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Parsley Crude Extract Cytotoxicity Against Breast Cancer Cells

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Abstract

Breast cancer is a lethal disease of females globally, generally, and in Iraq especially. Current cancer therapies are highly toxic and limited activity, leading to the search for effective and safe therapeutics. One of the safest treatments originates from herbs, and safer ones come from plants used in food. This study investigates the antitumor activity of parsley (Petroselinum sativum) crude extract consisting mainly of flavonoid compounds. Previously it exhibited a killing effect on several cancer cell line cultures. Furthermore, it has a high safety margin as humans and animals ingest it as part of the food chain. Methods, three continuous breast cancer cell lines of human origin were tested; CAL51, MCF7, and AMJ13, and one mouse mammary adenocarcinoma (AMN3) was used as an animal model. Normal breast epithelial cell line (HBL) was used to test the safety of the crude extract. Crystal violet cytotoxicity assay was applied to study the killing effect that the parsley extract may induce. Morphological changes were examined by crystal violet staining. Results: experimental work showed that the crude extract killed all cancer cell lines tested with the higher doses, but the mice-originated mammary adenocarcinoma AMN3 was more resistant. Moreover, parsley crude extract showed no cytotoxic effect on normal HBL cell lines. Cytological lesions are characterized by cell detachment and condensation of both cytoplasm and nucleus. Parsley crude extract diminishes cell viability and makes cytopathological alterations in breast cancer cells dependent on the concentrations used while sparing normal cells is encouraging results to move for more investigations.

Keywords: Cytotoxicity, Cancer cell line, herbal medicine, parsley.

Introduction

Globally, malignant tumors are the second cause of death (1). It has a high incidence in Iraq because of many environmental factors related to years of conflicts (2). One of the highest incidence types of cancer in Iraqi females is breast cancer, with a noted increase in incidence by more than two folds in recent years (3). Herbal medicine is a useful source for cancer drug discovery and prevention; it contains phytochemicals, extremely active ingredients for contemporary medicine in drugs utilized in cancer therapy (4). Ethanolic extract of Petroselinum sativum showed strong antitumor activity against

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oesophageal cancer Cell lines (5).For a long time, traditional remedies used plant extracts to cure different illnesses involving tumors (6). Experimental studies also revealed that plant crude extracts could act as an antiviral therapy (7), which were tested successfully in vitro to show antiviral activity against some RNA viruses (8). Parsley, with the scientific name Petroselinum sativum (P. sativum), is a part of the Umbelliferae family, which have antioxidant activity. It is further reported that parsley seed extracts induce death in human breast cancer cells MCF-7 (9). As parsley plant extracts possess multiple pharmacological properties, the present study investigated the killing induction in four breast adenocarcinoma cells due to parsley leaf extracts and studied its safety on normal breast tissue.

Materials and Methods

Crude extract preparation. Leaves of P. sativum were collected locally in Baghdad, Iraq. National herbarium of Iraq botany directorate in Baghdad- Iraq identified the leaves under the scientific name Petroselinum sativum, belonging to the umbilifera family. The leaves of parsley were dried in an oven at 40 ˚C, and after milling by an electric blender, the crushed leaves were sieved to an average particle of 105 mm and kept in a dry, dark glass container. The crushed parsley leaves were used to prepare the 70% ethanolic extract, following the protocol described earlier (10). Briefly, 100 gm of dried parsley leaves powder was soaked in 100 ml of 70% ethanol on an electrical magnetic stirrer for 72h at room temperature. Then, the mixture was filtered twice; the first time, we used a five-layer gaze of medical grade; the Whatman NO. 1 filter under a vacuum was used in the second step. The solution was subjected to dehydration, and the solvent was evaporated at 40 º C in a convection oven in a glass Petri dish.

Cell lines culture. The Iraqi patient-derived human breast cancer cell line named AMJ13 (11) and locally established AMN3 murine mammary adenocarcinoma cells were supplied with RPMI-1640 culture media (Elabscience, USA), and 10% fetal bovine serum (FBS) (Capricorn- Scientific, Germany), 100 units/ml pen-strep solution. Furthermore, estrogen-progesterone positive breast cancer cells MCF-7, triple-negative human breast cancer cell line CAL51 and human breast epithelial cell line (HBL) were growing in MEM medium (Elabscience, USA) with 10% fetal bovine serum (FBS) (Capricorn- Scientific, Germany), 2.5 µg/ml amphotericin B and an antibiotic mixture of 100 units/m penicillin, and 100 µg/ml streptomycin. The cells were allowed to grow as adherent confluent monolayers before subculturing and incubating at 37°C and 5% CO2 before trypsinization to detach the cells for the next experiment (11). Cell lines used in our study were evaluated regularly for mycoplasma.

