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Investigation of the cytotoxic effect of Lallemantia Fisch. & C.A. Mey. *species growing in Türkiye on various cancer cell lines*

Türkiye'de yetişen Lallemantia Fish. & C.A. Mey. *türlerinin çeşitli kanser hücre hatları üzerindeki sitotoksik etkisinin araştırılması*

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Abstract

The genus Lallemantia (Lamiaceae) is popularly known as "Dragonhead". The genus, which has been proven to have extremely rich compounds, is used in the treatment of many diseases among the people. The genus is represented by 5 taxa in the world and 3 taxa are naturally distributed in Türkiye. In this study, the cytotoxic properties of methanolic extracts of the above-ground parts of Lallemantia peltata, Lallemantia iberica and Lallemantia canescens taxa were investigated against BEAS-2B, SH-SY5Y, HCT 116 and Hep G2 cell lines. Changes in the viability of cancer cells were determined using the MTT method. The results showed that all three species had varying degrees of cytotoxic effect. This effect was maximum in L. canescens with 53.54% (p<0.001) on HCT 116, 37.53% (p=0.005) on SH-SY5Y and 32.03% (p=0.026) on Hep G2 at 800 µg/ml and minimum in L. iberica. In addition, the herbal extracts had no toxic effect on healthy lung cells (BEAS-2B).

Özet

Lallemantia cinsi (Lamiaceae) halk arasında "Ajdarbaşı" olarak bilinir. Son derece zengin bileşiklere sahip olduğu kanıtlanmış olan cins, halk arasında birçok rahatsızlığın tedavisinde kullanılmaktadır. Dünyada 5 taksonla temsil edilen cinsin 3 taksonu Türkiye'de doğal yayılış göstermektedir. Bu çalışmada, *Lallemantia peltata*, *Lallemantia iberica* ve *Lallemantia canescens* taksonlarının toprak üstü kısımlarının metanolik ekstraktının BEAS-2B, SH-SY5Y, HCT 116 ve Hep G2 hücre hatlarına karşı sitotoksik özellikleri araştırılmıştır. Kanser hücrelerinin canlılığındaki değişiklikler ise, MTT yöntemi kullanılarak tespit edilmiştir. Sonuçlar, her üç türün de değişen oranlarda sitotoksik etkiye sahip olduğunu göstermiştir. Bu etki; 800 µg/ml'de HCT 116 üzerinde %53.54 (p<0.001), SH-SY5Y üzerinde %37.53 (p=0.005) ve Hep G2 üzerinde %32.03 (p=0.026) ile bu etkinin *L. canescens*'te maksimum ve *L. iberica*'da minimum olduğu gözlendi. Ayrıca bitkisel ekstraktların sağlıklı akciğer hücreleri (BEAS-2B) üzerinde ise herhangi bir toksik etki yaratmadığı gözlemlendi.

INTRODUCTION

Cancer is a malignant tumour caused by uncontrolled division and proliferation of cells in an organ or tissue. Although cancer differs according to the region or organ where it is seen, nearly 100 types of cancer have been identified (Baykara 2016). According to the International Agency for Research on Cancer (IARC) GLOBOCAN database, 19.3 million new cancer cases were reported worldwide in 2020 and cancer is the leading cause of death worldwide with approximately 10 million (Bray and Møller 2006). According to the World Health Organisation, it is estimated that 17.5 million people may die from cancer and 27 million people may be diagnosed with cancer by 2050 (Siegel et al. 2023). Although some standard methods have been determined for the diagnosis and treatment of cancer patients, different approaches and treatment methods are applied specific to each cancer type (Baykara 2016). For this purpose, components obtained from medicinal plants are very important for the discovery and design of new therapeutic molecules with reduced side effects and high efficacy (URL-1).

