Cytotoxic Effect of *Brassica oleracea* Extract on two Tumor Cell Lines *in vitro*

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Abstract

This study was conducted to evaluate the cytotoxic effect of *B. oleracea* oil extract on cancer cell lines and normal cell line *in vitro*. Two types of tumor cell lines implanted *in vitro* which were (murine mammary adenocarcinoma, Ahmed-Mohammed-Nahi-2003 (AMN-3) cell line and human Rabdomyosarcama RD cell line). In addition, rat embryo fibroblasts (REF) was used as a normal cell line in this study. Tissue culture plates under sterile condition were treated with different concentrations of *B. oleracea* extract started from 2.3mg/ml to 300mg/ml for 72 hrs incubation. Results pointed out that *B. oleracea* extract had a cytotoxic effect depending on the type of cells and the concentration of the extract used for treatment. Results revealed the presence of significant cytotoxic effect on both RD and REF cell lines at concentrations (9.3, 18.7, 37.5 and 75) with percentage of inhibition rate as compared with control and there was not significant effect on AMN3 cell line. Also results showed that growth inhibition was significantly affected and variation in inhibition rate in the same concentration was detected between tumor cell lines and normal cells in this study.

Keyword: *B. oleracea* extract, Cytotoxic effect, Tumor cell lines, *in vitro*.

Introduction

In recent years, chemoprevention has attached two considerable attentions as a mean of blocking malignant transformation in its early stages and disease progression in later stages [1, 2]. Herbal medicine is still the most common source for primary health care of about 65- 80% of the world's population, mainly in developing countries, because of better cultural acceptability, better compatibility with the human body and fewer side effects. Leaves, flowers, stems, roots, seeds, fruit and bark can all be constituents of herbal medicines [3]. The medicinal values of these plants lie in their phytochemical components which produce definite physiological actions on the human body [4, 5]. The most important of these components are alkaloids, tannins, flavonoids and phenolic compounds [6]. *Brassica* genus is native in the wild in western Europe, the Mediterranean and temperate regions of Asia. In addition to the cultivated species, which are grown world wide, many of the wild species grow as weeds, especially in North America, South America, and Australia [7,8]. It is important genus in the Brassicaceae family, several species and types of *Brassicas* are significant oilseed crops, vegetables, forage crops, and are used in the production of condiments, such as mustard, *Brassica* species are widely used in the cuisine of many cultures and recognized as a valuable source of dietary.

Fiber and contain little fat, and source of vitamins and minerals [9].

They also contain a large number of novel phytochemicals, some of which protect against carcinogenesis. Hence, *Brassicas* are believed to be useful in the prevention of cancer [10, 11, 12].

The objectives of the study were to study the cytotoxic effects of *Brassica* extract against normal cell line and cancer cell lines.

Materials and Methods

Chemical reagents

RPMI-1640 Medium, Fetal Calf Serum (FCS), Phosphate Buffer Saline (PBS), Penicillin G, Streptomycin, Glucose, (HEPES) and Neutral Red were obtained from Sigma, USA. Trypsin, Ethylene Diamine Tetra-Acetic Acid (EDTA), Na_2HPO_4, Ethanol and Sodium Bicarbonate were obtained from BDH.

Extraction of plant material

A quantity of 30g of plant seeds were obtained from (College of Pharmacy/ Baghdad

University). Seeds were dried over night at 50°C and grinded in to powder then mixed with 150 ml hexane and the extraction carried out by soxhlet for 16 hrs. The extract was evaporated using a rotary evaporator. The residue (5g) was dissolved in 10 ml RPMI 1640 serum free culture medium. From this stock extract, the required concentrations were prepared for use in cytotoxicity assay [18].

Cell lines and Cell Cultures

Both tumor cell lines, [Ahmed-Mohammed-Nahi-2003 (AMN-3) and Rabdomyosarcoma (RD) and normal Rat Embryonic Fibroblast (REF) cell line were used in this they were kindly supplied by Biotechnology Research Center/ Al Nahrain University, Baghdad, Iraq. Cells were cultured in RPMI-1640 medium supplemented with 10% FCS, 50 mg/ml streptomycin, and 1000U/L penicillin. All cell lines were grown as a monolayer in humidified atmosphere at 37°C with 5% CO_2. The experiments were performed when cells in the logarithmic phase of growth [13].