Cytotoxicity assay. A Crystal violet cell viability assay measured Parsley crude extract's (PCE) ability to induce cell death. The cells were seeded in a 96-microwell plate (Spl life science, South Korea) at 10000 cells/well and incubated overnight to achieve a 60-70% confluent monolayer. Cells were treated with parsley crude extract at 2-fold dilutions (1000µg, 500 µg, 250 µg, or 125µg/ml) in serum-free culture media with sex replication for each concentration. After 72h of exposure, cell viability was determined by eliminating the culture media, and the cells were stained by crystal violet (50 µl) (Elabscience, USA) and incubated for 20 minutes at 37°C; later, we used tap water to remove the excess stain. The optical density reads were measured at 492 nm (test wavelength) on a microplate reader (MBG, Germany). Cytotoxicity percentages were estimated with respect to vehicle-treated cells (11, 12).

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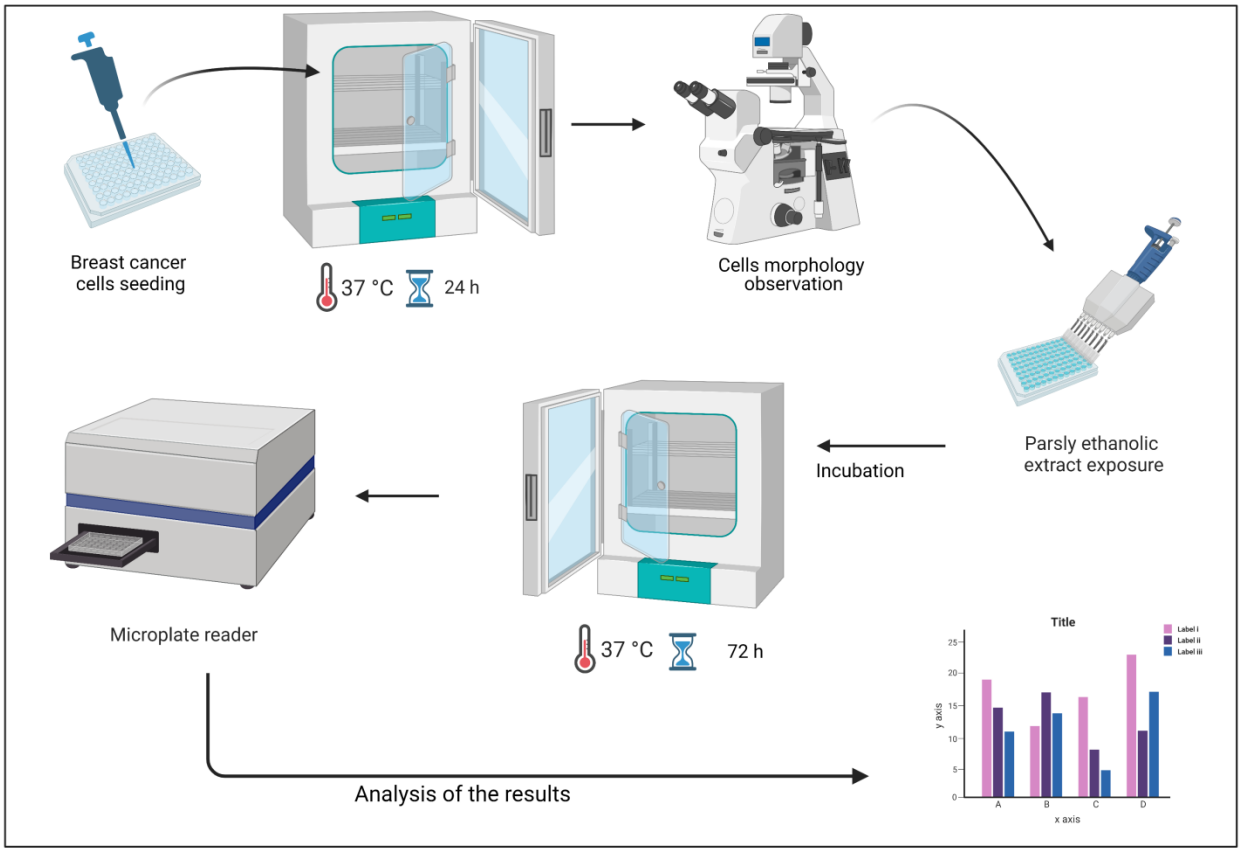

