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Effects of Solanum Nigrum Aqueous Extract on Normal and Cancer Cells Line

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Abstract: *This study was conducted to evaluate the cytotoxic effect of S.nigrum aqueous extract on cancer cell lines and normal cell line in vitro. Two types of tumor cell lines implanted in vitro which were (Hep-2 cell Line Human epidermoid larynx carcinoma) 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 S. nigrum extract for 24,48and 72 hrs incubation.Results pointed out that S.nigrum extract had a cytotoxic effect depending on the type of cellsand the concentration of the extract used for treatment. Results revealed the presence of significant cytotoxic effect on both RD, and Hep-2 cell Line and non significant againstREFcell ine. The results showed that growth inhibition was significantly affected and variationin inhibition ratein the same conce ntration was detected between tumor cell lines and normal cells.*

Keywords: *Solanum Nigrum, cancer cells line, aqueous extract*

I. Introduction

Natural Products, especially plants, have been used for the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient time and an impressive number of modern drugs have been developed from them. The first written records on the medi-cinal uses of plants appeared in about 2600 BC from the Sumerians and Akkaidians [1]. The "Ebers Papyrus", the best known Egyptian pharmaceutical record, which documented over 700 drugs, represents the history of Egyptian medicine dated from 1500 BC. The Greeks also contributed substantially to the rational development of the herbal drugs. Dioscorides, the Greek physician (100 A.D.), described in his work "De Materia Medica" more than 600 medicinal plants [1,2] . The World Health Organi-zation estimates that approximately 80% of the world's inhabitants rely on traditional medicine for their primary health care. Plants have long been used in the treatment of cancer [3]. The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer [4] . Of the 92 anticancer drugs commercially available prior to 1983 in the US and among worldwide approved anticancer drugs between 1983 and 1994, 60% are of natural origin [5].In this instance, natural origin is defined as natural products, derivatives of natural products or synthetic pharmaceuticals based on natural product models (Jaspars and Lawton, 1998). Solanum nigrum (Sn) Commonly known as Black Nightshade is a dicot weed in the Solanaceae family. Sn is an Annual branched herb of up to 90 cm high, with dull dark green leaves, juicy, ovate or lanceolate, and toothless to slightly toothed on the margins.

2 Flowers are small and white with a short pedicellate and five widely spread petals. Fruits are small, black when ripe[6]. S. nigrum is found mainly around waste land, old fields,ditches, and roadsides, fence rows, or edges of woods and cultivated land. It is a common plant found in most parts of Europe and the African continent. Sn is a popular plant in part due to its toxic content of Solanine, a glycoalkaloid found in most parts of the plant, with the highest concentrations in the unripened berries [7,8]. Although it is considered a rich source of one of the most popular plant poisons, it has proven also to be a reservoir of phytochemicals with pharmacological prospects [9,10].

The effect of crude polysaccharide isolated from S. nigrum linn. (SNL-P) was examined both in vivo and in vitro on U14 cervical cancer cells. Though exposure to SNL-P had no antiprolifreative effect in vitro at doses up to 1 mg/ml, it decreased the number of ascites tumor cells and survival time of U14 cervical cancer bearing mice which received between 90 - 360mg/kg bw. P.o. FACScan flow cytometer analysis showed that most of the ascites tumor cells were arrested in G2/M phase of cell cycle. This can be considered as the basis for its use as an anticancer agent [11,12,13] .

II. 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), Na2HPO4, Ethanol and Sodium Bicarbonate were obtained from BDH.

The Plant Solanim nigrum

The late herbalist Dr. ALI Al-Mosawai (Deparment of Biology, College of Science, University of Baghdad) supplied and identified the leaves of solanium nigrum which were the collection of June 2011. The plant was grown in a local garden in Baghdad. The leaves were left at room temperature to dry, and after dryness, they were powdered with a coffee grinder.

