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Cytotoxic Activity of Taraxacum officinale Ethanolic Plant Extract against Human Breast Cancer (MCF-7) Cells and Human Hepatic (WRL-68) Cells

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Abstract:

Taraxacum officinale (Dandelion) represent a potentially novel non-toxic alternative to conventional cancer therapy toward many cancers particularly breast cancer.

This study was conducted to detect the cytotoxic activity of T. officinale whole plant ethanolic extract on human breast cancer (MCF-7) cells in vitro compared to human hepatic (WRL-68) cells using MTT assay.

Graphed 6 prism program including non-linear regression with one –way ANOVA followed by Dennett's multiple comparison test to obtain data represent the mean± SD.

The results revealed high toxicity of the extract against MCF-7 cells with IC50 (190.5 µg.ml-1) and showed high-reduced activity toward WRL-68 cells with high IC50 value while the lowest viability (21.57±5.322) appeared in MCF-7 cells under treatment with 400 µg.ml-1 compared with high viability in WRL-68 cells.

The study concluded that T. officinale extract has a valuable source of anticancer drugs with safe and selective activity on cancer cells in comparison to human normal cells.

Key-words: *Taraxacum officinale; breast cancer; cytotoxicity; MTT assay; Hepatic cells.*

Introduction:

Breast cancer is the most common invasive female cancer worldwide (1-3). To find out drugs of natural origin without side effects and to find eas¬ily available and economically affordable therapies that can decrease the cost of treatment on the patient, the herbal medicines is the option, which can be used for the treatment of some cancers without or with less side effect. (2), (4, 5) As well as it offer high compatibility with the physiology of the body by selectivity inhibiting of cancer cells proliferation, increasing cytotoxicity, and decreasing of cell differentiation. (5-7), (3) One of the basic limitations of most chemotherapies is the side effects induced by severe toxicity. (5), (8, 9) The main objective of any cancer treatment is removing away all of the malignant cells and leaving the normal cells in intact form as possible (10) and Taraxacum officinale represent a potentially novel non-toxic alternative to conventional cancer therapy available. (11) Several studies investigated the potential activity of the plant extract on a number of cancers, including human melanoma cells (9), prostate cancer cells, (12) cervical cancer cells (13), (14) and human chronic myelomonocytic leukaemia (CMML) cells. (11) Furthermore, T. officinale leaves and roots extract is a fast-acting, and non-toxic chemotherapeutic, suitable anticancer drug, particularly for breast cancer (9), (13) and may be used in developing new agents to combat cancer. (12) As well as, the exploiting the single form of extract components may not be enough to accomplish a chemotherapeutic response, so that the components may work in a union or even synergistic way, which is possibly the reason by which it has been effective as extracts. (9), (15) As this plant can act to improve anti-cancerous activity relying on different mechanisms than standard chemotherapy. (16) Such as, blocking the proliferative activity of the cells (12), (17-19) or inducing programmed cell death (11), (13), (20) by affecting on a number of pathways that lead finally to reducing cancer cells viability. The aim of this study was to investigate the cytotoxic activity of T. officinale whole plant extracts against MCF-7 human breast cancer cells in vitro and to demonstrate

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its safe, selective, and efficient activity on human cancer cells without affecting human normal cells, by comparing its activity on WRL-68 human hepatic cells.

Materials and Methods:

The study was conducted in May (2017) in The Centre of Biotechnology/ Al Nahrain University.

1. Extraction of plant materials from T. officinale plant:

T. officinale plants were collected from different locations of Baghdad city. Plant samples were dried until desiccation using a shaker incubator (Hirayama, Japan) at 40 oC and then ground into fine powder by Electric Grinder (Sanyo, Japan). 26 gm of the powder was dissolved in 130 ml ethanol (BDH, England). 5:1 dilution was placed in shaking incubator (Hirayama, Japan) that adjusted at 40 o C, and kept for 24 hours on 100 cycles/min. The mixture was filtered using filter paper; the supernatant was condensed with a rotary evaporator (Hedolph, Germany) on 100 cycles for 30 min at 40 oC. The condensed solution was then poured into Petri dishes and left until all the ethanol totally evaporated to obtain solid material, which weighed. Finally, the product of the extraction procedure was 3 gm of weight. To prepare a stock solution, 8 mg of the dried extract was dissolved in sterile distilled water; the volume was completed to 10 ml and mixed with vortex (Giffin, England) until complete dissolving. The mixture was sterilized using 0.22 µm millipore filter (Sigma Aldrich, United States), and then kept in a refrigerator until using for cytotoxicity experiment.