Figure 1 illustrates the crystal violet cell viability assay methodology to determine the cell-killing effect of parsley crude extract (PCE).

Statistical analysis. The data are presented as tumor growth inhibition rate. Statistically significant differences at $P < 0.05$ were considered. One Way ANOVA analysis was done using GraphPad Prism 7.04 software (GraphPad Software, Inc. San Diego, California, USA. IC50 Analysis was done using the same software, choosing nonlinear regression, building equation: Dose-Response -- Special, X is log(concentration). "Absolute IC50, X is log(concentration)". (https://www.graphpad.com/guides/prism/latest/curve-fitting/reg_absolute_ic50.htm).

Ethical Approval

There were neither humans involved in the study to obtain their consent nor animals involved in this study. This work is supported by the Iraqi center of cancer and medical genetics research (ICCMGR), Mustansiriyah University- Baghdad, Iraq.

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Results

Cytotoxicity of Parsley crude extract.

Measuring the killing effectiveness of parsley crud extract (PCE) was conducted in a panel of four breast cancer cell lines (CAL51, MCF7, AMN3, and AMJ13) and normal epithelial breast cells (HBL) (Figure 2). The results presented in Fig. 3 specified that parsley crude extract effectively reduced the viability of CAL51 breast cancer cells (with significant effects) at 1000 and 500 µg/ml. In addition, PCE had less effect on MCF7 cell growth as only 1000 µg/ml is effective (with significant effects) in inducing 50% cancer cell cytotoxicity (Fig. 3). Moreover, The PCE had a more significant therapeutic efficacy for AMJ13 cancer cells as all concentrations used were effective to induce more than 50% cytotoxicity (Fig. 3). While there was no considerable decline in cell viability in all concentration on AMN3 (Fig. 3). There is no significant cytotoxicity of the first three concentrations on HBL cells. It has cytotoxicity in higher 1000 ug/ml concentrations on the HBL breast normal cells (fig. 3).

Safety of Parsley extract.

The comparison between the IC50 values for Parsley extract in tested cancer and normal cells is illustrated in figure 4. The results show that cancer cells are more sensitive than normal cells, proving extract safety. Also, we can see that human cancer cells are more sensitive than mouse cancer cells.

Morphological changes induced by Parsley crude extract in treated breast cancer cells.

The treated and untreated cells were stained with crystal violet to facilitate cell examination. Most cytopathological changes that noticed at a concentration of 100ug/ml. Breast cancer cells showed similar lesions of cell detachment due to cell death, cytoplasmic shrinkage, and nuclear condensation. Cell debris can be noticed in all observed fields (Figure 5). HBL normal cells showed no significant lesions as observed under an inverted microscope. (Figure-5).