Cytotoxicity Assay

Cytotoxic assay was carried out according to Freshney [14]. Cell suspension was prepared for each types of cell lines and 1×10^5 exponentially growing cells seeded in a 96 well tissue culture plates as (200 µL) in each well and incubated at 37 ºC for 24hrs. After incubation, the wells examined to inspect the formation of cell monolayers and 200 µL/ well from each concentration (2.3, 4.6, 9.3, 18.75, 37.5, 75, 150 and 300 µg/ml) of three fold concentrated filtrate were added to the wells as three replicate for each concentration. Three replicates were made for control which contained only the cells with growth medium. After 48 hrs, 50 µL/ well of neutral red dye was added and incubated again for 2 hrs. After incubation, the contents of the plate were removed by washing the cells with PBS to remove the excess dye followed by the addition of 20 µl/well of extraction dye solution that draw out the dye from the viable cells that had stained. The results were read using ELISA reader at wave length 492 nm. [15]. The percentage of growth inhibition (PGI) was calculated according to the following equation [16].

$$\text{Growth inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of treated cell} * 100}{\text{Absorbance of control}}$$

Statistical Analysis

The values of the investigated parameters were given in terms of mean ± standard error, and differences between means were assessed by analysis of variance (ANOVA) and Duncan test, using SPSS computer program version (7.5). Differences in results were considered significant at probability value equal or less than 0.05 [17].

Results

Cytotoxic effect of *B. oleracea* extract on REF

Results in Table (1-1) revealed that the *brassica* extract showed a significant inhibition effect on REF cell line. The highest inhibition rate shown at concentrations (2.3, 4.6, 150 and 300 mg/ml) while the lowest cytotoxic effect was recorded at (9.3, 18.7, 37.5 and 75) and this effect was not significant compared with control cells.

Cytotoxic effect of *B. oleracea* extract on (RD).

Results displayed in Table (1-1) showed significant difference in viable cell number compared with control RD cells, the highest inhibition rates were shown at concentrations (300, 150 and 75 mg/ml), also there were significant differences between the different used extract concentrations .

Cytotoxic effect of B. *oleracea* extract on AMN- 3

Result in Table (1-1) showed significant differences in inhibition rates compared with AMN3 control cells. The highest inhibition rate (46%) occurred at the concentration 2.3 mg/ml. Result also revealed no significant differences between the different concentrations compared with AMN3 control cells.

Table (1-1)
Cytotoxic effect of different concentrations of B. oleracea extract on growth of both tumor and normal cell lines after 72 hrs incubation time.

Concentrations	*IR*	*REF OD (Mean±SD)*	*IR*	*RD OD(Mean±SD)*	*IR*	*AMN3 OD(Mean±SD)*
Control		BC, a 0.900±0.05		E, b 1.016		A, c 0.1300
2.3	36.6	A, a 0.573±0.0462	23	D, b 0.773±0.0252	46	A, c 0.07±0.0200
4.6	23.3	D, a 0.693±0.0603	7	C, b 0.936±0.0321	30.7	A, c 0.090±0.0100
9.3	5.5	B, c 0.856±0.0404	9	C, b 0.913±0.0321	28.4	A, c 0.093±0.0154
18.7	-5.5	C, a 0.953±0.0451	10	C, a 0.903±0.0451	23	A, b 0.10±0.0265
37.5	00	BC, a 0.900±0.0200	11	C, a 0.896±0.0551	4.6	A, c 0.124±0.0434
75	5.5	B, a 0.850±0.0458	35	B, b 0.650±0.0436	-2.3	A, c 0.133±0.0577
150	32.2	AD, a 0.616±0.0289	51	A, b 0.496±0.0208	38.4	A, c 0.080±0.0173
300	38.8	A, a 0.553±0.0351	55	A, b 0.456±0.0513	34.6	A, c 0.085±0.0050

Differences A, B, C are significant (P<0.05) to compression columns.
Differences a, b, c are significant (P<0.05) to compression rows, IR inhibition rate, OD Optical Density, (-) sign refer to growth more than the negative control.