2. Cytotoxicity assay for *T. officinale* extract on human breast cancer MCF-7 and human hepatic WRL-68 cell lines

2.1. Cancer Cell Line

Two cell lines, MCF-7 (Human Breast cancer cells) and WRL-68 (human normal liver cells) were used in this study for screening the cytotoxicity of T. officinale crude extract. They were supplied by Pharmacology Department/ Medicine College/ Malayia University. The cell lines were maintained using tissue culture media (Roswell Park Memorial Institute-1640 Medium, Sigma Aldrich). 450 ml of the media was supplemented with 50 ml of 10 % Fetal Bovine Serum (Sigma Aldrich, United States) and 0.5 ml Penicillin/Streptomycin stock solution (10,000 U/ml penicillin, 10 µg/ml streptomycin; Thermo Fisher Scientific, United States). The prepared media were kept in a refrigerator (Thermo Scientific, United States).

2.2. Maintenance of cell lines

Cryopreserved cells were thawed using water path at 37 o C for (1-2) minutes and then transferred to sterilized Laminar Air Flow Hood (Sanoy, Japan). The container was wiped with 70% ethanol (BDH, England) to remove any possible external contamination, after that, it was transferred into a blue cap centrifuge tube and kept at 37° C water bath for 5min. To form cell stock solution, the cryomedia was excluded by centrifugation (BIO-RAD, Korea) at 200X for 5 minutes. The supernatant was removed and the cell pellets re-suspended in complete fresh medium and incubated in a CO2 incubator (Sanoy, Japan) at 37 ° C and 0.5 CO2 until using. Then, the cell lines were sub cultured when monolayer was confluent. The growth medium was decanted and the cell sheet washed once with 2ml trypsin-versene solution (Thermo Fisher Scientific, United States). Then, 2 to 3 ml of trypsin-versene was added to the cell sheet and the flask rocked gently, part of it was decanted again to obtain about one millilitre of trypsin-versene solution covering cell surface. The cells were incubated for 1-2 min at 37° C until they had detached from the flask. Complete media were then added to the flask and re-incubated at 37ºC for 24-48 hr to be ready for performing viability test. The procedure was performed according to Jennie and Penelope. (21)

2.3. Cell viability

The cell viability was determined before assessing the cytotoxic effect of the extract on the cell lines, which should be in the range of (104–105) cell/well as mentioned by Freshney, (22) viable cell counting was accomplished by using trypan-blue exclusion. Trypsinized and suspended cells for the cell lines (for MCF-7 and WRL-68 cell) were seeded in a microtiter plate for the cytotoxicity assay; with 1:10 dilution (3.6 ml media + 0.4 ml stock). The diluted cells were counted with haemocytometer by mixing 0.2 ml of cell suspension and 0.2 ml of trypan-blue with 1.6 ml of PBS; then 20µl of the mixture was added to haemocytometer. After 1-2 minutes, viable and dead cells numbers were calculated and cell concentration (cell/ml), total cell count, and cell viability (%) were calculated as in following equations:

$C \equiv n \times d \times 10000$

Where C (Cell concentration (cell/ml)), n (number of counted cells), d (dilution factor=10)

Total cell count = C (cell/ml) × the original volume of fluid from which the cell sample was taken.

Cell viability (%) =(total viable cells(unstained))/(total cells counted (stained&unstained)) × 100

2.4. MTT (Methyl Thiazolyl Tetrazolium) Cytotoxicity Assay:

2.4.1. Preparation of cells for MTT assay:

The cultured cells in the microtiter plate (96 wells) were exposed to the extract solution sterilized that with millipore filter (0.45µm) with different concentrations to determine their cytotoxic effect after the end of the exposure time. 200 µl/104-105 cells/well from single cell suspension were added to all of the 96 wells of the microtiter plates, shook and incubated in a humidified chamber at 37˚C, 5% CO2 until the cells reached 70% confluence. Then, it was exposed to seven concentrations of ethanolic extract of T. officinale (400, 200, 100, 50, 25, 12.5, 6.25 µg/ml) which added to each well (three replicates for each concentration) for MCF-7 and WRL-68 cell lines; also, 200 µl of maintenance medium to each well of the control group. After 24 hr. of incubation, the cytotoxic activity was evaluated for the two cell lines.

