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Cytotoxicity of Ethanolic Extract of *petroselinum sativum* against Esophageal Cancer Cell Lines

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Abstract:

Digestive system tumors, including esophageal carcinoma considered one of the fatal tumors with a high mortality rate. The search for effective therapeutics is necessary to improve cancer patients' survival. The classical cancer therapy showed many health problem during long time treatment, therefore alternative medicine needful to improve cancer treatment and reduce side effects.

Herbal medicine is the origin of the most effective current cancer therapeutics. parsley (Petroselinum sativum) plant possess multiple pharmacological properties that drive attention to be tested for cancer therapy.

This study aimed to evaluate the anti- tumor efficacy of 70% ethanolic extract of parsley (Petroselinum sativum) leaves against human esophageal cancer cell line (SK-GT-4) and normal rat embryonic cells (REF). The assessment of the cytotoxicity effect of the extract was carried out by crystal violet assay for determining viability of cells CV.

Parsley ethanolic extract has reduced the viability of esophageal cancer cells, and the maximum inhibition rate was 39.68 % at the dose of 500 µg/ml. The corresponding values for the normal embryonic (REF) cell (as inhibition rate) were 82% under the same experimental conditions and doses.

In conclusion, the result revealed that, although the ethanolic extract has a cytotoxic effect on both esophageal cancer (SK-GT-4) and normal embryonic (REF) cells, but the effectiveness against cancer cells was less evident.

Keywords: *Phytotherapy, digestive system tumors, herbal medicine, Petroselinum sativum, crude extract.*

Introduction:

Cancer is the second cause of death globally (1). In Iraq, it is widely distributed due to pollution and other unknown factors (2). Esophagus cancer is one of the common types of cancer in Iraq. And according to the available data incidence is relatively high and trends are up going in terms of quantity and variables related age and sex (3). Lung and gastrointestinal cancers showed constantly rising trends especially after 2007 (4) and the total incidence of patients with cancer increased by over 2X between 2013 and 2019 in Erbil and Duhok (Kurdistan Regional government, Iraq) (5).

In traditional remedies world wide plant and herbs extracts to have been used extensively to treat various diseases, including cancer (6). Herbal medicine is now considered a promising tool in cancer prevention and treatment. Natural products are a very effective modality for modern medicine in drugs that are used in cancer treatment as cytotoxic therapy (7). Experimental studies revealed that plants crud extract could work as an anti-viral effect (8), and protect against cancer cells (9, 10). However, current work was conducted to study the cytotoxic effect of the parsley leaf extract against human esophageal cancer cell lines and study its potential effect on normal tissue. The anticancer activity of ethanolic extract of parsley leaves, seeds and its active ingridents such as flavonoids and polyphenols have been documented by many reserchers (11) and the cytotoxic effect of apigenin extracted from parsley seeds was confimed in AMN3 bearing mice (12).

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Materials and Methods:

Parsley leaves were purchased from local market in Baghdad and authenticated at the National herbarium of Iraq Bot-

any directorate under the scientific name petroselenium sativum belongs to the family umbilifera. Parsley leaves dried in a vaccum oven at 40˚C. The dried leaves crushed by electric blender and stored in a dark and dry glass bottle.

The preparation of 70% ethanolic extract of parsley leaves was carried out according to (13) as follows 100 g of crushed leaves was suspended in 100 ml of 70% ethanol in a screw capped conical flask and kept for stirring using electrical magnetic stirrer at 25 º C for 72h Once the process was finished the mixture was subjected to two steps filtration, in first step five layer of medical gaze was used to filtrate the mixture, while in the second step the filtration was carried out under vacuum using Whatman NO. 1 filter was dropped in glass Petri dish and the solvent was evaporated at 40 º C in air ventilation oven, however figure (1) show the summery of the conducted work.