The use of medicinal plants in traditional medicine is widespread and still leads to the development of new pharmacological agents. One of the common plant families traditionally used for this purpose is Lamiaceae and this family is rich in plants with various biological activities (URL-2). The genus *Lallemantia* Fisch. & C.A. MEY. belongs to the family Lamiaceae and has a total of 5 species, 3 of which are in Türkiye. *Lallemantia* is a medicinal plant from which essential oil can be obtained from leaves and herbaceous parts, mucilage can be extracted from seeds, both edible and industrial oil can be obtained, and all parts have economic use (Baykara 2016). In addition, it has been demonstrated by various studies that it has strong antimicrobial and antioxidant effects. Studies with different species of *Lallemantia* plants have shown that these species have high antimicrobial and antioxidant activity due to their high secondary metabolite content (Altın et al. 2021). On the other hand, there is no study in the literature on the cytotoxic activity of *Lallemantia* species growing abundantly in Türkiye.

In this study, it is aimed to fill the gap in the literature by comparatively investigating the cytotoxic activities of methanol extracts of flowers and leaves of *Lallemantia peltata* (L.) Fisch. & C.A. Mey., *Lallemantia iberica* (M.Bieb.) Fisch. & C.A. Mey. and *Lallemantia canescens* (L.) Fisch. & C.A. Mey. species, which have natural distribution in Türkiye. In line with this objective, we determined the cytotoxic activity of *Lallemantia* species on Human colon cancer (HCT 116), Human brain cancer (SH SY5) and Human liver cancer (Hep G2) cell lines as well as the toxic or proliferative effect on Human healthy lung (BEAS-2B) cell line. This study is the first study that investigate the cytotoxic effects of *Lallemantia* species grown in Türkiye.

MATERIAL AND METHOD

Collection of the Plant Material

The plant specimens were collected and identified from their natural habitats by M. Kursat and preserved under suitable conditions by drying in the shade. Detailed localities where the plants were collected, and images of the species are given below (Table 1 and Figure 1).

Table 1. Detailed location information of the studied taxa

	Species	Locality description	Date
1	*L. peltata*	B9, Bitlis, Bitlis Eren University, Rahva campus, northern slopes, 2600 m.	12.07.2022
2	*L. iberica*	B7, Elazığ, Baskil, Bolucuk village, 1480 m.	25.07.2022
3	*L. canescens*	B9, Bitlis, Mount Nemrut, steppes, 2290 m.	15.07.2022

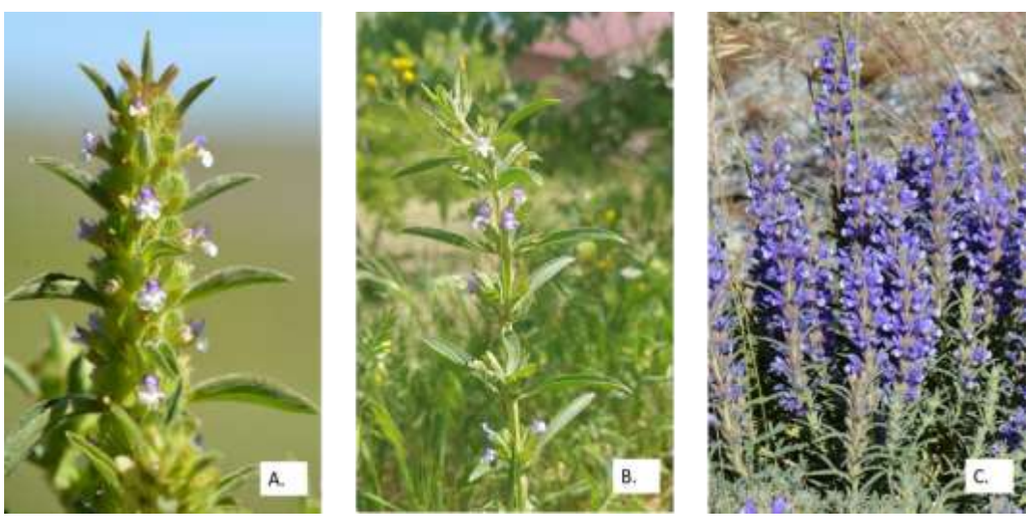

Figure 1. General appearance of *Lallemantia* species: (A. *L. peltata*, B. *L. iberica*, C. *L. canescens*)