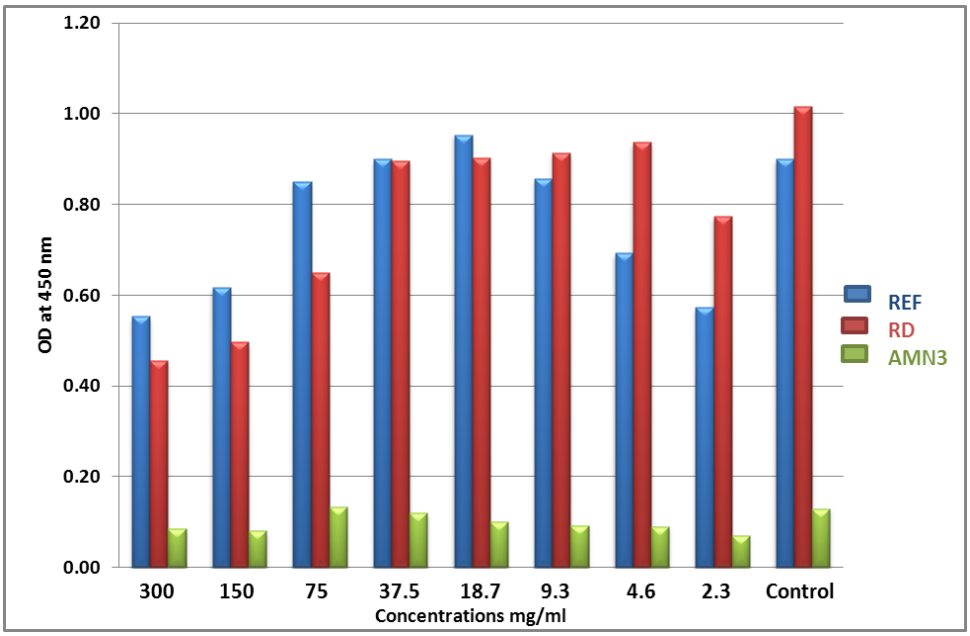

Fig.(1-1) The OD of REF, RD and AMN3 cell lines after exposure to different concentrations of B. oleracea extract for 72hrs, the difference in OD indicate the viability the more OD which means the more viable cells.

Discussion

Results showed that oil extract of *brassica* had the ability to reduce the viability of both tumor cell lines (RD and AMN3) and normal cell line (REF). The reduction in cell growth may be related to the effect of some compound especially some isothiocyanates such as sulforaphane, Erucin (4-methylsulphin3-butenyl) which are associated with induction of Phase II detoxification enzymes that termed mono- functional inducers, which are hypothesized to be of health benefits because of their role in detoxification of carcinogens [19, 20, 21]. Several other mechanisms of sulforaphane were also demonstrated, such as inhibition of tumor cell proliferation, induction of apoptosis, protection of DNA from damage induced by different xenobiotics and antimetastatic potential [22]. Erucin (4-methylsulphinyl-3-butenyl), which is an analogue of sulforaphane, is also of interest to health researchers [23]. Erucin has been shown to arrest cell cycle on Jurkat T-leukemia cells and holds a promise for future development as a chemo-preventive agent [24]. This isothiocyanates can decrease the risk of developing different cancers such as breast cancer gastric cancer and skin cancer [25]. The results agree with [26] who reported that the anticancer activity of *Brassica* extract was assessed against A-549 cell line (Human lung carcinoma)**.** 3, 3'-Diindolylmethane in *Brassica* vegetables is a potent modulator of the innate immune response system with potent anti-viral, anti-bacterial and anti-cancer activity, several studies indicated that some compounds naturally present in *Brassica* such as indoles, induce apoptosis in various types of cancer[27]**.** In conclusion there was a good evidence that the crude *Brassica* extract have cytotoxic effect toward the tumor cells and this a good indication that the *Brassica* extract contains compounds which have cytotoxic effect against the tumor cells, at different concentration the extract showed cytostatic, some of the extract concentrations showed hormosis effect which inhibit the cell growth at low concentrations but not at high concentration.

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الخلاصة

هدفت الدراسة الى تقييم تأثير المستخلص الزيتي لنبات السلجم في الخطوط الخلوية السرطانية والطبيعية خارج الجسم الحي. استخدم خطين خلويين سرطانيين مزروعة في الزجاج *in vitro* وهي خط خلايا سرطان الثدي الفأري AMN3 وخط خلايا سرطان الحوض البشري RD بالمقارنة مع مع خط خلايا طبيعي متحول مولد لالياف جنين الجرذREF باستخدام تقنية الزرع النسيجي. عوملت الخطوط الخلوية بتراكيز مختلفة من التركيز mg/ml 2.3 الى mg/ml 300لمدة تعريض بلغت ٧٢ ساعة. اشارت النتائج الى ان للمستخلص تأثير سمي يعتمد على نوع الخلايا ومقدار التركيز واظهر المستخلص تأثيرا تثبيطيا معنوياً لكل من الخطين الخلويين REF و RD في التراكيز (٩.٣,١٨.٧,٣٧.٥,٧٥ ملغم/مل) مع نسبة التثبيط بالمقارنة مع قيم السيطرة وكذلك اظهرت النتائج تأثيرا تثبيطياً غير معنوي في الخط الخلوي AMN3 بالمقارنة مع السيطرة .واوضحت النتائج معدل التاثير التثبيطي في النمو اذ كان تاثيرا معنويا ضمن التركيز الواحد بين الخطوط الخلوية السرطانية والخط الطبيعي.