



CYTOTOXICITY OF PARSLEY EXTRACT ON ORAL CANCER CELL LINES

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ABSTRACT

Aim : To study the cytotoxicity activity of Parsley extract on oral cancer cell lines .

Objective : To analyse the cytotoxicity of Parsley extract on oral cancer cell line

Background: Parsley, in addition to being a source of certain vitamins and minerals, has been used in the treatment of prostate, liver and spleen diseases, as well as anemia, arthritis, and microbial infections. It has also been found useful as a diuretic and laxative. Cytotoxicity is the quality of being immune to cells.

Reason: Cytotoxicity analysis of Parsley extract will pave way to make use of the medicinal properties of the herb in cancer therapy and its potential as an anti cancer

Result: The cytotoxic effect of Parsley extract in comparison with that of PC has been recorded. The graph shows that increasing concentration of Parsley extract has an increasing anti cancer activity against KB cells.

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INTRODUCTION

Hepatocellular carcinoma, is the sixth most common cancer worldwide, continues to have high prevalence in many Asian countries. Due to the poor prognosis, hepatocellular carcinoma is the fourth fatal cancer in the world. It is also the third most frequent cause of cancer deaths among men worldwide. Usually, males are more affected than females and, are most common between the 30-50 years of age. Chemotherapy is the most common treatment for the cancer. Unfortunately, many of the chemotherapeutic drugs are non-specific and cause severe side effects. Therefore, searching for new alternative strategies for the treatment and prevention of hepatocellular carcinoma is necessary^{1,2}. Natural products have been considered as a valuable source for the anticancer drug discovery. There are over 100 different known cancers that affect humans.⁶ Cancer is often treated with some combination of radiation therapy, surgery, chemotherapy, and targeted therapy.⁷ Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend the body.⁵ The *Petroselinum sativum* (PS) or parsley, a member of the family of Umbelliferae, has also been reported to have antioxidant, antidiabetic, antimicrobial and cytotoxic activities. Recently we have also reported that seed and oil extracts of *Petroselinum sativum* induced cytotoxicity against human breast cancer cells.

It is the most frequent cancer in both developed and developing regions, but the rate of human breast cancer is higher in developing countries in compared to developed nations. Alternative and complementary medicines are now considered to play an emerging role in the cancer prevention. Natural products are an extremely promising strategy for chemoprevention to block the development of cancer in human. Many natural plants have furnished modern medicine with the drugs that are used in cancer therapy as cytotoxic agents. *P. sativum* has also been found to reconstruct kidney tissue after nephrotoxicity. Due to the diverse pharmacological and preventive properties of *P. sativum* plant extracts, present investigation was carried out to investigate the cytotoxic activities of alcoholic extract and seed oil of *P. sativum* against human breast adenocarcinoma cells (MCF-7).^{1,2} Anticancer and antioxidant property of certain medicinal herbs can be used to treat trauma over a longer period of time which is always very promising. Phytochemicals present in this herb have anticancer property.

The “phyto-” of the word phytochemicals is derived from the Greek word 'Phyto', which means plant. Hence these are plant chemicals. Phytochemicals are defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic diseases.³ Thousands of phytochemicals have been identified so far, and scientists have only begun to investigate their promise. Natural dietary phytochemicals have been widely used in vitro, in vivo, and preclinical cancer prevention and treatment studies. Some of these clinical trials have shown various degrees of succession. This is because phytochemicals have the potential to stimulate the immune

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system, block substances we eat, drink and breathe from becoming carcinogens, reduce the kind of inflammation that makes cancer growth more likely, prevent DNA damage and help with DNA repair, reduce the kind of oxidative damage to cells that can spark cancer, slow the growth rate of cancer cells, trigger damaged cells to commit suicide before they can reproduce and help to regulate hormones thereby resisting the risk of cancer.¹ Cytotoxicity is the degree to which an agent has specific destructive action on certain cells. It is the possession of destructive action, particularly in reference to lyses of cells by immune phenomena.⁴ Cell proliferation rates or viability levels are good indicators of cell health. Proliferation or viability analysis which is crucial for cell growth and differentiation studies, and are often coupled with metabolism analysis. Cytotoxicity is the degree to which an agent has specific destructive action on certain cells. It is the possession of destructive action, particularly in reference to lyses of cells by immune phenomena.¹⁰

MATERIALS AND METHODS

Preparation of extract

Parsley was commercially bought. The dried powder (50 grams) was extracted with 100ml of chloroform. The extraction was followed by the filtration process.

Maintenance of cell lines

The vial containing the KB cell lines procured from ATCC (CCL-17) was removed from liquid nitrogen freezer and immediately placed in a 37°C water bath. It was agitated continuously until the medium thawed. Then it was centrifuged at 1000rpm for 5 minutes at room temperature. The supernatant was discarded and cells were washed with fresh medium to remove residual DMSO. The cell pellet was re-suspended in 3ml of DMEM with 10% FBS. It was then incubated in a CO₂ incubator at a humidified 37°C. The medium was changed every 2-3 days or when ph indicator (e.g. Phenol red) in medium changed colour. The culture was kept in a medium with 10% FBS until cell line were re-established.

MTT Assay

The MTT assay (Mossman, 1983)⁹ is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37°C in humidified atmosphere with 5% CO₂. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2X 10⁴ cells /well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the samples (100, 200 & 300 µg) for 24 hours. After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtitre plate reader. Cyclophosphamide was used as a positive control.

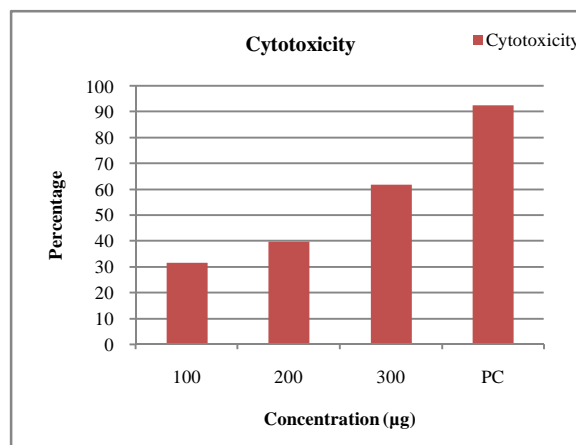
Cell survival was calculated by the following formula

$$\text{Viability \%} = (\text{Test OD} / \text{Control OD}) \times 100$$

$$\text{Cytotoxicity \%} = 100 - \text{Viability \%}$$

RESULTS AND DISCUSSION

Cytotoxicity analysis using various concentrations of Parsley extract (100, 200, 300 micrograms) was performed. The viability of the KB cell lines shows a gradual decrease as the concentration of the extract is increased. This exhibits the Cytotoxicity of Parsley extract with increasing concentration which paves the possibility for the usefulness of Parsley as an anti cancer agent in the near future. Graph (Figure 1) shows percentage cytotoxicity of Sample and PCPC – Positive Control



Graph shows percentage cytotoxicity of sample and PC

Table 1 shows percentage cytotoxicity of sample and PC

Concentration (µg)	Cytotoxicity %
100	31.58
200	39.70
300	61.73
PC	92.43

CONCLUSION

The use of natural products for treatment is generally a more preferred option due to the adverse side effects which accompany other treatment plans and is widely preferred and accepted for the reason stated.⁵ The presence of these phytochemicals reveals the anticancer property of the extract. The study exposed the cytotoxic potential and antitumor and anti cancer properties of Parsley. The extract presented cytotoxic effect in high concentrations, leading to increased cell death in the KB cell culture.⁵ Further research is required to know the exact mechanism of action of Parsley as an anticancer drug.

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