Preparation of the Extract

For each species separately, leaf and flower parts of the plants were ground into powder and weighed on a precision balance, and extracts were obtained from 1 g of plant samples by the methanolic extraction method. For this purpose, approximately 1 g of plant sample was weighed and kept in 100 ml of 80% methanol in a shaking oven at 35°C for 72 h for extraction. Then the mixture was filtered using Whatman No 1 filter paper, and this process was repeated 3 times. The extracts were then poured into sterile petri dishes and dried in a sterile cabinet until the solvent evaporated. The dried extracts were dissolved in 99% dimethylsulfoxide (DMSO) (molecular grade) and the concentrations were adjusted with dulbecco's modified eagle medium (DMEM) and the stocks were stored at +4°C (Kirbag et al. 2021).

Cell Culture

Healthy lung cell line (BEAS-2B), Human brain cancer (SH-SY5Y), Human colon cancer (HCT 116) and Human liver cancer (Hep G2) cell lines were used in this study. For this purpose, cells were grown in 25cm² flasks in DMEM suplemented with 2.5 mM L-Glutamine, 1% Penicillin-Streptomycin and 10% fetal bovine serum (FBS) at 37˚C under 5% CO_2 atmosphere conditions. When the bottom of the flask was covered with at least 95% cells, the cells were taken into the experiment. A 0.25% trypsin-EDTA solution was used to remove the cells from the surface (Yener et al. 2018).

Determination of Cytotoxic Activity

96-well plates were seeded with 100µl medium containing 10^4 cells per well, and cell dilution was accomplished using standard medium. After that, it was incubated for 24 hours at 37˚C in with 5% CO_2. Following the incubation period, the wells' media was discarded and three replicates of the *Lallemantia* plant's methanolic extract (800, 400, 200, 100, and 50 µg/ml) were applied to the cells. Healthy lung cell line (BEAS-2B) was used as positive control and untreated cell lines were used as negative control. Then, the cells were incubated with 5% CO_2 at 37˚C for 72 hours. After the incubation period, 20µl (3-(4.5-Dimethylthiazol-2-yl)-2.5-Diphenyltetrazolium Bromide) MTT solution (5mg/ml) was added to the wells containing the cells and incubated at 37˚C for 4 hours in a dark environment. Following exposure, the medium was discarded and 100µl of DMSO added to resolve formazan crystal (Freimoser et al. 1999, Riss and Moravec 2004, van Meerloo et al. 2011, Van Tonder et al. 2015, Tekin et al. 2017). Then the colour change was measured by absorbance measurements at 570 nm wavelength with ELISA micro-plate reader (KHB ST-360) and % viability levels were calculated according to the following formula:

$$\%\ \textbf{viable cell} = \frac{\textbf{absorbance of the test samples} - \textbf{absorbance of the blank}}{\textbf{absorbance of control well} - \textbf{absorbance of the blank}} \times 100$$

Statistical Analysis

The study's findings were evaluated using SPSS statistical software (version 22.0 - IBM Corp. 2020). The details was displayed as mean ± SD. The numerical data's mean ± SD was obtained via the SPSS analyses. In this research, the dispersed data in the comparisons between control and tested groups was assessed by the student t-tests. P-values below 0.05 were regarded as statistically significant.

RESULTS

In this study, the cytotoxic activity of methanolic extracts of 3 species belonging to the genus *Lallemantia* growing naturally in Türkiye was tested against different cell lines.

L. peltata

The graph showing the cytotoxic activity of *L. peltata* on Healthy lung (BEAS-2B), Human brain cancer (SH-SY5Y), Human colon cancer (HCT 116) and Human liver cancer (Hep G2) cell lines is given below.

