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Cytotoxic Activity of *Onobrychis Megataphros* Leaf Extract

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Abstract: This study aims to determine the cytotoxic effects of *Onobrychis megataphros* plant leaves. Therefore, firstly, the *O. megataphros* samples included in our study were collected from Şanlıurfa and the surrounding areas (Siverek-Hilvan) during the vegetation periods between 2021-2022. The surface parts of the plants were dried in the shade, in the open air, and then pulverised to a suitable size with the help of a scale mill. The plant material was extracted three times separately with methanol at room temperature. After filtration, the samples were subjected to methanol extraction in Soxhlet apparatus for 6-8 hours. The extract was then filtered with Whatman blue band filter paper and evaporated at 40°C to remove the solvent. The cytotoxic activity of plants is associated with the presence of bioactive compounds such as flavonoids, alkaloids and saponins. Studies have shown that these sustances have anticancer effects by inducing apoptosis that inhibits cell proliferation and preventing angiogenesis. In our study, the cytotoxic activity of *Onobrychis megataphros* was investigated. The focus of our study is to fully understand the mechanisms underlying the cytotoxic activity of *O.megataphros* and to determine its potential as a therapeutic agent in cancer treatment. In vitro cytotoxicity experiments that were carried out in the laboratory, PC-3 prostate cancer cell line (CRL-1435) was obtained commercially from ATCC. Cytotoxic activity of the *O.megataphros* was detected with MTT (3-4,5-dimetil-tiyazolil2,5-difeniltetrazolyum bromid) test. The results have shown that the sample has not decreased vitality in all cell tests. The vitality was 100%. In conclusion, *O. megataphros* is a promising plant as a natural source of cytotoxicity with potential anticancer activity, and further research on this topic may contribute to the development of new cancer therapies that are both safe and effective.

Keywords: *Onobrychis megataphros*, Cytotoxicity, Cytotoxic activity

Introduction

Phytotherapy, which means the use of natural compounds found in plants, vegetables and roots on patients and is considered among complementary medicine methods, has been practiced since ancient times. The use of complementary medicine has increased steadily in recent years in many developing and industrialized countries. In developed countries, the use of complementary medicine is 42.1% in the USA, 48.2% in Australia, 49.3% in France, 70.4% in Canada, 71% in Chile, 70% in China, 40% in Colombia and 80% in African countries (Ozcelik & Toprak, 2015).

In our nation, as in the rest of the world, many plant species have long been utilized by the populace for a variety of reasons (Awuchi, 2019; Muvakit & Ozaslan, 2023). Roughly 20,000 medicinal plants are utilized for therapeutic purposes, according to publications on medicinal plants, 91 nations' pharmacopoeias, and the World Health Organization (WHO). Since 1926, researchers have studied the crucial aspects of plants for human health in the lab (Mazzoleni & Nelson, 2005).

There are two subgenera within the genus Onobrychis, which grows natively in Turkey: Onobrychis and Sisyrosema. Dendobrychis, Laphobrychis, and Onobrychis are the three known divisions of Onobrychis among

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these subgenera. With a variety of applications among humans, the Fabaceae family is the second largest family in Turkey (Muvakit and Ozaslan, 2023), following Asteraceae. For instance, Vicia faba is used to treat gastrointestinal disorders, Onobrychis gracilis to cure colds and flu, Vicia cracca subsp. stenophylla to treat colds, and Vicia ervilia to treat diabetes (Demirci & Ozhatay, 2012; Sargın et al., 2013; Hayta et al., 2014).

Plant extracts are compounds obtained from different parts of the plant (root, leaf, flower, etc.) using various solvents. These extracts can contain many bioactive compounds, including various compounds such as alkaloids, flavonoids, terpenoids, phenolic compounds and lignans. These compounds can inhibit cell growth, trigger apoptosis (programmed cell death) and stop the proliferation of cancer cells (Ingle et al., 2017).

Cytotoxic activity refers to the ability of a substance to inhibit the growth of cells or trigger cell death. By targeting cancer cells, this activity can stop tumor growth or kill cancer cells. Plant extracts may have cytotoxic activity and are therefore being investigated as potential therapeutic agents for cancer treatment (Habli et al., 2017).

Although studies have revealed that plant extracts are extremely important for health, studies in this direction continue rapidly. Although studies on the determination of these potentials of plants belonging to the genus Onobrychis, which are widely available in our country, have accelerated in recent years, studies on Onobrychis megataphros species have been limited.. The aim of this study was to determine the cytotoxic activity of *Onobrychis megataphros* leaf extracts.

