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Anticancer activity of bee venom components against lung cancer

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ABSTRACT

This study aims to determine the effects of bee venom on the proliferation capacity of Calu-3 cells and the migration ability of the cells. For this purpose, bee venom samples were collected from *Apis mellifera anatoliaca* in Muğla (Türkiye) provinces and Calu-3 cells were exposed to this bee venom. A test for cell viability using Calu-3 given bee venom in varied doses (20 µg/mL, 18 µg/mL, 15 µg/mL, 12 µg/mL, 10 µg/mL, 9 µg/mL, 7.5 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.625 µg/mL and 0.312 µg/mL) was conducted. And scratch assay was performed on cells treated with the doses (15 µg/mL, 10 µg/mL, 7.5 µg/mL, 1.25 and 0.312 µg/mL) and imaged every two hours for 24 hours. According to the results of our study's cell proliferation and scratch assays, bee venom had a cytotoxic and proliferative effect on Calu-3 cells which had a dose-dependent cytotoxic and proliferative effect. The study's outcomes how crucial dosage adjustment is in the use of bee venom in lung cancer studies due to its cytotoxic effect. Even though we have achieved a better understanding of how bee venom components work, our knowledge might still be improved by looking at how bee venom affects Calu-3 cells when combined with other substances or by developing the purification method for bee venom.

Arı zehiri bileşenlerinin akciğer kanserine karşı antikanser etkisi

ÖZET

Bu çalışma ile arı zehrinin Calu-3 hücrelerinin proliferasyon kapasitesi ve hücrelerin migrasyonu yeteneği üzerindeki etkilerini belirlemeyi amaçlanmaktadır. Bu amaçla, Muğla ilinde yetiştirilen anadolu bal arısı (*Apis mellifera anatolica*) kolonilerinden arı zehri örnekleri toplanmıştır ve Calu-3 hücreleri bu arı zehirine maruz bırakılmıştır. Değişen dozlarda (20 µg/mL, 18 µg/mL, 15 µg/mL, 12 µg/mL, 10 µg/mL, 9 µg/mL, 7,5 µg/mL, 5 µg/mL, 2,5 µg/mL, 1,25 µg/mL, 0,625 µg/mL ve 0,312) arı zehri verilen Calu-3 kullanılarak hücre canlılığı testi gerçekleştirilmiştir. Çeşitli dozlarda (15 µg/mL, 10 µg/mL, 7,5 µg/mL, 1,25 µg/mL ve 0,312 µg/mL) arı zehrine maruz bırakılan Calu-3 hücrelerinde yara iyileştirme deneyi yapılmış ve hücreler 24 saat boyunca her iki saatte bir görüntülenmiştir. Çalışmamızın hücre proliferasyonu ve yara iyileşmesi deneyi sonuçlarına göre, arı zehirinin Calu-3 hücreleri üzerinde doza bağlı olarak değişebilen sitotoksik ve proliferatif etkisi olduğu görülmüştür. Çalışma, akciğer kanseri çalışmalarında arı zehrinin sitotoksik etkisinden dolayı kullanımında doz ayarlamasının ne kadar önemli olduğunu ortaya koymaktadır. Arı zehiri bileşenlerinin nasıl çalıştığını daha iyi anlamış olsak da arı zehirinin diğer maddelerle birleştirildiğinde Calu-3 hücrelerini nasıl etkilediğine bakılarak veya arı zehiri için saflaştırma yöntemi geliştirilerek arı zehri hakkında bilinenler geliştirilebilir.

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1. Introduction

Honeybees, which are hymenopteran insects falling under the Apis genus, are renowned for their production and storage of valuable substances, including honey and various chemicals beneficial to humans (1). These bee-derived products used for human purposes encompass honey, propolis, bee pollen, bee bread, beeswax, and bee venom.

Bee venom (BV), also referred to as apitoxin and secreted by bee venom glands, is one of these bee products with a broad range of biological functions. It is a transparent and scentless liquid featuring a mixture of proteins with an acidic pH ranging from 4.5 to 5.5. Bees employ BV as a defense mechanism against potential threats. BV consists primarily of water, with a mere 0.1 gram of dry venom per drop (2). Its composition includes peptides like melittin, adolapin, apamin, and mast cell degranulating peptides. Additionally, it contains enzymes, notably Phospholipase A2, as well as low-molecular-weight compounds such as bioactive amines like histamine and adrenaline, along with minerals (3).

Since research on apitherapy began in the early 20th century, BV has been found to have numerous therapeutic applications for various diseases. Thanks to its anti-inflammatory properties, different forms of traditional BV therapy have been employed to alleviate pain and manage chronic inflammatory conditions like rheumatoid arthritis and multiple sclerosis (4). Moreover, BV has also been explored for its potential in neurodegenerative diseases such as Parkinson's Disease and Alzheimer's Disease (3). Beyond medicinal use, bee products have found applications in cosmetics (5,6). Besides all these, BV has been widely used in the treatment of tumors. BV peptides like melittin and phospholipase A2 have the potential to target various cancer cell types for anticancer or antimetastatic effects, including those found in the lung, kidney, liver, prostate, bladder, breast (mammary), and even leukemia (7,8,9).