Preparation of plant extract

The plant powder of S. nigrum was macerated with distilled water in ratio of 1:7(50 gr of the powder in 350 ml of sterilized distilled water).During extraction the mixture was shacked continuously for 6-7 hours at room temperature in a shaking incubator, the suspension was filtered by Whatman no.1 filter paper and the filtrates concentrated by using vacum rotary evaporator. The crud extract was stored in dark sterile screw bottle at 4°C until use [14,15].

Cytotoxicity Assay

Cytotoxic assay was carried out according to Freshney [14].

Cell suspension was Growth inhibition (%)= Absorbance of control - Absorbance of treated cell*100\ 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

III. Results and Discussion

Effect of S. nigrum extract on HEp-2 Cell Line

When the cancer cell line (HEp-2) treated with the extract the result showed significant cytotoxic effect of extracts in a dose and time dependent manner in comparison with the control..

Table (1). Effect of S. nigrum aqueous extract on Hep-2 cancer cell line after three period of exposure (24,48,72)hr.

Exposure time / Concentration of extract	IR% (mean±SE)		
	24 hr.	**48 hr.**	**72 hr.**
0.1mg/ml	A,a 16.120±2.826	A,a 20.703±0.687	A,b 33.173±2.645
0.25mg/ml	A,a 17.100±1.986	B,b 24.547±2.098	A,c 33.590±1.372
0.5mg/ml	B,a 22.453±2.494	C,b 29.657±2.299	A,b 34.987±2.443
1mg/ml	C,a 27.027±2.064	D,a 38.557±1.559	B,b 42.137±2.202
2.5mg/ml	D,a 31.717±1.811	E,a 36.613±2.968	BC,b 45.583±2.571
5mg/ml	E,a 35.400±1.565	F,b 44.050±2.285	C,c 48.477±1.618
10mg/ml	F,a 37.497± 3.507	G,a 48.353±1.781	D,b 56.720±2.262
25mg/ml	G,a 46.973±3.363	H,a 52.313±2.159	E,b 61.553±1.524
50mg/ml	H,a 49.397±3.678	I,a 59.120±1.307	F,a 66.463±1.500
100mg/ml	H,a 51.603±0.743	I,a 63.380±2.114	G,b 70.577±1.625

Effect of S. nigrum aqueous extract on RD cells line.

Table.(2). Effect of S. nigrum aqueous extract on RD cancer cell line after three period of exposure (24,48,72)hr.

Exposure time / Concentration of extract	IR% (mean+SE)		
	24 hr.	48 hr.	72 hr.
0.1mg/ml	A,a 15.160+1.065	A,a 16.623+0.401	A,b 27.693+1.308
0.25mg/ml	B,a 19.533+2.032	B,a 19.347+1.112	B,b 33.257+1.046
0.5mg/ml	C,a 27.410+1.735	C,b 22.040+0.243	C,c 36.320+0.115
1mg/ml	D,a 37.563+1.106	D,b 29.867+1.086	D,a 40.470+0.925
2.5mg/ml	D,a 36.530+0.792	E,a 33.360+1.574	E,b 43.583+0.608
5mg/ml	D,a 39.753+0.368	F,a 41.987+0.356	F,b 46.900+0.135
10mg/ml	E,a 45.357+1.060	F,a 42.893+0.210	G,a 47.053+0.302
25mg/ml	E,a 46.657+0.938	F,a 43.533+0.393	G,b 48.077+0.349
50mg/ml	E,a 44.010+1.591	G,a 45.403+0.496	G,b 50.680+0.805
100mg/ml	D,a 41.483+1.698	G,a 45.587+1.029	G,b 51.677+0.397

The comparison of the cytotoxic effect of S. nigrum extract on the growth of RD cells line, notes that there is a significant difference in the growth inhibition rat .The toxicity of these extract was,time and dose dependent manner. the significant difference in cells viability ,the inhibition rat started (15%) with a concentration (0.1mg/ml)at 24 period of exposure increased to the maximum levels (51%) at 72 exposure period with a concentration (100mg/ml) .From the comparison the effect of S.nigrum extract acts in a linear response when high dose with high time give high inhibition rat . 6

Effect of S. nigrum extracts on REF cells Line:

The normal fibroblastic primary cells (REF) were cultured and used for evaluation the toxicity of S.nigrum. The result showed a slight toxicity in each extracts but with non-significant difference (P>0.05) on that particular primary cells in all three period of exposure as shown in table (3).