Method

Collection and Extraction of Plant Samples

The Onobrychis megataphros plant utilized in the study was gathered in the vegetative phases of 2021 and 2022 from Şanlıurfa and the surrounding area (Siverek-Hilvan). The plant samples were dried in the shade, and then the leaves were ground into a fine powder. In a sokslet device, the samples were exposed to methanol extraction for six to eight hours. Subsequently, the extract was filtered through Whatman blue band filter paper and the solvent was evaporated at 40 °C.

Cell Culture Studies

Cultivation of Cells

In our study, PC-3 prostate cancer cell line (CRL-1435) was obtained commercially from ATCC. Cells stored at -80 °C were thawed before the study and placed in medium (DMEM containing 10% FBS, 1% penicillin-streptomycin and 1% L-glutamine). Centrifuged at 800 rpm for 5 min, the cells settled to the bottom. The pellet was dissolved in 5 mL of medium, transferred to a 25 cm2 cell culture dish and incubated at 37°C with 5% CO2. Cultured cells were observed daily under an inverted microscope and the medium was changed every other day. When the cells covered 80% of the cell culture dish, they were trypsinized and seeded into new culture media and cultured until sufficient cell numbers were reached.

For trypsinization, the medium of the cells was removed. Cells were washed with DPBS to remove serum and dead cells. Afterwards, DPBS was aspirated and 0.5 mL of 0.1% Trypsin-EDTA solution was added to separate the cells from the culture dish and incubated at 37 °C with 5% CO2 for 4 min. At the end of 4 min, the cells were checked under a microscope to see if they had detached from the culture dish. After making sure that all cells were removed, 5ml of medium was added to the cells and this cell suspension was transferred to a falcon tube. After centrifugation at 800 rpm for 5 min, 5 mL of medium was added to the pellet to ensure homogeneous mixing of the cells and then the cell suspension was transferred to 25cm2 cell culture dishes.

Cell Counting

For cell counting, trypsinized cells were used. After trypsinization, the cells were centrifuged and the pellet was thawed in 5 mL medium. A 10µL volume of the cells was taken and an equal amount of 0.5% trypan blue dye was added. After pipetting, 10 µL of this mixture was placed on a Thoma slide and cell counting was performed

under an inverted microscope. The number found was multiplied by the dilution coefficient and the number of cells in 1 mL of medium was calculated.

Cytotoxic Activity Test

The cytotoxic effects of the sample were determined by MTT (3-4,5-dimethyl-thiazolyl-2,5-diphenyltetrazolium bromide) test. The MTT assay is a standard colorimetric assay that measures cell proliferation. It is based on the principle that the yellow MTT dye is converted into purple formazan crystals by the dehydrogenase enzyme in the active mitochondria of living cells and then this color change is measured spectrophotometrically (Mosmann, 1983). With this method, it is aimed to determine the growth profiles of cell cultures and to determine the cytotoxic effect depending on the application times and doses of the substances.

For MTT assay, PC-3 cells were grown in medium and counted before the study. Each well of the 96-well plate was seeded with 5×103 cells and left to incubate for 24 hours at 37 °C containing 5% CO2. After incubation, the sample was added to the cells at different doses (500-0 µg/mL) in 3 replicates. At the end of 48 hours of incubation, 40µL of MTT dye was added to each well and the cells were incubated for another 4 hours to ensure the formation of formazan crystals. After the presence of blue-violet formazan crystals was detected on the microscope, the medium was replaced with 80 µL DMSO to solubilize these crystals and formazan crystals were dissolved for 20 min. The color intensity of the cells was measured using a spectrophotometer at 570 nm wavelength. The results were calculated as mean ± standard deviation and % viability of the cells was determined.

Results and Discussion

According to the results of cytotoxic activity, it was observed that the sample did not decrease the viability from the highest dose to the lowest dose as seen in Figure 1. It is seen that the viability is above 100% at all doses.

