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Determination of the cytotoxic effect of *Prangos ferulacea* (L.) Lindl. which grows naturally in the foothills of Bayburt Kop Mountain, on non-small lung cancer

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Abstract

The genus *Prangos* has been used for centuries as an antibacterial and antidiabetic agent. In this study, *Prangos ferulacea* (L.) Lindl. species, which is frequently encountered in various regions of our country, especially in Northeast Anatolia, and known as "çaşır or çakşır" among the people, were collected from the flora of Bayburt Kop Mountain, and methanol extract of the stem parts of this plant was obtained. Afterward, the cytotoxic effect of this extract on the non-small cell lung cancer cell line (H-460) was determined and compared with the cytotoxic activity on the lung fibroblast cell line (MRC-5). The results obtained using the Wst-8 method, it was determined that no toxic effect occurred in the MRC-5 cell line after both 24 and 48 hours of methanol extract treatment, while a toxic effect occurred in the H-460 cell line (125 µg/ml and 62.5 µg/ml) after 48 hours of application. However, this toxic effect was not statistically significant.

Keywords: Prangos ferulacea (L.) Lindl., lung cancer, H-460, MRC-5, cytotoxicity

Introduction

Plant floras, which are distributed in different geographical regions of the world, have been used traditionally and scientifically for centuries for the nutrition, protection, shelter, warmth and treatment of living things. The plant flora in Turkey is also rich in medicinal aromatics due to its different geographical characteristics and climates. The genus *Prangos*, which can grow spontaneously in many regions of the world and our country and is popularly known as "Çaşır or Çakşır" that consists of about 30 species and many of these species are used by local people in traditional and alternative treatment methods [1]. *Prangos ferulacea* (L.) Lindl. (Apiaceae) is a perennial plant that grows in the Northeastern Anatolia Region, especially in around Erzurum [2], and is widely used for therapeutic purposes. Its fruits and roots have been reported to be used to treat digestive disorders, bleeding and wounds. Ethnobotanical literature reports that eating the roots of *Prangos ferulacea* (L.) Lindl. grated with honey or consuming the above-ground parts by boiling them is an aphrodisiac. A decoction of the young shoots has also been used as a treatment for diabetes, while an infusion of the leaves has been used to manage hypertension. The use of the softened above-ground parts as an infusion has been reported for kidney and bladder inflammation, while topical application has been used to treat hemorrhoids [3].

Umbelliferae family (Apiaceae), to which *Prangos ferulacea* (L.) Lindl. belongs, is remarkable for isoprene and furanocoumarins. Its chemical components include essential oils, terpenes, benzoids, coumarins, ozones, flavonoids, myrosinase enzyme, alcohols, peroxidase enzyme, esters, vitamins, benzaldehydes, minerals, ketones, glycosides, phenolic acids and other compounds. From this plant, some coumarins have been isolated, such as ferudenol, prangon, scopoletin, ostenol, herniarin, oxypeusedanine, ostol,

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imperatorin, bergapten, isoimperatorin, meranzine hydrate, oxypeusedanine hydrate, hydroxypeusedanine, xanthotoxin, pranthimgin, pranferol, l-isopeusedanine, meranzin, psoralen and pranusine [2].

Badzar et al. (2018) reported that the chemical contents of these organs are different from each other, and in the studies carried out by some other researchers, it was suggested that the quality and quantity of essential oils of plants collected at altitudes higher than 2800 m compared to 1210 m and 1726 m above sea level, and it was stated that the quality and quantity of essential oils may be affected by many factors such as climatic conditions, genetic structure, cultivation methods, altitude and geographical conditions [4].

The rate of spread of the first six cancer types published by the International Agency for Research on Cancer in 2002 is as follows; lung, stomach, bladder, colorectal, larynx, prostate, breast, colorectal, stomach, ovarian, lung cancers and leukemias in men and breast, colorectal, stomach, ovarian, lung cancers and leukemias in women. Although many antioxidative, antimicrobial, antitumoral and antiproliferative studies have been conducted on *Prangos ferulacea* (L.) Lindl. species in the literature, the effect of this species on lung cancer has not been sufficiently elucidated. In this study, we aimed to determine the cytotoxic effects of different concentrations of methanol extract obtained from *Prangos ferulacea* (L.) Lindl. which grows naturally in the foothills of Bayburt Kop Mountain, on non-small cell lung cancer cells and healthy lung fibroblast cells after 24 and 48 hours of treatment.

Material and Methods

Preparation of methanolic plant extract

The plants were collected from the foothills of Kop Mountain and identified by Professor Meryem Sengul Koseoglu, member of Atatürk University, Faculty of Science, Department of Botany. Stem of plant samples were dried at room temperature in the dark. After the dried plants were pulverized with the help of a grinder, 10 mL of 80% methanol was added for each gram sample and mixed with the help of vortex. After these processes, it was extracted in soxhlet extractor and the methanol solvent was removed with the help of a vacuum evaporator. After the plant extract was dried in a lyophilizer, it was dissolved with DMSO and transferred to an amber bottle. The samples were stored at +4°C until used in studies [5].