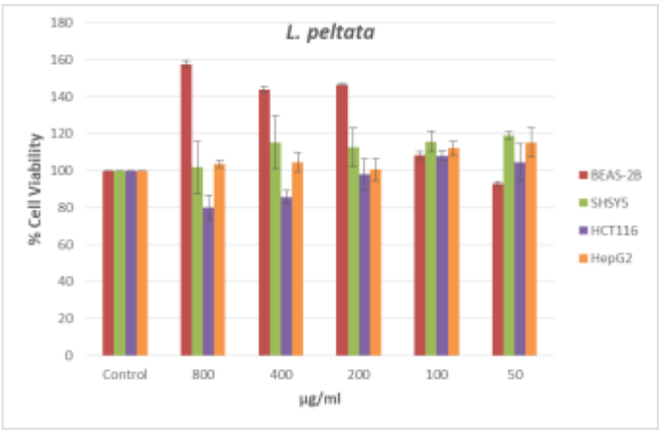

Figure 2. Viable cell percentage diagram of *L. peltata*

The methanolic extract of *L. peltata* shown a noteworthy cytotoxic effect on the human colon cancer-HCT 116 cell line. This effect showed the strongest effect at a concentration of 800 µg/ml with a 19.82% (p=0.036) cell death. Furthermore, cytotoxic effect was seen to diminish at a dose of 200 µg/ml, while the cell survival rate rose. In other words, the cytotoxic activity decreased with decreasing concentration and a cytotoxic activity directly proportional to the concentration was observed.

This effect is not significant in the same way on other cell lines. For example, high concentration samples of the plant extract (800-400 and 200 µg/ml) showed a strong proliferative effect especially on healthy lung cells, increasing the number of living cells by almost 60%-40%. According to the results obtained, the plant extract has no toxic effect on healthy cells, while this effect tends to decrease with decreasing concentrations.

In studies with human brain cancer (SH-SY5Y) and human liver cancer (Hep G2) cell lines, a proliferation of ~20% was observed on both. The plant extract served as a nutrient for the cell groups studied and was predicted to suppress cell proliferation and even kill the cells, but it was observed that it encouraged them to proliferate. The results obtained are shown in Figure 2 with the percentage of living cells.

L. iberica

The graph showing the cytotoxic activity of *L. iberica* on healthy lung (BEAS-2B), human brain cancer (SH-SY5Y), human colon cancer (HCT 116) and human liver cancer (Hep G2) cell lines is given below.

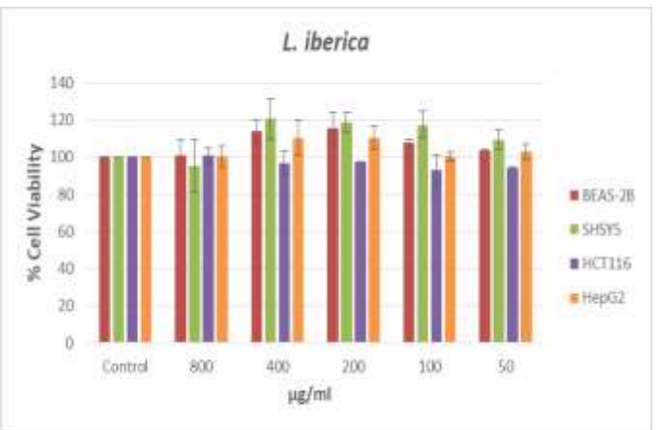

Figure 3. Viable cell percentage diagram of *L. iberica*

The cytotoxic activity of the methanolic extract of *L. iberica* on the cell lines studied has a weak cytotoxic activity on brain and colon cancer cell lines at 800 and 100 µg/ml with 4.73% and 6.82% mortality respectively. This weak effect persists in the colon cancer cell line at all concentrations, while the effect on brain cancer disappears with decreasing concentration. In addition, a proliferation was observed in both healthy lung cell lines and brain and liver cancer cell lines in parallel with the decrease in concentration, including 400 µg/ml. The plant extract acted as a nutrient for the cell groups studied and was predicted to suppress cell proliferation and even kill the cells, but it was observed that it encouraged them to proliferate. The results obtained are shown in Figure 3 with the graph of % living cell percentage.