2.4.2. MTT Assay:

Cell viability was measured after 24 h. of exposure by removing the medium, adding 20 µl/well solution of MTT, and incubating for 4 h at 37°C. The crystals were solubilized by the addition of 40 µl/well of Dimethyl Sulphoxide (DMSO)

followed by incubation at 37°C for 15 min with shaking. Then, the optical density of each sample of 96-well plate was determined on a microplate reader at 620 nm. The Percentage of cell viability was calculated according to Betancur- Galvis (23) and Gao (24) as follow:

Viability rate = (mean of control-mean of treatment)/(mean of control) × 100

3. Statistical analysis

Graphed 6 prism program was used in the study with one –way ANOVA followed by Dennett's multiple comparison test to obtain data representing the mean± SD for cytotoxicity experiment (25). The data of optical density taken from plate reader were then subjected to non-linear regression analysis to calculate the concentration of compounds required to cause a 50% reduction (IC50) for each cell line.

Results:

The results appeared in (Table 1) indicated that (400, 200, 100, 50, and 25 µg/mL) concentrations gave results vary between the two types of cell lines, while the rest concentrations results remain at convergent values between them.

1. IC50 values of T. officinale extract on human breast cancer and human hepatic cell lines:

The study results showed that T. officinale extract has cytotoxic effect on breast cancer cells in a dose-dependent manner. The Inhibitory concentration of 50% of human breast cancer cell line (MCF-7) was 190.5 µg.ml-1, while The inhibitory concentration on the human hepatic cell line (WRL-68) was very high where its value is 1807000 (~1.807e+006) µg.ml-1. These results indicated that T. officinale have a high inhibitory effect on human breast cancer cell, without effect on normal human hepatic cells, which represent a promising therapy for targeting breast cancer without affecting the normal cells.

Table 1. The viability of human breast cancer cell line and human hepatic cell line under Taraxacum officinale extract exposure represented with mean and standard deviation.

T. officinale Extract Concentration	MCF-7	WRL-68
400	21.575.322	85.033.041
200	40.015.685	92.711.219
100	60.8812.20	98.155.535
50	74.654.518	99.234.514
25	87.382.630	96.221.972
12.5	94.684.011	96.841.772
6.25	94.750.4064	95.521.718

*** MCF-7: human Breast cancer cell line, WRL-68: human hepatic cell line.**

1.1. Cytotoxicity effect on human breast cancer cell line:

The lowest breast cancer cell viability resulted from T. officinale extract concentration of 400 µg/ml was (21.57±5.322); it increases (94.75±0.4064) with reducing the extract concentration to (6.25 µg/mL) as indicated in (Table 1) and (Figure 1).

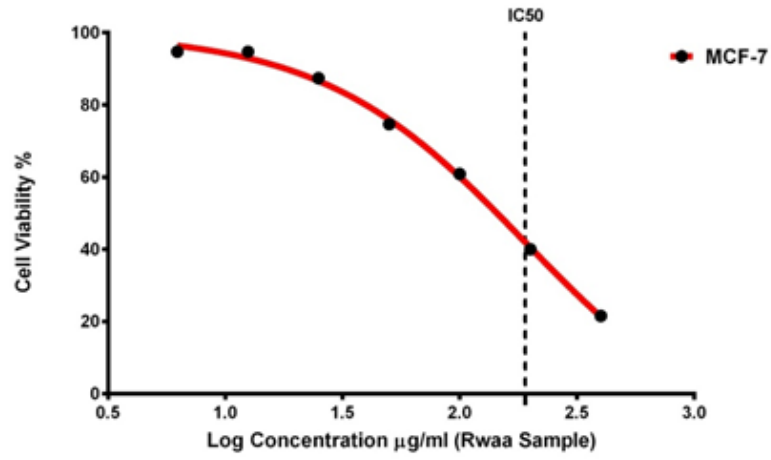

Figure 1. Percent of human Breast cancer cell line viability under T. officinale Extract exposure representing IC-50 value.

1.2. Cytotoxicity effect on human hepatic cell line:

The highest viability of human hepatic cells was (99.23±4.514) with 50 µg.ml-1 concentration. The lowest viability was (85.03±3.041) which observed in 400 µg.ml-1 (Table 1 and Figure 2).

