.53%, 12.5%, and 20.26% at concentrations 125, 250, 500, and 1000 µg/ml respectively.

Table 5: Growth inhibitory rate of different concentrations of sesame extract on REF cell line after 48 and 72 hrs of exposure.

Concentration (µg/ml)	Inhibition % (mean ± SE)	
	48 hrs	72 hrs
125	- 12.06 ± 0.50 c	7.80 ± 0.11 d
250	- 12.60 ± 0.49 c	8.53 ± 0.23 c
500	4.00 ± 0.36 b	12.50 ± 0.17 b
1000	15.03 ± 0.42 a	20.26 ± 0.17 a

* ($p \leq 0.05$). Different letters refer to a significant differences between treatments

Discussion:

Ethanol extract of sesam seeds contain a variety of secondary metabolites and the presence or absence of these active compounds depend on the type, polarity and dielectric constant of solvents used in extraction(6,19). On the other hand the differences in retention time of sesamin

was attributed to mobile phase of HPLC analysis(16).

The cytotoxic effects of phenolic compounds may depend on lipophilicity of the compound in question, which is very important for the penetration into the target cells (20). On the other hand, lipids and proteins present in biological membranes facilitate the solubility of polyphenols, and differences in cell membrane structures and metabolic

activation of chemicals can also affect the activity of polyphenols (21), also it is possible to suggest that the chemical constituents of sesame extract, especially phenolic acids and flavonoids, may have a selective cytotoxic effect against cells and such effect is determined by the type of cells under investigation.

The cytotoxic activities of lignan against Hep-2 (larynx epidermoid carcinoma), HeLa (human cervix carcinoma) and C6 (rat glioma) cell lines was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay at several concentrations for 24 hr, and observed high reduction in cancer cells number that the lignan was a high influence on cells viability (22).

Sesamin causes cell cycle arrest at G1 phase in human breast cancer MCF7 cells and decrease Rb protein phosphorylation. Furthermore, sesamin reduces cyclin D1 expression, which can be inhibited by proteasome inhibitor. The reduced expression of cyclin D1 by sesamin is found

in lung cancer, kidney cancer, keratinocyte cancer, melanoma and bone cancer cells (23).

Human cell line HL-60 and Molt-4 treated with sesamin produced ROS, but in condition of combined treatment with H₂O₂, sesamin has dual effect, as an antioxidant or a prooxidant depending on the concentrations of sesamin and type of leukemic cells. Taken together, the sesamin-induced apoptotic death pathways involved mitochondrial transmembrane potential reduction, caspase-3 activation and increase expression of GADD153 (endoplasmic reticulum stress protein) (24).

In conclusion *S. indicum* defatted ethanol seed extract showed high toxic activity on Hep-2 cell line and low toxicity on AMN-3 and RD cells with less effect was found on Ref cell line. Further investigations on the efficacy of purified sesamin are required to get better understanding of the mechanism of anticancer activity.

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الفعالية السمية للمستخلص الايثانولي لبذور السمسم Sesa- mum indicum على الخطوط الخلوية السرطانية خارج الجسم الحي

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الخلاصة:

أجريت الدراسة الحالية بهدف التحري عن الفعالية السمية في المستخلص الايثانولي لبذور نبات السمسم Sesa mum indicum متزوع الدهون على ثلاث أنواع من الخطوط الخلوية السرطانية و هي Hep- 2 و AMN- 3 و RD و خط الخلايا الطبيعية لجنين الجرذ Ref . بينت نتائج الكشف الكيميائي لمكونات المستخلص الايثانولي لبذور السمسم وجود الفينولات , و التانينات , و الصابونينات و الكلايكوسيدات و القلويدات و الكومارينات و الفلافونيدات . استخدمت تقنية كروماتوغرافيا السائل ذو الأداء العالي للكشف الكمي و النوعي عن محتوى مركب Sesamin الفعال مقارنة مع المركب القياسي , إذ بلغ تركيز المادة الفعالة 226.6 مايكرو غرام / مل و يمثل نسبة % 79.93 من محتوى المستخلص الخام . بينت النتائج الفعالة السمية لمستخلص بذور السمسم خارج الجسم الحي على الخطوط الخلوية حدوث تثبيط معنوي في نموها مقارنة بخلايا السيطرة (غير المعاملة) و كان التأثير معتمدا على كل من الجرعة ووقت المعاملة لكل من الخلايا Hep-2 و AMN- 3 و Ref و RD إذ أظهر المستخلص فعالية سمية واضحة معنويا بعد مدة تعرض 72 ساعة عند التركيز 1000 مايكرو غرام / مل و التي بلغت % 85.83 و % 40.06 و % 20.20 على التوالي بينما بلغت نسبة التثبيط خلال مدة التعرض 48 ساعة عند التركيز 1000 مايكروغرام / مل لخلايا RD % 31.03.