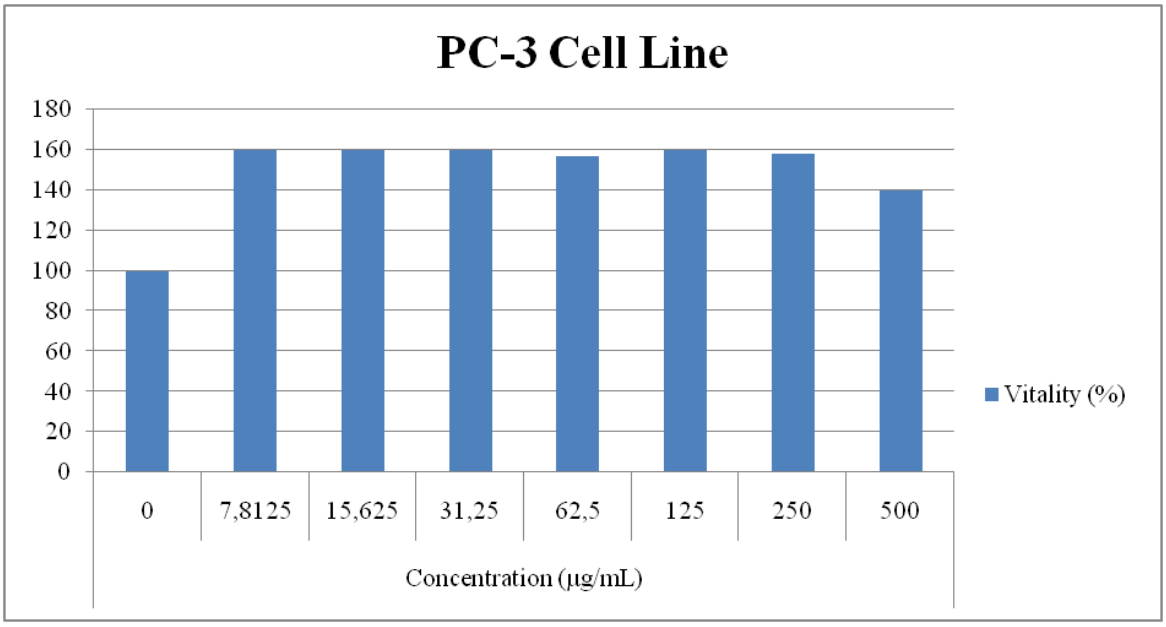

Figure 1. The vitality (%) of the sample in PC-3 cell line

The term "cytotoxic activity" describes a substance's capacity to stop cell growth or cause cell death (Akter et al., 2014). This action can inhibit tumor growth or destroy cancer cells by specifically targeting them. Because plant extracts may be cytotoxic, they are being studied as possible therapeutic agents for the treatment of cancer (Suffredini et al., 2006). Numerous investigations have assessed the cytotoxic properties of various plant extracts against cancerous cells. Both in vitro (cell culture) and in vivo (on living organisms) models are typically used in these investigations (Itharat et al., 2004; Prakash and Gupta, 2013 Liang et al., 2017). Plant extracts have lethal effects on cancer cells through a variety of methods, such as cell cycle regulation, antioxidant activity, induction of apoptosis, and blockage of cellular signaling pathways (Almatroodi et al., 2021). In studies on the determination of cytotoxic activity on different species belonging to the genus

Onobrychis (Karakoca et al., 2015; Clericuzio et al., 2020; Amin et al., 2023; Yeniçeri et al., 2024;), it was observed that Onobrychis species have cytotoxic activity and the results obtained from our study are compatible with the literature.

Conclusion

In conclusion, *O. megataphros* is a promising plant as a natural source of cytotoxicity with potential anticancer activity, and further research on this topic may contribute to the development of new cancer therapies that are both safe and effective.

Recommendations

Further mechanistic studies should be conducted to determine the effective mechanisms for the anticancer activity of O. megataphros. This is necessary to understand which components of the plant target cancer cells and how they act. Chemical analyses should be carried out to identify the active components responsible for the plant's anticancer activity. This is important to isolate the plant's potential anticancer agents and obtain their pure forms. Dose response studies should be conducted to evaluate the cytotoxic effects of different doses of the plant against cancer cells. In addition, toxicity studies in animal models are necessary to evaluate the toxicity of the plant on humans. Clinical trials should be conducted to evaluate the anticancer efficacy and safety of the herb. This is important to assess the usability of the herb in the treatment of cancer in humans. Besides the potential anticancer activity of O. megataphros, other biological activities of the plant should also be investigated. This would allow us to more comprehensively evaluate the potential health benefits of the plant. Methods for collecting and using the plant in a sustainable manner should be developed. This helps to preserve the plant's natural habitat and make it accessible for future generations. These recommendations could enable progress in the evaluation of O. megataphros as a natural source of cytotoxicity with potential anticancer activity and help us better understand the plant's usability in cancer treatment.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

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