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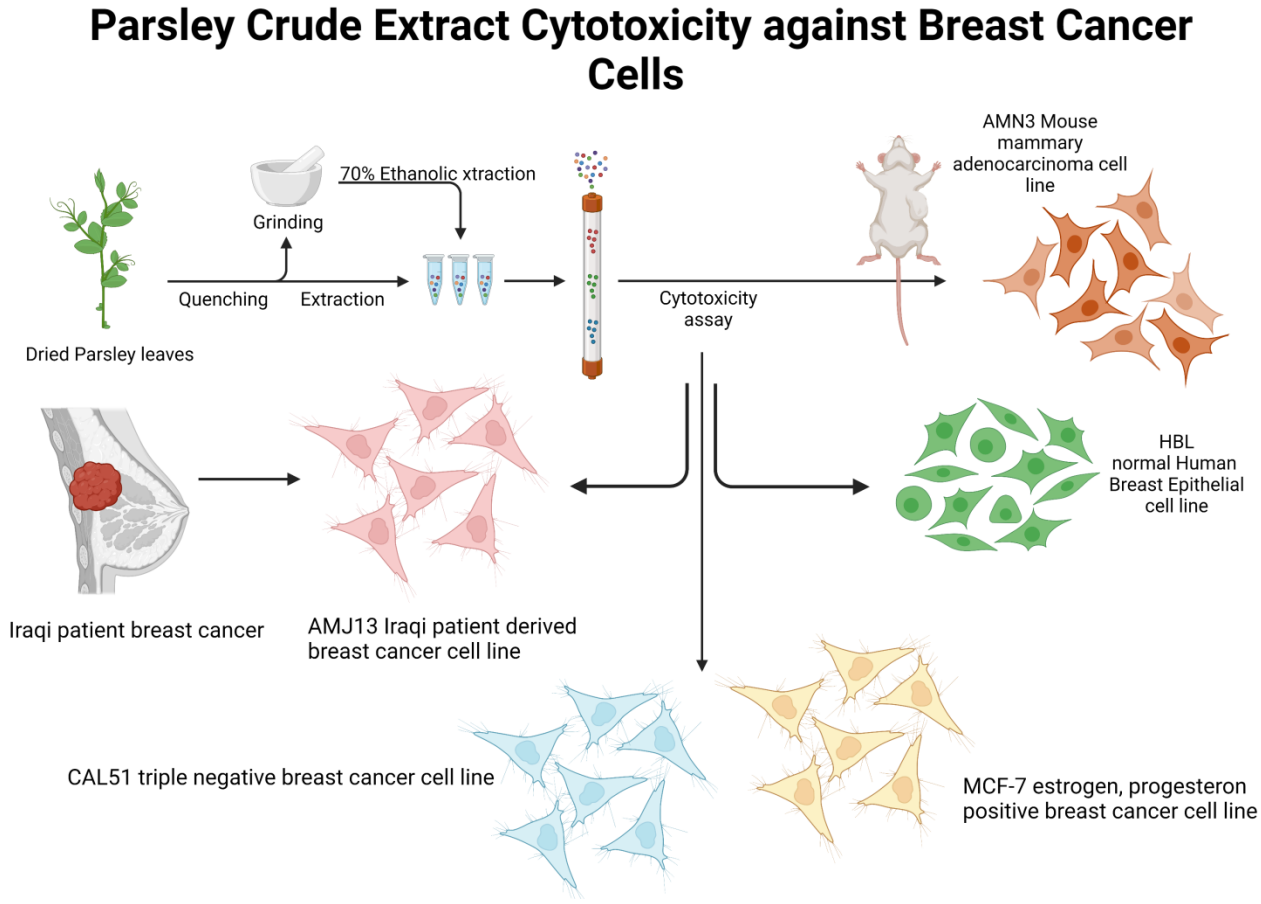

Figure 2, In vitro antitumor activity of Parsley crude extract. The therapeutic efficacy of parsley crud extract (PCE) was tested in a panel of four breast cancer cell lines (AMN3, CAL51, AMJ13, and MCF-7) and normal breast cells (HBL)