Ethical approval

In this study, no living tissue was isolated from humans or animals, and the cells used were commercially purchased. The authors of this article declare that the materials and methods used in this study do not require ethical committee approval and/or legal-specific permission. Ethical approval was approved by the Bayburt University Non-Invasive Clinical Research Ethics Committee (2023-7/2).

Evaluation of *In-vitro* cell viability

The H-460 (non-small cell lung cancer) and MRC-5 (lung fibroblast) cell line was selected for cell culture. The medium prepared in 25 ml flasks by adding RPMI (GIBCO) medium and 10% FBS (GIBCO) was used for cell proliferation. During cell transfer, the trypsin-EDTA solution (GIBCO) was used after washing with PBS buffer (Sigma) in order to obtain viable cells at the base of the flask. The flasks were incubated for 24-48 hours in an incubator containing 5% CO_2 at 37°C. Cells were checked for viability with trypan blue dye (Sigma) before each passage. After the cells in the flask were centrifuged, the suspension was poured and the cells were counted on the thoma slide by staining with trypan blue dye [6,7].

To determine cell viability, CVDK-8 kit based on the WST-8 method was used which purchased from Ecotech Biotechnology® (Erzurum - Turkey). Initially, H-460 and MRC-5 cells were seeded in 96-well plates and incubated for 24 hours for cells to attach to these plates. Then, plant extract dissolved with PBS (Sigma) prepared at 4 different concentrations (125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.625 µg/ml) was added to these wells and the plates were kept in the incubator for 24 hours again. After incubation period (24 and 48 hours), cell viability was determined according to the guidelines outlined in the kit. 10 µl of CVDK-8 reagent (Ecotech Biotechnology®) was added to the cells treated with different doses of *Prangos ferulacea* (L.) Lindl. stem of plant methanol extract and the cells were incubated for 4 hours. After the experiment was completed, the absorbance of each sample was measured at 450 nm [8,9].

Statistical analysis

The study was performed in 3 repetitions and the data are given as mean±standard deviation. Data were analyzed by the GraphPad Prism statistical program using Student's t test. A p value of equal to or below .05 was accepted as significant.

Results

Four different concentrations of plant methanol extract (125 mg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.625 µg/ml) applied to both healthy lung fibroblast cells and non-small cell lung cancer cell line for 24 hours were not found to be cytotoxic (Table 1).

Table 1. Cell viability results of 24th hour treatment

24th hour	MRC-5	H-460	
	Mean±SD	Mean±SD	
Control	100±7.34	100±8.18	
125 µg/ml	102.04±5.67	96.57±6.43	
62.5 µg/ml	104.41±3.81	96.61±1.65	**(% Viability)**
31.25 µg/ml	109.38±6.81	98.35±9.07	
15.625 µg/ml	122.91±6.11	99.94±3.91	

SD: standart deviation, % Viability: cell viability detection Kit-8 (CVDK-8) results, Mean: the average of the test results performed with 3 repetitions, p value should be <0.05

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According to the results obtained after 48 hours of treatment, the toxicity in healthy cells was negligible, while the toxicity in cancerous cells was much higher. In addition, it was concluded that the concentrations applied were more lethal compared to the 24 h treatment. However, similarly, it was observed that the 48-hour application was not statistically significant (Table 2).

Table 2. Cell viability results of 48th hour treatment

48th hour	MRC-5	H-460	
	Mean±SD	Mean±SD	
Control	100±1.36	100±49.12	
125 µg/ml	93.29±30.64	60.63±23.91	
62.5 µg/ml	95.72±32.29	66.77±36.09	(% Viability)
31.25 µg/ml	96.31±27.49	95.93±22.29	
15.625 µg/ml	99.44±7.09	96.71±2.61	

SD±: standart deviation, % Viability: cell viability detection Kit-8 (CVDK-8) results, Mean: the average of the test results performed with 3 repetitions, p value should be <0.05

Discussion

Biological activity studies on *Prangos ferulacea* (L.) Lindl. plant and its active ingredients have been carried out for many years and many scientific data have been brought to the literature. For example, the antimicrobial effect of this plant has attracted the attention of researchers and many important data have been obtained in this context. In one study, plant species with four different solvents (ethanol, methanol, water, n-hexane) were found to be effective against gram-positive bacteria such as *Bacillus cereus, Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, Escherichia coli,* The antibacterial effect against several gram-negative bacteria such as *Klebsiella pneumoniae, Proteus mirabilis and Salmonella enteritidis* was examined and ethanol and methanol extracts were shown to have strong antibacterial effect. In another study, the antibacterial properties of the fruit extract against positive and negative gram bacteria such as *Eschrechia coli, Staphylococus aureus and Pseudomonas aeroginosa* were revealed and the presence of some compounds such as limonene, apinene and humulene were suggested as the reason for the antibacterial properties of this plant [10].