L. canescens

The graph showing the cytotoxic activity of *L. canescens* on Healthy lung (BEAS-2B), Human brain cancer (shsy5), Human colon cancer (HCT 116) and Human liver cancer (Hep G2) cell lines is given below.

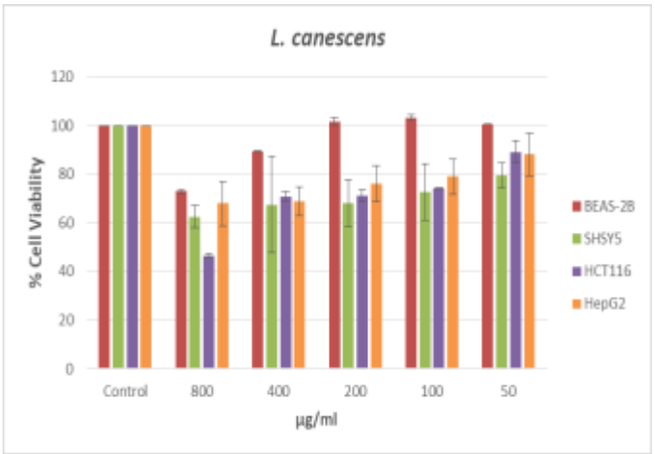

Figure 4. Viable cell percentage diagram of *L. canescens*

Among the plant groups studied, the plant extract with the strongest cytotoxic activity was *L. canescens,* which produced certain effects on all cell lines.

The human colon cancer (HCT 116) cell line was the subject of the most extensive cytotoxic action of the methanolic extract of L. canescens on the cell lines examined. This effect was realised at a concentration of 800 µg/ml with 53.54% (p<0.001) cell death. In second place is the brain cancer (SH-SY5Y) cell line with a cell death rate of 37.53% (p=0.005). This was followed by human liver cancer (Hep G2) with a cell death rate of 32.03% (p=0.026).

Although this effect of the methanolic extract of *L. canescens* on the cell lines studied decreased in parallel with the decrease in concentration, it still maintained its cytotoxic activity with a cell death rate of 20.47% on human brain cancer (SH-SY5Y) cell line and 10.88% on colon and liver cancer cell lines even at a concentration of 50 µg/ml.

The 800 µg/ml concentration of *L. canescens* also showed toxic effect in healthy cell group and killed 27.08% (p<0.001) of the cells. This effect decreased in parallel with the decrease in concentration and disappeared after 200 µg/ml concentration.

In addition, the cytotoxic activity observed after 200 µg/ml concentration decreased and the survival rate of the cells increased. In other words, the cytotoxic activity decreased with decreasing concentration and a cytotoxic activity directly proportional to the concentration increase was observed. The results obtained are shown in Figure 4 with the percentage graph of % viable cells.

DISCUSSION

Vitamins, polyphenols, carotenoids, and flavonoids are examples of secondary naturally occurring chemicals that have positive therapeutic or promoting impacts on human health. The primary biological activity of these compounds is attributed to their antioxidant properties (Badu-Gyan and Owusu 2017, Çakıroğlu and Uçar 2018). Over time, free radicals and oxidant substances build up in cells, damaging them by disrupting in normal cell cycle progression and metabolism. They can either encourage cancer or trigger cell death by damaging DNA by inhibiting the efficiency of the cell cycle regulatory mechanism (Devasagayam et al. 2003, Sarma et al. 2010). For this reason, determining the biological activity properties of plants that grow spontaneously in nature will be both a natural and environmentalist approach for future use as drug raw materials. One of the most often used methods for understanding the biological activity of natural products is to determine the cytotoxic activity of the plant (Wamidh and Mahasneh 2010).