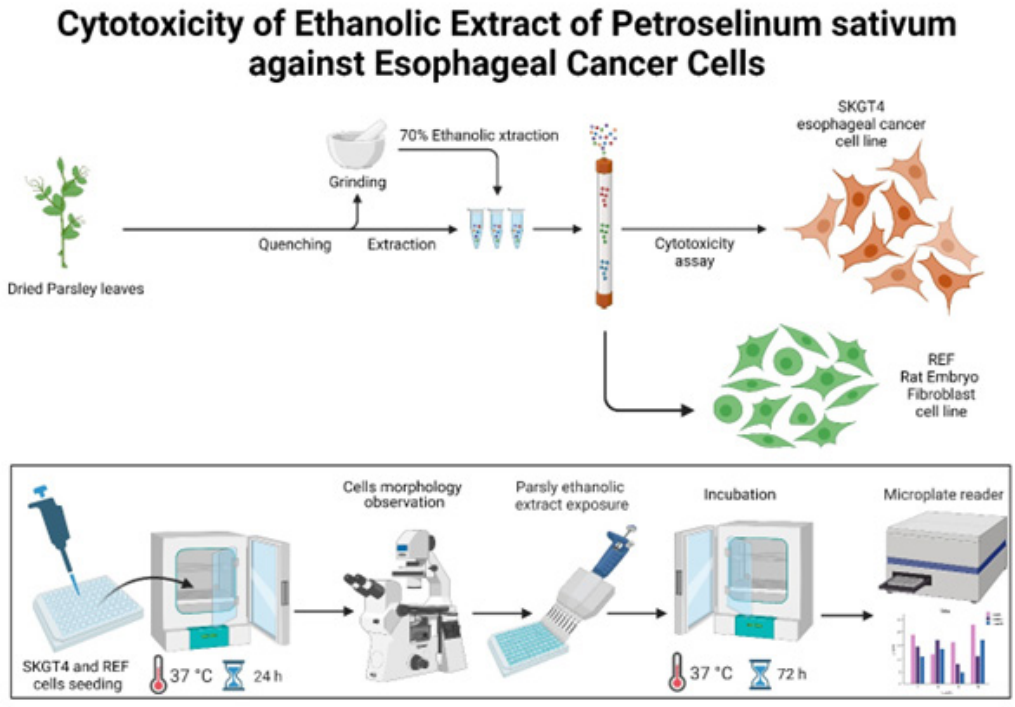

***Figure (1)** showing overall summery of the research work investigating the parsley extract against esophagial carcinoma as well as normal cells.*

Maintenance of Cell Cultures

The oesophageal cancer cell lines SK-GT-4 and normal Rat embryonic fibroblast (REF) were cultured and maintained at a humidified atmosphere of CO2 5% (at 37°C). MEM medium (Usbiological, USA) was used for SK-GT-4 cell line, and RPMI-1640 was used for REF cells. Both cells' lines medium contains 10% FBS (fetal bovine serum) (Capricorn- Scientific, Germany), penicillin (100 units/ml), and streptomycin (100 µg/ml) and then incubated at 37 °C. All cell lines were cultured as adherent confluent monolayers and maintained in a humidified Co2 incubator (5%) at 37 oC. According to standard procedure (14, 15), cells were harvested after trypsinization (with trypsin-EDTA) (Capricorn-Scientific, Germany).

Cytotoxicity assays

Crystal violet assay was conducted to evaluate the cytotoxicity of parsley crud extract against human oesophageal cancer cell line (SK-GT-4), and normal Rat embryonic fibroblast cells (REF) at a concentration of 10000 cells /well were cultured for 24h (confluence cells monolayer), then treated with the extract at duplicate dilution (125-1000) µg/ml in culture media. Cell viability was determined after 72h of exposure through discarding the medium, adding 50 µl of Crystal violet solution (Bio-world, USA)(9), and incubating for 2h at 37°C. Later, the stain was removed, and wells were washed with tab water. The absorbency was measured using MBG microplate reader (Germany) at the test wavelength of 492 nm. The growth inhibition percentage was calculated for treated and untreated cells (three replication for each concentration) as follow equation (16).

$$\text{Inhibition rate (\%)} = \frac{\text{mean of control} - \text{mean of each concentration}}{\text{mean of control}} x100$$

Statistical Analysis:

The experiments data were analyzed using Graph Pad Prism Programs V. (7.0). Significance between control and samples was measured using students T- test. Statistically significance was considered at P value of ≤ 0.05.

Results:

***In vitro* antitumor activity of Parsley extract**

We conducted a crystal violet viability assay to evaluate the therapeutic efficacy of parsley ethanolic extract and its possible cytotoxic effect against esophageal cancer cells and normal embryonic fibroblast (REF). The results in Fig. 1 indicated that parsley crud extract significantly decreased the number of SK-GT-4 esophageal cancer cells at all concentrations tested. The most killing effect was noticed at the concentration of 500 µg/ml (39.68%).