In another scientific study, a comparative experiment was designed in patients with bacterial vaginosis and a group was given *Prangos ferulacea* (L.) Lindl. vaginal cream along with oral metronidazole and it was found that this cream accelerated the recovery of the disease and suppressed the symptoms. In addition, considering that there is a close connection between the development of cancer in organisms and the mechanisms of action of oxidative stress and antioxidants, it has gained importance to elucidate the antioxidant mechanism of *Prangos ferulacea* (L.) Lindl. and various studies have shown that this plant is a rich source of antioxidants. It has been stated that the significantly high antioxidant activity of *Prangos ferulacea* (L.) Lindl. extracts may be caused by various flavonoids, coumarins, alkaloids and terpenoids in the plant. It has been reported that the protective and antioxidant effects of *Prangos ferulacea* (L.) Lindl. species are higher than that of α-tocopherol (vitamin E) and the effect of glutathione-S-transferase (GST) has been demonstrated [11]. Razavi et al. (2009) investigated the cytotoxic, phytotoxic, antimicrobial and antioxidant effects of quercetin 3-O-glucoside (Q3G) isolated from the aerial parts of *Prangos ferulacea* (L.) Lindl. by HPLC. They stated that it makes it strong for defense and therefore they stated that Q3G can be considered as a natural anticarcinogenic agent [12].

In another study, it was stated that a drug called doxorubicin (DOX), which has a wide chemotherapeutic effect in many cancer types, was used and that this chemical had a strong side effect due to its prooxidant effect, and PC12 (rat pheochromocytoma-derived neuroendocrine cell line) was used as a neural model. The connection between ostol and DOX-mediated apoptosis isolated from *Prangos ferulacea* (L.) Lindl. in the cell line was examined and it was determined that DOX caused cell death in the PC12 line and increased caspas-3 activation compared to the control group. It was concluded that pretreatment with Ostol inhibited DOX-mediated oxidative stress and apoptosis [13]. In a different study for the prevention of DOX-mediated oxidative stress and apoptosis, oxypeusedanine and isoimperatorin isolated from *Prangos ferulacea* (L.) Lindl. were used in PC12 cell line. The PC12 cell line was pretreated with the isolated coumarins and then treated with the IC50 dose of DOX. As a result of Bax and Bcl-2 mRNA expressions, reactive oxygen species (ROS), mitochondrial membrane potential (MMP) and Caspas-3 measurements, it has been proven that ROSs are inhibited and show protective activity against apoptosis [14]. In another study using nanoparticles, silver nanoparticles (Iso-AgNP) were synthesized from isoimperatorin coumarin isolated from *Prangos ferulacea* (L.) Lindl. based on the fact that metallic silver nanoparticles (AgNP) have anticancer and antimicrobial properties and can cause apoptosis [15].

In the literature, there are many cytotoxicity studies with active ingredients of *Prangos ferulacea* (L.) Lindl. species. According to a study, the efficacy of ostol, isoimperatorin, oxypeusedanine and braylin active substances isolated from *Prangos ferulacea* (L.) Lindl. species in H1299 (Non-small cell lung cancer), PC3 (Prostate cancer) and SKNMC (Neuroepithelioma) cell lines were determined and cytotoxic effects of oxypeusedanin and braylin agents were determined. Although no effect was found, it was determined that isoimperatorin had a high cytotoxic effect on SKNMC and PC3 cell lines, and ostol on PC3 cell lines [16]. In another study conducted with ostol, isoimperatorin, oxypeusedanine, psoralen, oxypeusedanine hydrate, gospherol, oxypeucedanin methnolate and pranferol isolated from plant roots, no cytotoxic effect was observed in monkey kidney epithelial cell line. effect was found. However, isoimperatorin did not show any cytotoxic effect neither in cancer cell lines nor in healthy cells [17].

As seen in the literature, although cytotoxicity studies on the active components of *Prangos ferulacea* (L.) Lindl. have

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been carried out in different cancer lines, there are not many cancer studies using the extraction of the stem parts of the plant consumed by the public. In this study, we prepared the methanol extract of *Prangos ferulacea* (L.) Lindl. using soxhlet extraction method and evaluated the cytotoxic effects of some concentrations (125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.625 µg/ml) of the plant extract. Concentrations are not kept too high to avoid solvent toxicity. Proliferation assay was determined by WST-8 method using H-460 and MRC-5 cell lines and our results show that after 48 hours of treatment, 125 µg/ml and 62.5 µg/ml concentrations show cytotoxic effects, but this toxicity is not statistically significant (p value not <0.05).

Conclusion

A statistically significant cytotoxicity can be obtained if all the plant organs are studied separately or if the extraction is carried out with different solvents such as ethanol, acetone, n-hexane. In addition, since the physiological processes may be different in each cancer type, the concentrations used in our study should be studied in different cancer types to obtain more different results.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical Approval
Ethical approval was approved by the Bayburt University Non-Invasive Clinical Research Ethics Committee (Protocol No: 2023-7/2).

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