Cytotoxic effect of the aqueous extract of *Portulaca Oleracea* L. on some cell lines

السميه الخلوية للمستخلص المائي لنبات البقلة *Portulaca Oleracea* L. لبعض الخطوط الخلوية

Maeda H. Mohammad Zaynab S. Abdul-Gany Aymen A. Hassan
Iraqi Center of Cancer and Medical Genetic Researches \ AL Mustansyria University

مائده حسين محمد زينب سعد عبد الغني ايمن علي حسن
المركز العراقي لبحوث السرطان والوراثة الطبية/ الجامعة المستنصرية

Abstract

The cytotoxic effect of the aqueous extract of *Portulaca oleracea* L. was tested *in vitro* against the cancer cell AMN3 (Murine Mammary Adenocarcinoma cell line) and the normal cell REF (Rat Embryo Fibroblasts). Proliferation was significantly reduced at high concentrations (300-1000 µg/ml) during the first 24 hrs. of contact on AMN3. while the extract showed no significant effect during different period time of exposure as compared with control against REF cells.

المستخلص

تم اختبار تأثير السميه الخلوية للمستخلص المائي لنبات البقلة *Portulaca oleracea* L. خارج الجسم الحي في خلايا سرطان الغدة اللبنية الفأري (AMN3) وتأثيره في خلايا الـ REF الطبيعية. اظهرت النتائج وجود فروقات معنوية باستخدام تراكيز مرتفعة من المستخلص (300-100 مايكروغرام/ مل) خلال الـ 24 ساعة الاولى من تعريض الخلايا للخط السرطاني AMN3، بينما اظهرت النتائج عدم وجود فروقات معنوية خلال فترات التعريض مقارنة بمعاملة السيطرة لخلايا الـ REF الطبيعية .

Introduction

Herbal remedies are widely used for the treatment and prevention of various diseases and often contain highly active pharmacological compounds. Toxicity related to traditional medicines is becoming more widely recognized as these remedies become popular in the Mediterranean region as well as worldwide. Most reports concerning the toxic effects of herbal medicines are associated with hepatotoxicity although reports of other toxic effects including kidney, nervous system, blood, cardiovascular and dermatologic effects, mutagenicity and carcinogenicity have also been published in the medical literature [1].

Portulaca oleracea (Family Portulaceae), commonly known as pursalane, pig weed, little hogweed, postelijn, pussley, perpine and berbin. *P. oleracea* contains vitamins A, C and E, and minerals like calcium and potassium salts, phosphorus, manganese, silicone, copper and high levels of potassium and iron. Also contains fatty acid like glutathione and omega-3. It is also a good source of co- enzyme Q10. All this makes the plant a great source of antioxidants [2].

P. oleracea has been used throughout history for many different medicinal purposes; the plant is rich in pectin, which lowers cholesterol. It has been used as a hypo-lipidemic agent, as an anti-oxidant, treatment of chronic cough, ulcerative colitis, urinary tract infections mastitis, diarrhea [3].

The objective of this study was to study cytotoxic effects of *P. oleracea* extract on cancer and normal cell.

Material and Methods

Extraction preparation

P. oleracea (leaves and stems) that collected from local markets was shed by distilled water, and dried at room temperature, stored in dry situation. According to [4], aqueous extract of plant was prepared by dissolving (40g.) powdered plant (for all plant) in 160 ml of distilled water in (1: 4 w\ v), then crashed by mortar in ice bath, the mixture was stirrered by blender for 1\ 2 hrs. to destroy cell wall, then filtered by Watmann filter paper (1) and the extract was evaporated to dryness 40 ˚c on dry heat Incubator, the extract then was kept at -4 ˚c until the time of use for following experiments.

One gram of the powder extract dissolved in 100 ml distilled water, sterile by filtered (0.22 µm.).

Reagents for Chemical Tests

According to [5] reagents for chemical testes were used to detect active (secondary metabolites) compounds in aqueous extract of the plant. The extract tested by the fallowing reagents:-

Test of Alkaloids (mayers reagent) [5]

Test of phenoles- Tannins (Fecl2 reagent) [5]

Test of Flavonoids (PbHCO3) [6]

Test of Terpenoids- Saponins (Saponins, HgCl2 reagent) [6].