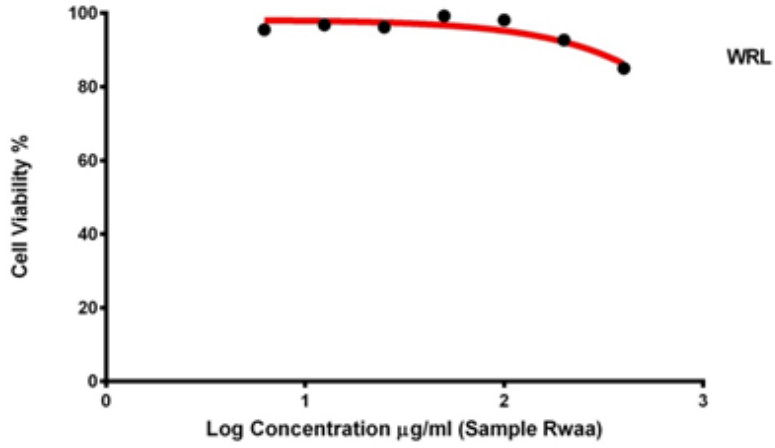

Figure 2. Percent of human hepatic cell line viability under Taraxacum officinale Extract exposure.

Discussion:

Taraxacum officinale plays an important role in the treatment of cancer because of its anticancer properties. (12), (14), (18), (26), (36) Which has been attributed to the plant as an extract of the whole plant or specific plant parts. (12) so that, the study accomplished to prove the cytotoxicity of T. officinale toward MCF-7 Human Breast cancer cells, according to the availability of limited scientific data on this effect. (12)

In the study, the plant ethanolic extract of T. officinale was used to detect its inhibitory effect on human breast cancer cell line (MCF-7) in contrast with human hepatic normal cell line (WRL-68). The results revealed high cytotoxicity against MCF-7 (in a dose-dependent manner) in contrast with high-reduced effect on WRL-68 cells. This agrees with some studies that showed an anti-cancerous effect of T. officinale on a number of cancers, including breast cancer; in particular, Sigstedt (12) who proved T. officinale anticancer activity toward MCF-7/AZ, because it reduced the growth of breast cancer cells. Also, Gonzalez-Castejon (27) indicated 26% viability reduction at 0.2 mg/ml concentration with time-dependent and partially dose-dependent reduction of cell viability on HepG2 human hepatic cancer cell line cells by using the aqueous extract of dandelion. Moreover, Menk, Tettey and Appiah-Opong studies investigated dose-dependent anti-proliferation effect of T. officinale extract. (16-18).

In this study, the potential effect of T. officinale extract on MCF-7 cells indicated that IC50 value occurred at 190.5 µg.ml-1 concentration for 24 hours and led to inhibition of 50% of breast cancer cells. In addition, the highest potential effect of T. officinale whole plant extract on MCF-7 cells occurred at 400 µg.ml-1; because it exert the highest cytotoxicity (21.57±5.322 viability), which mean 79% inhibition in comparing with WRL-68 cells that showed (85.03±3.041 viability). In comparison to Sigstedt (12) who indicated that MCF-7/AZ cells viability was unaffected by flower extract at concentrations higher than 500 µg.ml-1, but T. officinale root extract showed more toxicity than T. officinale leaves extract, leaving only 50% viable at a concentration of 50 µg.ml-1 as compared to 180 µg.ml-1 of the leaf extract indicating that the effect origin from leaves and roots components. On the contrary, Koo (26) showed an increasing in apoptosis of Hep G2 at 200 µg.ml-1, without any effects at doses larger or less than 2 mg.ml-1 and 20 µg.ml-1. While Chatterjee (9) revealed 2.5 mg.mL-1 root IC50 value of root extract on Human Melanoma Cells within 24 hours, which is higher than our results, Moreover, Trinh (13) results showed a higher antiproliferative effect of methanolic extract of T. officinale than ethanolic extract on breast cancer stem cells (BCSC) depending on doses used, which increased with time. So it is clear that using whole extract of the plant can give high potential activity as a result to the synergistic effect of the phytochemicals components of plant parts which lead to take the whole plant component extract in the study in compare to the recent study, which indicates that components of T. officinale extracts act either alone or synergistically to induce cancer cell death by inducing complex signalling. (9), (15), (27) Because all parts of this plant are pharmaceutical materials (roots, shoots and flowers). (28) And those parts contain variable components as well as different concentrations for the components, which indicate the importance of using plant phytochemicals as extract for cancer therapies.