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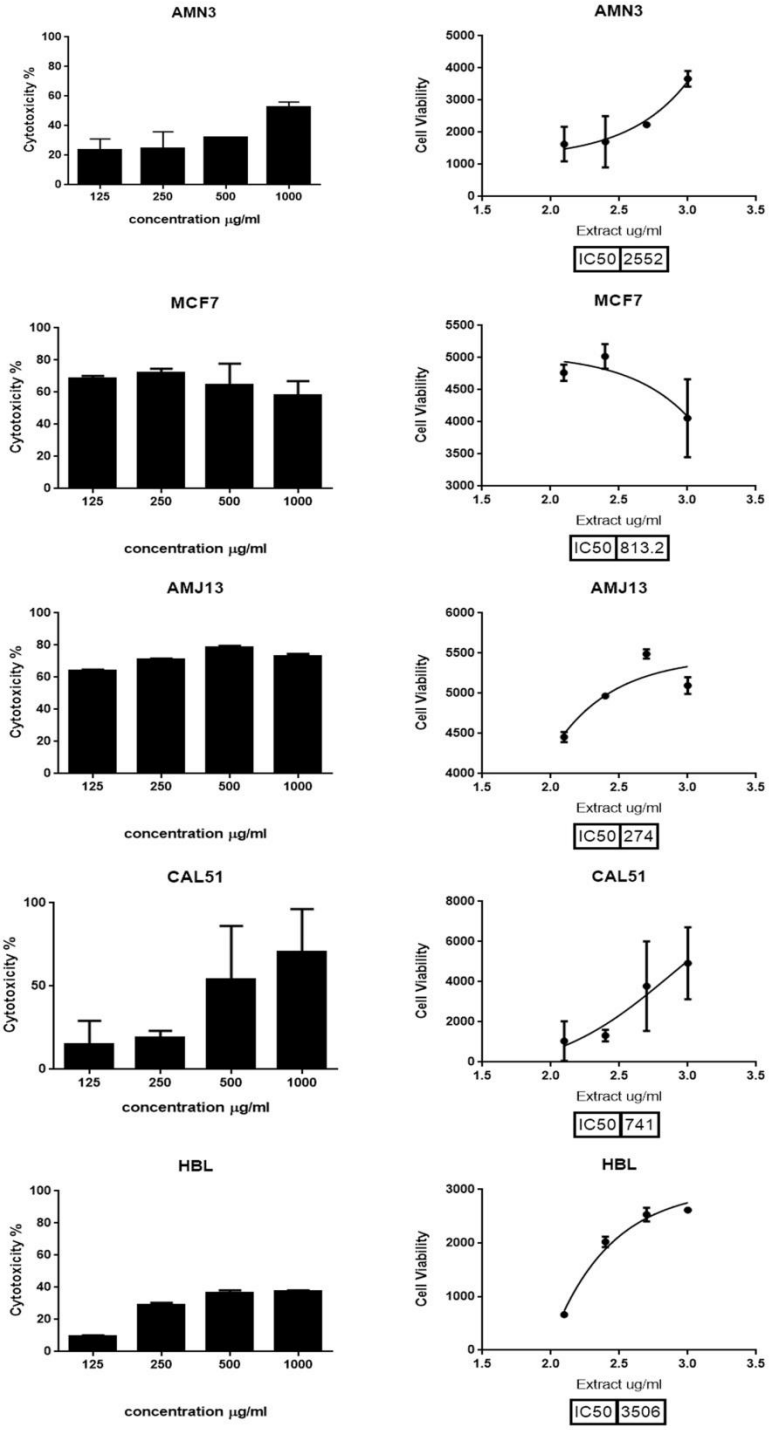

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Figure 3: The killing effect of parsley crude extract in a pnel of four breast cancer cell lines comparing to epithelial normal breast tissue cell line (HBL). The results showed the highest cytotoxic effect at 1000 and 500 µg/ml in most concentrations assessed. The error bars represent the standard deviation.

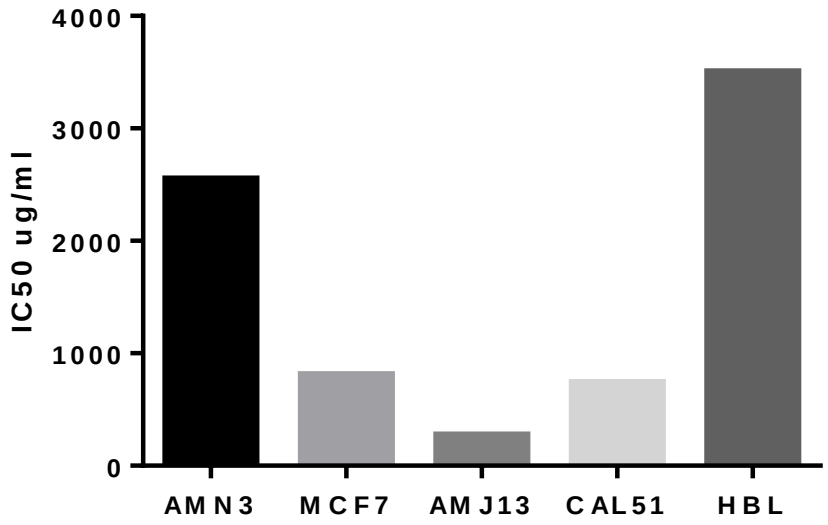

Figure 4, compares the IC50 values for Parsley extract in tested cancer and normal cells. The results show that cancer cells are more sensitive than normal cells, which proves extract safety. Also, we can see that human cancer cells are more sensitive than mouse cancer cells