Cell Line and Culture

This *in vitro* method was used to investigate the effect of the aqueous extract of *P. Oleracea* on two types of cells grown on RPMI- 1640 medium (Rosswell Park Memorial Institute) (Sigma chemicals) and supplemented with 5% of Fetal Bovine Serum (FBS):

Ahmmed- Mohammed- Nahi- 2003 (AMN3) cell line. This murine mammary adenocarcinoma cell line was derived from a spontaneous mammary adenocarcinoma of female BALB\c mice [7]. Passages 131 of AMN3 cell line was used throughout this study and cells were maintained using RPMI- 1640.

Rat Embryo Fibroblasts (REF) cells of this normal murine cell line were a mixture of fibroblastic and epithelial cells with normal chromosomal picture [7]. Passage 59 of this cell line was used in this study and the cells were maintained using RPMI- 1640.

Cell line Preparation for Cytoxicity Study

The growth medium was decanted off. Two to three ml of trypsin- versene was added to the cell sheet and the flask recked gently. After approximately 30 seconds most of the tyrosine- versene was poured off and the cells incubated at 37 ˚C until they had detached from the flask. Cells were further dispensed by pipetting in the growth medium [8].

Afterwards, 200 µl of cells in growth medium were added to each well of sterile 96-well microtitration plate. The plates were sealed with a self adhesive film, lid placed on and incubated at 37 ˚C.

When the cells are in exponential growth, i.e. after Lag phase, the medium was removed and serial dilutions of aqueous extracts in serum free media (SFM) (1000, 500, 250, 125, 62.5, 31.25, 15.625 or 0.0) µg/ml were added to t

replicates were used for each concentration of each extract. Afterwards, the plates were re- incubated at 37 ˚C. for the selected exposure times (24, 48 or 72) hrs.

Cytotoxicity assay

Supernatants were removed from the wells of the microtitration plate at the end of each exposure period while maintaining sterile conditions. 100 µ l of MTT solution (3- (4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl-tetrazolium bromide] (1mg\ml) and 50 µ l of SFM in (2: 1 v/ v) was added to each of the wells in the microtitration plate, then covered and incubated for 4 hrs. at 37 ˚C. At the end of this incubation period 200 µ l of Dimethyl Sulfocside (DMSO) was added to each well to dissolve the formazan crystals. The Optical density was determined at 550 nm. using ELISA reader [8].

Statistics

Experiments data were analyzed using Statistical Package Social Sciences (SPSS) Programs V. (10.0.1) (ANOVA) by statistical Last Significant Design (LSD). Significance between control and samples was determined using students F- test. A P value ≤ 0.05 was considered statistically significant.

Results

Chemical Tests

Depending on the chemical reagents used the result showed the presence of the following active compound in the aqueous extracts of *P. oleracea*. The extract contains alkaloid, phenoles, tannins, flavonoids and terpenoids Table (1).

Table(1): Phytochemicals detected in the crude extracts of P. *oleracea*

Phytochemicals to be detected		Results of aqueous extract
Alkaloids	Mayers regent	+
phnoles	Tannins/ Fecl2	+
	Flavonoids/ KoH	+
Terpenoids	Saponins	+
	Hgcl2	+

+: The extract contains the designated phytochemical

Effect on AMN3 cell line

Figure (1) shows the effect of aqueous extract of *P. oleracea* on cancer (AMN3) with concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.625 , 0.0) µg/ml and exposure period (24, 48 , 72) hrs..

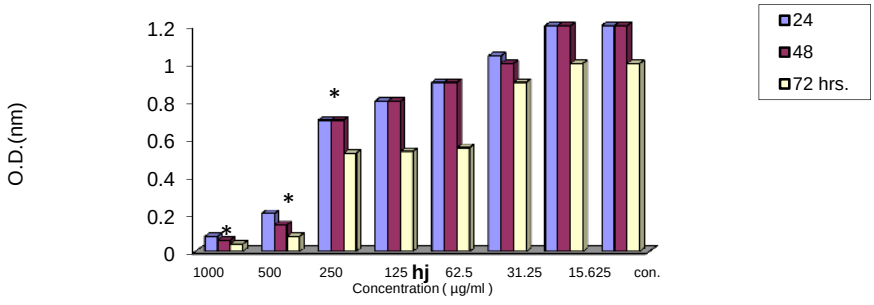

Fig (1): effect of aqueous extracts of *P. oleracea* on cancer cell line (AMN3) after 24, 48 and 72 hrs.