Many studies related with our study in an important points that involve safety, selectivity, and significantly reduced activity toward normal human cells. The study results indicated little effect on human hepatic WRL-68 cell line ranged between (85.03±3.041 at 400 µg.ml-1 extract concentration to 99.23±4.514 in 50 µg.ml-1); this agrees with Ovadje (11)

and Chatterjee (9) who indicated that the toxicity of T. officinale root extract toward non-cancerous peripheral blood mononuclear cells (ncPBMCs) and normal human fibroblasts (NHFs) does not induce apoptosis or reduce cell viability of those cells, even at high doses and after a long exposure. Which supported also by Tettey (17), who shows only 7% inhibiting effect on RAW 246.7 macrophage cells. But, Menke (16) study results inconsistent with our study and appear 128 mg.ml-1 IC50 value on normal fibroblasts. In spite of that, those observations demonstrated the selectivity of Dandelion extract on cell cultures. (11) Where It is clear that cancer cell metabolism differs from that of normal cells, and this difference makes tumorigenic cells vulnerable to the drugs that interfere with cell metabolism (29) Furthermore; it was not unaffected on normal cells only but it can induce its viability like what observed in García-Carrasco (30) study, where root extract produced no effect significantly on adipocyte cell viability; but surprisingly, the leaf extract caused increasing in adipocyte viability with dose-dependent manner. Moreover, T. officinale extracts have been exploited for centuries as recipes for different uses, (9) therefore; it is assumed that normal cells are unaffected by it.

This high and selective activity on breast cancer cells can result from different suspected mechanisms that lead to selective cytotoxic activity toward cancer cells, like apoptosis; (8), (31) A recent study indicates that components of dandelion extract act to selectively induce extrinsic apoptosis specifically with low concentration of root extract without affecting noncancerous cells. (9), (27) In addition Sudiarta (14) study proved significant increasing in the cell apoptosis by treatment with (25, 50, and 100) mg.mL-1 on MCF-7 cells with a dose-dependent manner. Which converge with the effective concentrations in our study, at concentrations higher than our study (0.2 mg.mL-1), Koo (26) elucidated increasing of apoptosis by increased enhancing of cytokines production including tumor necrosis factor alpha (TNF-a) and of Interleukin-1 (IL-1). While previous works done by Ovadje (11) and Chatterjee (9) showed that T. officinale extract induced apoptosis effectively by rapidly activating the death-receptor mediated extrinsic pathway apoptosis leading to rapid activation of caspase-8. This selective induction may be due to the increasing in apoptosis antigen-1 (Fas) receptors expression in cancer cells than in normal cells, (8) as well as, increased expression of TNF-related apoptosis-inducing ligand (TRAIL), Death receptor 4 (DR4), and Death receptor (DR5) that represented by Trinh. (13) In addition, Sigstedt (12) study investigated T. officinale extract trigger anti-invasive effect by blocking MCF7/AZ invasion resulted from many components in the roots and leaves.

Moreover, Sudiarta study proved T. officinale anticancer effect through induction of retinoic acid receptor β2 (RARß2) gene expression by demethylation effects. When it's exposed to concentrations ranging between (6.25 and 100) µg.ml-1 which represent a part of our study for the same period (24 hour), even under low concentrations (12.5, 25, and 50 mg.mL-1) which show a significant effect. (14) Finally, the cytotoxicity activity of T. officinale can result from inhibition of extracellular signal-regulated kinases (ERK) activity, like what investigated by Sigstedt (12) study. Those can be considered as some of the strategies to accomplish the extract activity in our study.

In addition to what previously mentioned, those mechanisms of cytotoxicity toward breast cancer cells approved to T. officinale extract were resulted from the activity of the Phytochemicals component of the extract, which needed to work synergistically to exert its efficiency with quietly high doses. The inhibition of tumor cell proliferation by T. officinale extracts was attributed to triterpenoids (taraxasterol and taraxerol) and sesquiterpenes. (12), (27), (32), (33) Furthermore; it may result from phenolic compounds. (12), (33) such as luteolin, which induce apoptosis and regulate DR5 receptor expression in human cancer cells, (34) and flavonoids which responsible on inhibition of protein kinase activity, apoptosis, matrix metalloproteinases (MMP) 2 and 9 secretion, tumor cell invasion, and inhibition of adhesion leading to cells' spreading, (35) which investigated above as the major mechanisms of T. officinale extract efficiency.

Conclusions: The study concluded that T. officinale complete plant extract exert a great activity on human breast cancer cell MCF-7 line in contest with high reduced activity on human hepatic normal cell WRL-68 line, representing safe, selective therapeutic agent.

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