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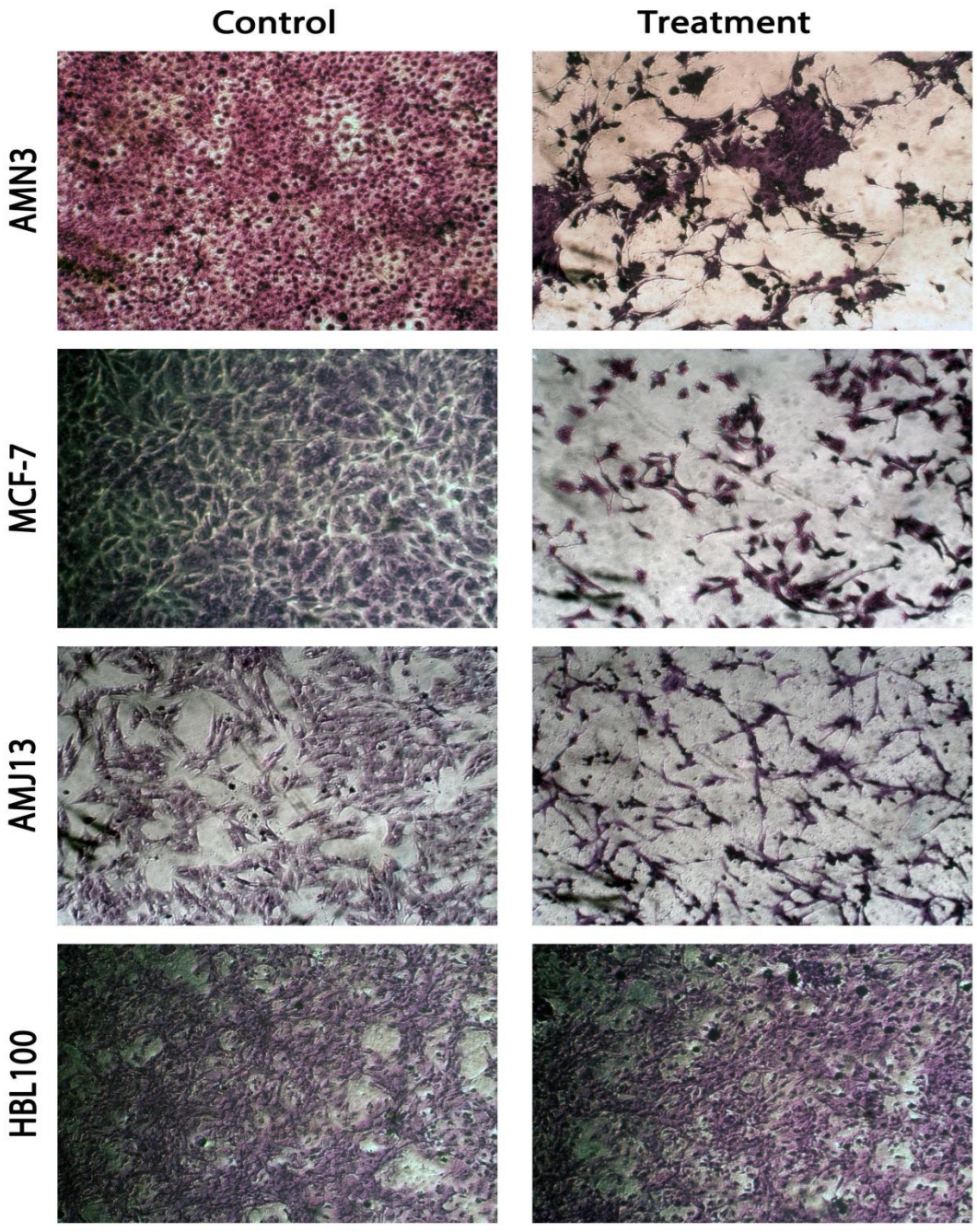

Figure-4, Morphological changes induced by Parsley crude extract in treated breast cancer cells. Treated cancer cells are characterized by cell detachment and nuclear and cytoplasmic condensations. In contrast, normal HBL cells showed no Signiant lesions or cell detachment