Table(3). Effect of S .nigrum aqueous extract on REF normal cell line after three period of exposure (24,48,72)hr.

Exposure time / Concentration of extract	IR% (mean+SE)		
	24 hr.	48 hr.	72 hr.
0.1mg/ml	A,a 0.5133+0.2438	A,a 0.5900+0.2663	A,b 0.2233+0.0503
0.25mg/ml	A,a 0.4967+0.1002	B,b 1.2900+0.3747	B,b 0.8733+0.2444
0.5mg/ml	A,a 0.2967+0.1305	C,b 2.0200+0.1670	B,b 1.0800+0.1836
1mg/ml	B,a 1.0967+0.1206	DE,b 3.1700+0.3297	C,a 1.7233+0.1557
2.5mg/ml	AB,a 0.9300+0.2651	E,b 2.8100+0.1744	D,b 2.9800+0.1389
5mg/ml	A,a 0.4567+0.0987	D,b 3.4967+0.2026	E,b 3.6867+0.0929
10mg/ml	C,a 2.0800+0.4058	D,b 3.9067+0.2011	F,c 4.8133+0.0379
25mg/ml	B,a 1.3133+0.1002	F,b 4.4233+0.1665	G,b 5.5667+0.3995
50mg/ml	B,a 1.3300+0.4557	F,b 4.0967+0.3421	G,c 5.9433+0.3585
100mg/ml	B,a 1.0467+0.3329	F,b 4.6633+0.5401	G,c 5.8400+0.1652

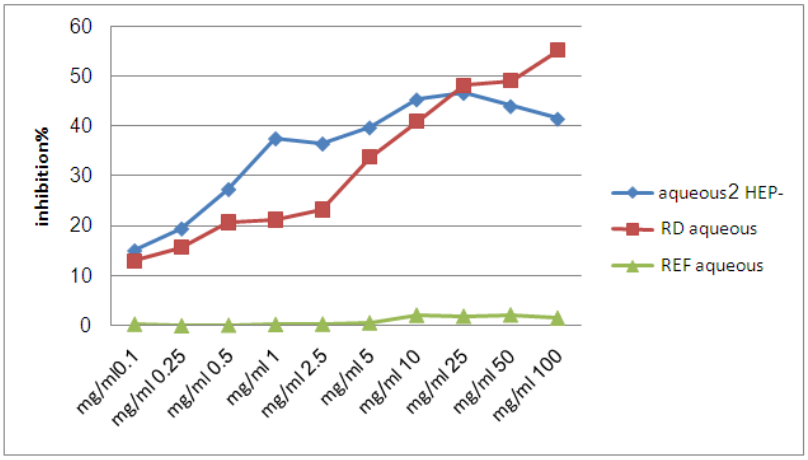

Figure (1). Camper between the effect of S.nigrum aqueous extract on three cells lines (HEP-2,RD,REF)after 24 hour incubation period