*** Significant differences**

The cytotoxicity of the aqueous extract was increase in high concentrations and it has a dose- dependent effect on viability of AMN3 cells, and with significant effect at level ($P < 0.05$) to show cell- lysis from 24 hrs. on 250, 500 and 1000 µg/ml compared with control. And there is no significant difference at level ($P > 0.05$) on 48 and 72 hrs period time of exposure. The median growth inhibitory concentration (IC_{50}) on (24, 48 , 72) hrs was 300 µg/ml.

Effect on REF cell

The effect of aqueous extracts of *P. oleracea* on normal (REF) time of exposure was (24, 48 , 72) hrs. is illustrated in Figure (2). All the concentrations (1000, 500, 250, 125, 62.5, 31.25 , 15.625) µg/ml of the extract showed no significant effect at level ($P > 0.05$) during different time of exposure as compared with control.

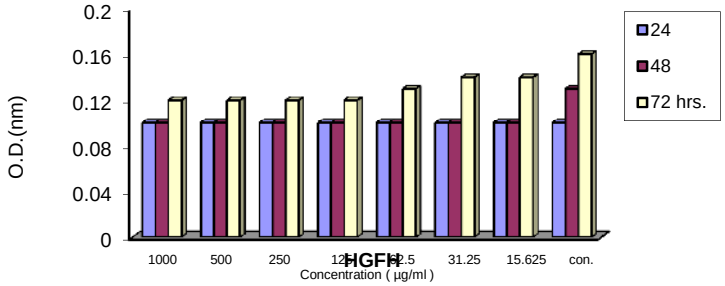

Fig (2): effect of aqueous extracts of *P. oleracea* on normal cell line (REF) after (24, 48 and 72) hrs.

The reduction in growth of REF cells even at the highest concentrations was less than that of the malignant cell lines used in this study; this could be considered as further indication of the relative safety of crude extracts of *P. oleracea* towards normal cells.

The results showed positive reactions to alkaloids, flavenoids, saponines, glycosides and tannins. This indicates that those phytochemicals are soluble solvent used and the targeted therapeutic activity of *Portulaca oleracea* crude extract may result from the synergistic effects of those phytochemicals [9]. Purification of those phytochemicals from the crude extract to homogeneity may lead to lose biological activity [5]. Fruits and vegetables generally possess phytochemicals responsible for antioxidant and anticancer activities, and the benefit of a diet rich with fruits and vegetables was attributed to the complex mixture of phytochemicals present in whole foods [10].

P. oleracea contains many biologically active compounds including alkaloids, coumarins, flavonoids, saponin, tannin, threonine, valine, tryptophane that may be plays a role as an active compound for the antitumor effect [11].

Portulaca oleracea has alkaloid pigments, the reddish β-cyanine (visible in the coloration of the stems), this pigments are potent antioxidant and have mutagenic properties. And contain large amount of dopamine and may possibly play a role as antitumor. Dopamine may inhibit the production or release of endogenous factors required for cell viability and proliferation [12]. *Portulaca oleracea* also rich in vitamin E (α- tocopherol) which might produce beneficial protective effect in cancer as postulated, including the following inhibition mechanism, of cancer formation by the quenching of free radical, direct on tumor cells such as control c

through induction of differentiation, cycle inhibition or induction of apoptosis [13]. Alpha- tocopherol extract has antitumor activity and acted by inhibiting tumor cell proliferation in nude mice with colon cancer [14].

The most characteristic event recognized in the cytological study was the occurrence of cellular degeneration cell death and cytolysis in a dose- dependent manner. The cytological findings of Hep-2 and AMN3 cell line were consistent with that obtained by [15]. They investigated components of aqueous extract of *P. Oleracea* on KATO III (human gastric carcinoma cell line) and COLO 320 HSR cells (human colon adenoma cell line) that showed a tumoricidal activity at 24 hrs of exposure, but not against the non- tumorous cell lines, L929 (murine lung connective tissue) and W138 (human lung diploid cell) cells [16].

The most accepted explanation for the cytotoxic effect of plant extract is the ability of plants to induce the programmed cell death in cancerous cells, as attempt to arrest their proliferation. A number of food items as well as herbal medicine have been reported to produce toxic effects by inducing programmed cell death [17].

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