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Discussion

The current work aimed to investigate the ability of parsley crude extract to kill breast cancer cells with different genetic mutations and receptor statuses. The AMJ13 breast cancer cell line is derived from Iraqi patients who suffer from ductal carcinoma, and the cancer cells are estrogen-progesterone negative status and with functional BCA1 and BRCA2 genes. MCF-7 cells are estrogen-progesterone receptor-positive, while CAL51 is triple-negative breast cancer cells. AMN3 mouse breast cancer cells are estrogen-progesterone receptor-positive. So this study covered all types of breast cancer regarding receptor status.Folklore medicines that utilize herbs are found to be used by 80% of the population globally, as reported by the World health organization (13). Medicinal plants possess novel phytochemicals that may treat several health issues (6). In order to show the important role of herbal and phytomedicine, the current investigation is designed to estimate the promising therapeutic effects of anticancer properties of Petroselinum sativum (P. sativum) crude extract against a panel of different types of breast cancer cell lines. These breast cancer cell lines cover the major types of breast cancer according to the hormonal receptors' status. CAL51 is a triple-negative breast cancer cell line, MCF-7 estrogen-progesterone positive breast cancer cell line, and AMJ13, which is Iraqi patient-derived breast cancer that is estrogen and progesterone negative cell line. The mouse mammary adenocarcinoma cells AMN3 are positive estrogen progesterone receptors. These cells are important for further in vivo studies in immunocompetent mice. The cytotoxic activity of the extract was evaluated by viability assay in normal human breast cells HBL. The results indicate that leaf P. sativum extract has a significant broad-spectrum antitumor inhibitory effect on the growth rate of breast cancer cells in vitro in certain concentrations. The results demonstrate that parsley leaf extract reduced the cell viability of AMJ13 cells more efficiently in higher concentrations. Parsley leaf extract has shown anti-breast cancer activity on MCF7 (14). Our results confirm earlier discoveries that the parsley extracts induce cell death in human breast cancer T47D cells (15) as a result of the cancerous cells' sensitivity towards the death flavonoids. Petroselinum sativum seed extracts were observed to have a cell death effect against human hepatocellular carcinoma cells (16). Moreover, there was encouraging anticancer efficiency of parsley seeds flavonoid (apigenin) against mice mammary adenocarcinoma (17). As described by (18), the proposed mechanism of action shows the presence of a compound named apiole, which has been isolated from Petroselinum sativum that induces an elevation in cell cycle regulators, for instance, some tumor suppressor proteins such as p53, p21/Cip1, and p27/Kip1 in treated cancer cells.

Conclusions

Our results show that PCE significantly decreases cell number and induces breast cancer morphological lesions in increased concentrations of the extract. Normal cells are not affected, which proves safety. Additional molecular-level experiments are undertaken to interpret the cell death mechanisms of parsley crude extract on human breast cancer cells.

Conflicts of Interest

The authors declare that no conflicts of interest are associated with this study.