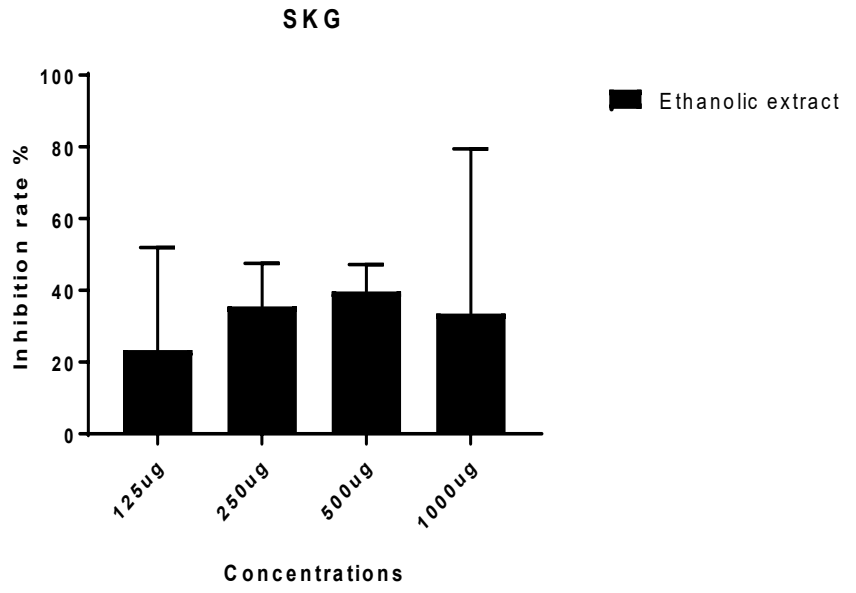

Figure (2): *Inhibition rate (%) of different concentrations of 70% ethanolic extract of parsley leaves on esophageal cancer cells.*

Furthermore, the surprising result was the presence of a significant decrease in cell viability of all concentrations on REF cells.

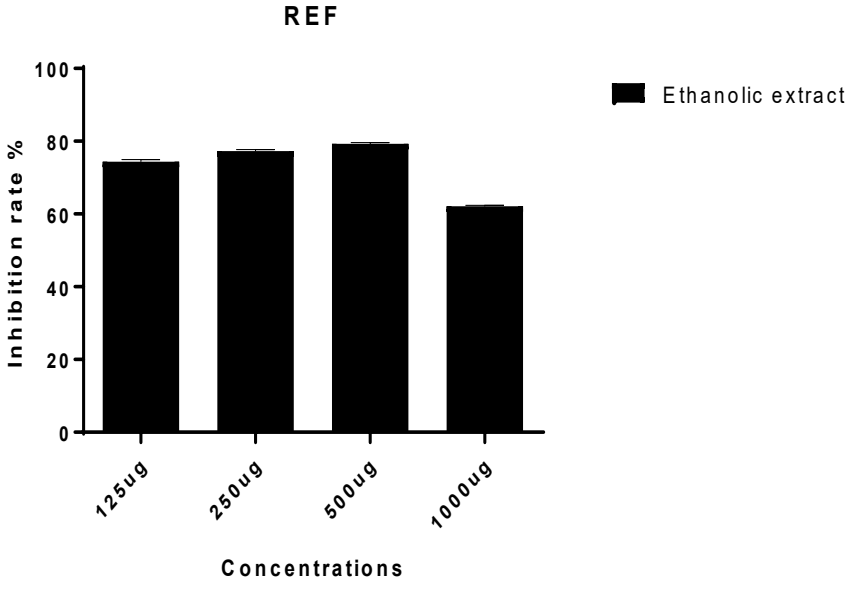

Figure (3): *Inhibition rate (%) of different concentrations of 70% ethanolic extract of parsley leaves on normal embryonic fibroblast cells (REF).*

Discussion:

The current study showed effective antitumor activity of the ethanolic parsley extract against oesophageal cancer cells with no harmful effect in normal cells.

The Petroselinum sativum was used in many clinical trials for hormonal cancer therapy; in a study of 259 people who take Petroselinum sativum or have cancer hormonal therapy, the results showed that no report of hormonal cancer therapy is found in people who take Petroselinum sativum (17).

Also, this plant has an anti-cancer effect on breast cancer as a decent nutritional supply of vitamin C, folate, and vitamin K (20, 21) and for Hepatocellular Carcinoma (16, 17) and many other cancer types (18, 19).

Our results show that Petroselinum sativum Parsley extract decreases normal cell viability and alters esophageal cancer cells' cellular morphology at all concentrations. Further molecular studies are undergoing to elucidate the mechanism (s) of action of these extracts on human digestive system cancer cells.

Finally, it is worth mentioning that very limited information is currently available in the literature concerning the impact of parsley leaf extract on the esophageal cancer cell line. In conclusion, the result of this study can be considered the first practical proof in this regard.

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