In the literature review of the genus *Lallemantia,* no previous cytotoxic study on the genus was found. This study is the first study in this field. It has been proven by previous studies that the species belonging to this genus have a very rich phytochemical content (Sefidkon et al. 2006, Karami et al. 2017, Rahimi et al. 2019, Alan et al. 2019). In a study, 14 different flavones and two different flavonols were found in *Lallemantia* species (Jamzad et al. 2003). It is known that these components have antitumoral, antiviral, antithrombotic, anti-inflammatory, antiallergic, atherosclerosis, vasodilatation effects and protection against chronary heart diseases as well as antioxidant effects on living organisms (Kahraman et al. 2002). *Lallemantia* species may be a good source of bioactive substances due to their high flavonoid and phenol content (Semnani 2006).

The findings obtained from studies using *Lallemantia* species are as follows Rahimi et al. (2019) analysed the essential oil of *L. iberica* species collected from 5 different regions of Iran and reported that they had a rich content

of phenols and flavanoids. In addition, the essential oils of the members of the same species grown in different climatic and soil conditions were found to be different from each other in terms of quality. Sefidkon et al. (2006) analysed the essential oil of *L. peltata* and reported the main components of the oil as germacrene D (42.5% and 49.9%) and β-caryophyllene (20.6% and 26.0%). Alan et al. (2019) determined that *L. peltata* and *L. canescens* plant extracts have rich antioxidant content, in parallel with this effect, plant extracts exhibited in vitro antioxidant activity close to standards. In addition, it was determined that the extracts showed stronger antimicrobial effect especially on fungi but had limited DNA protective activity. Karami et al. (2017) observed that the seed extract of *L. iberica* has antibacterial effect on various microorganism groups. Başak and Candan (2008) examined the antioxidant properties of methanol extracts obtained from tissue culture samples of *L. canescens* and found that the samples have antioxidant activity by scavenging free radicals and inhibiting lipid peroxidation.

As a result of our study, the strongest cytotoxic activity was found to belong to the extract obtained from *L. canescens* species. According to the findings obtained, it was observed that the extract had cytotoxic activity against 3 cell lines, but this effect varied according to the cells and the dose applied. At the same time, high doses of the plant extract showed toxic effect in healthy cells. This is a disadvantage, but with decreasing concentration, this effect disappears and cytotoxic activity continues. The literature records of *L. canescens*, which showed activity on all cell lines tested, also showed that it contains very dense flavanoids and sesquiterpenes. In this respect, the cytotoxic activity of *L. canescens* extract may be thought to be due to secondary metabolites such as flavanoids and sesquiterpenes.

L. peltata was active only on HCT 116 cell line at 800 and 400 µg/ml concentrations. *L. iberica* had the lowest cytotoxic activity with ~5-10% cell death rate against HCT 116 only at all concentrations.

CONCLUSIONS AND RECOMMENDATIONS

Herbal extracts have demonstrated variable rates of anticancer and antitumor effects on cancer cell lines. In this study, for the first time, a cytotoxic study was carried out on *Lallemantia* species with natural distribution in Türkiye. The data obtained will contribute to the development and design of new anticancer and antitumour drugs, especially in the field of cancer research and pharmacology, and will be the basis for the establishment of larger-scale projects.

Additionally, it is unclear the type, quantity, and effects the biocompounds in species. Therefore, further chemical and molecular tests are required. The therapy with extracts from the *Lallemantia* species shown a strong cytotoxic impact on cancer cells and was found to be a natural growth inhibitor in accordance with the results of this research. Its effects in vivo, however, are still unknown. Therefore, further research should be done to clarify its impacts on organisms and introduce them to the fields of science and medicine.

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