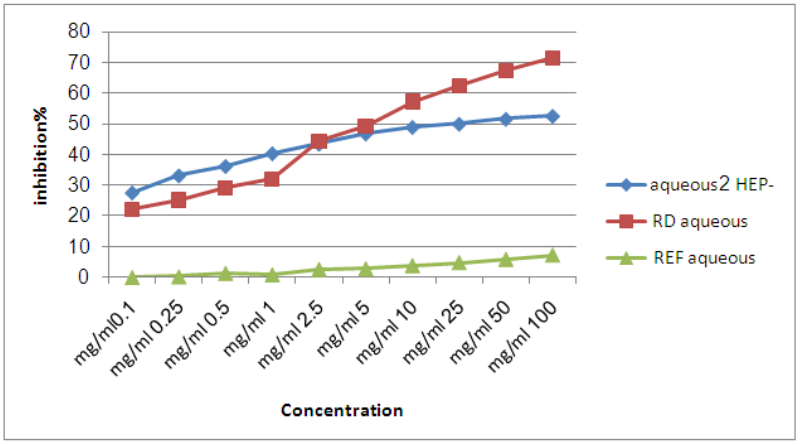

Figure (2). Camper between the effect of S.nigrum aqueous extract on three cells lines (HEP-2,RD,REF)after 48 hour incubation period

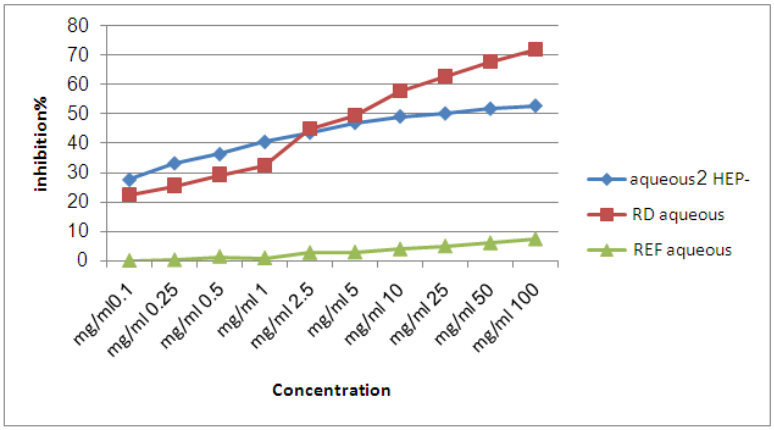

Figure (3) . Camper between the effect of S.nigrum aqueous extract on three cells lines (HEP-2,RD,REF)after 72hour incubation period.

Statistically, it can be documented that the two types of S. nigrum extract possess a cytotoxic effect toward cancer cell lines, but the severity of this toxicity was varied from one cell line to another at different exposure period . However, these extracts showed non-significant difference on normal fibroblastic cells.

From the results were a variable cytotoxic effects with significant differences on two types of cell lines (HEp-2, RD) in a dose and time dependent manner. Figures (1, 2, 3,) compare between the percentages of inhibition rat in cells viability for cancer cells lines (Hep_2,RD) and normal embryo fibroblast cells line (REF)

caused by S.nigrum aqueous extract at three exposure period (24,48,72)hour . From these figures we can observed the clear effect of S. nigrum plant extracts on cancer cells line and there's no or lees effect on normal cells line .

The Hep-2 cells line appear to be more effected with lower concentrations of extract at all exposure period than RD cells line, while in high concentrations the RD cells line more affected. The S.nigrum extracts possesses a high cytotoxic effects on different types of cancer cell lines and this may be attributed to the presence of some specific compounds in these extracts, and the significance was varied form cell type to another [17,18] .

Several reports have shown that alkaloids (one of the S .nigrum active groups as cited by Duke, 1992) from Lobelia inflate capable to reverse P-gp dependent multidrug resistance property of two types of cancer cell lines, Caco-2 (human colon carcinoma) and CEM ADR 5000 (leukemia cell line)[19,20]. The alkaloids fraction affect the P-gp expression by enhancing rhodamine 123 retention in both Caco-2 cells and CEM ADR 5000. Because the rhodamine 123 is a substrate of P-gp, alkaloids thus inhibits P-gp function probably by substrate competition [21,22,23,24].

In conclusion there was a good evidence that the crude S.Nigram extract have cytotoxic effect toward the tumor cells and this a good indication S.Nigram that the extract contains compounds which have cytotoxic effect against the tumor cells, at different concentration.

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