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References

1. Siegel RL, Miller KD, Fuchs HE, Jamal A. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7-33.https://doi.org/10.3322/caac.21708
2. World Health Organization. Eastern Mediterranean region: key statistics from WHO global health observatory, country office website, Iraq 2020. WHO, Office for the Eastern Mediterranean. Availablefrom: https://www.who.int/countries/irq. Accessed 25 Oct 2020.
3. Al-Shammari AM. Environmental pollutions associated to conflicts in Iraq and related health problems. Rev Environ Health. 2016; p. 245.https://doi.org/10.1515/reveh-2015-0024
4. Rochaniawan A. Herbal Medicine as cancer treatment. Open Access Indonesian J Med Rev. 2021;1(5).https://doi.org/10.37275/oaijmr.v1i5.49
5. Almzaien AK, Mohammad MH, Al-Shammari AM, Hasan AA, Ahmed AA, Shakir HK. Cytotoxicity of Ethanolic Extract of petroselinum sativum against Esophageal Cancer Cell Lines. Iraqi J Cancer Med Genet. 2022;15(1).https://doi.org/10.29409/ijcmg.v15i1.324
6. Briskin DP. Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. Plant Physiol. 2000;124(2):507-514.https://doi.org/10.1104/pp.124.2.507
7. Salih R, Odisho SM, Al-Shammari AM, Ibrahem O. Antiviral effects of olea europaea leaves extract and interferon-beta on gene expression of Newcastle disease virus. Adv Anim Vet Sci. 2017;5(11):436-445.https://doi.org/10.17582/journal.aavs/2017/5.11.436.445
8. Abdul Jalill RD, Hussein MF, Hussein, Al-Shammari AM. GC/MS ANALYSIS OF RHEUM RIBES RHIZOMES. Mintage J Pharm Med Sci. 2015:29-34.
9. Farshori NN, Al-Sheddi ES, Al-Oqail MM, Musarrat J, Al-Khedhairy AA, Siddiqui MA. Anticancer activity of Petroselinum sativum seed extracts on MCF-7 human breast cancer cells. Asian Pac J Cancer Prev. 2013;14(10):5719-5723.https://doi.org/10.7314/APJCP.2013.14.10.5719
10. Kafi AL, Hussein SM, Jumaa AH. Study the in vitro effect of alcoholic extract of Prunus armeniaca kernels, Methotrexate, amygdalin and the combination between them on HeLa cancer cell line. Iraqi J Cancer Med Genet. 2015;8(2).https://doi.org/10.29409/ijcmg.v8i2.152
11. Farshori NN, Al-Sheddi SA, Al-Oqail MM, Musarrat J, Al-Khedhairy AA, Siddiqui MA. Cytotoxicity assessments of Portulaca oleracea and Petroselinum sativum seed extracts on human hepatocellular carcinoma cells (HepG2). Asian Pac J Cancer Prev. 2014;15(16):6633-6638.https://doi.org/10.7314/APJCP.2014.15.16.6633
12. Salih F, Al-Shammari AM, Al-Khafaji AS. Photodynamic action of low power He-Ne laser on photosensitized human Hep-2 and AMN3 cell lines with hematoporphyrin derivative in vitro. Iraqi J Canc Med Gen. 2010;3(1):54-60.
13. World Health Organization (WHO). WHO strategy on traditional medicine 2014-2023. Available from:https://www.who.int/medicines/publications/traditional/trm-strategy14-23en. Accessed 7 Feb 2019.
14. Slighoua M, Mahdi I, Amrati FE, Di Cristo F, Amaghnouje A, Grafov A, Boucetta N, Bari A, Bousta D. Assessment of in vivo estrogenic and anti-inflammatory activities of the hydro-ethanolic extract and polyphenolic fraction of parsley (Petroselinum sativum Hoffm.). J Ethnopharmacol. 2021;265:113290.https://doi.org/10.1016/j.jep.2020.113290
15. Abd Almohammadi M, Abdolmohammadi MH, Fouladdel Sh, Shafiee A, Amin Gh, Ghaffari SM, Azizi E. Anticancer effects and cell cycle analysis on human breast cancer T47D cells treated with extracts of Astrodaucus persicus (Boiss.) Drude in comparison to doxorubicin. DARU J Pharm Sci. 2008.
16. Al-Oqail MM, Farshori NN, Al-Sheddi ES, Al-Massarani SM, Siddiqui MA, Al-Khedhairy AA. Petroselinum sativum protects HepG2 cells from cytotoxicity and oxidative stress induced by hydrogen peroxide. Mol Biol Rep. 2020;47:2771-2780.https://doi.org/10.1007/s11033-020-05380-z
17. Alol LH, Al-Mzaien KA, Hussein SM. The promising anticancer efficacy of parsley seeds flavonoid (apigenin) in induced mammary adenocarcinoma (AMN3) mice. J Physiol Biomed Sci. 2012;25(1):5-12.
18. Wei PL, Tu SH, Lien HN, Chen Li-C, Chen C-S, Wu CH, Huang C-S, Chang HW, Chang CH, Tseng H, Ho Y-S. The in vivo antitumor effects on human COLO 205 cancer cells of the 4, 7-dimethoxy-5-(2-propen-1-yl)-1, 3-benzodioxole (apiole) derivative of 5-substituted 4, 7-dimethoxy-5-methyl-l, 3-benzodioxole (SY-1) isolated from the fruiting body of Antrodia camphorata. J Cancer Res Ther. 2012;8(4):532.https://doi.org/10.4103/0973-1482.106529