Lung cancer remains the leading cause of cancer-related deaths worldwide despite significant advances in technology and treatment options. Among the various subtypes of primary lung cancer, adenocarcinoma, which arises from the body's mucus-producing cells, is the most commonly diagnosed. In fact, it accounts for approximately 40% of all lung cancer cases (10). Adenocarcinoma is known for its high aggressiveness and is often diagnosed in advanced stages, resulting in lower survival rates. Treatment of adenocarcinoma poses several challenges as it tends to be resistant to conventional treatments such as radiotherapy and chemotherapy. Overcoming these barriers often requires the integration of more than one therapeutic approach (11). We have concentrated our research on the Calu-3 cell line as our preferred in vitro model for studying the response of the airway epithelium against BV. It is believed that the Calu-3 cell line, which originates from human bronchial epithelium, possesses characteristics resembling serous cells (12). It has emerged as a widely used model for investigating a variety of aspects, including cellular responses to oxygen and ventilator-induced injuries, viral infections, bronchial epithelium-specific functional barrier properties, and drug transporters (13, 14, 15). In this study, we investigated the toxicity capacity of BV on Calu-3 cells exposed to different doses of BV. It is thought that determining the dose-dependent effect of BV on Calu-3 cells, which has not been determined before, will be advantageous in facilitating lung cancer treatment in both veterinary medicine and human medicine.

2. Material and Methods

Bee venom collection, preparation and determination

The bee venom used in the study was collected in September 2021 from bee colonies in a producer-owned apiary (BeeSas Beekeeping) in Muğla, Türkiye. A sharp lancet was used to scrape the glass plates off after the bees' venom had dropped on them in order to collect the BV. The BV was freeze-dried and then stored until analysis in a freezer at 20 °C. The Muğla Sıtkı Koçman University Food Analysis Application and Research Center examined the BV content. Component analysis of apamine, phospholipase A2, and melittin was performed using an Agilent 1260

HPLC with a binary pump and degasser unit, together with an Agilent 1200 Series autosampler, autoinjector, column oven, and variable wavelength detector.

Cell viability assay

The effect of BV on the cytotoxicity of Calu-3 cells was investigated using methyl thiazolyl tetrazolium (MTT) as a substrate. Calu-3 cells were seeding density in 96-well plates at a density of $5x10^3$ cells per well. Various BV concentrations were administered to cells for 24 hours, including 20 µg/mL, 18 µg/mL, 15 µg/mL, 12 µg/mL, 10 µg/mL, 9 µg/mL, 7.5 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.625 µg/mL, and 0.312 µg/mL. Cells were washed with fresh media and 100 µL of MTT (5 mg/mL) was added to the wells following incubation. After the formazan salt produced after 4 hours of incubation was solubilized with sodium, the number of formazan salts at 570 nm was counted using a microplate reader (Sunrise, Tecan GmbH, Austria).

Scratch assay

A density of between 70% and 80% confluence was attained after 24 hours of cell culture incubation of $10x10^4$ Calu-3 cells in 6-wells. The monolayer cells were scraped across the center of the well with a 1 mL pipette tip. PBS was used to gently wash the cells twice. Five different BV concentrations (15 µg/mL, 10 µg/mL, 7.5 µg/mL, 1.25 and 0.312 µg/mL) were given to fresh medium. Under the conditions of cell culture, cells were incubated for 24 hours. Cells were imaged every two hours and Leica Application Suit software (10X) was used to measure the size of every group's wound size.

Statistical analysis

A two-way analysis of variance was utilized to assess the impact of BV concentrations on the outcomes of the MTT analyses. The factors that were determined to be significant underwent an advanced examination known as the Tukey test. Data were analyzed using the GraphPad Prism program (10th version), and they are shown as mean standard deviation. The statistical significance was denoted by the following symbols: * P 0.05, ** P 0.01, *** P 0.001, and **** P 0.0001.

3. Results

Determination of bee venom

The percentages of apamin, phospholipase, and melittin in the sample of BV were determined. The quantities of apamin, phospholipase, and melittin in BV were estimated by HPLC-VWD to be 4.05%, 14.36%, and 70.98%, respectively.

Cell viability assay

Cell viability test was performed on Calu-3 cells exposed to 20 µg/mL, 18 µg/mL, 15 µg/mL, 12 µg/mL, 10 µg/mL, 9 µg/mL, 7.5 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.625 µg/mL and 0.312 µg/mL concentrations of BV. The absorbance values that correspond to the 1000, 2000, 4000, 6000, 8000, 10000, and 12000 Calu-3 cell numbers were used to build a calibration curve. The cell numbers have been calculated using the calibration curve. The group exposed to BV at a concentration of 1.25 µg/mL had the greatest number of Calu-3 cell viability, as was found from the results of the viability tests. There was a significant difference in the number of cells in the 1.25 µg/mL group compared to the control group; however, no such variation was observed among the 0.312 µg/mL, 0.625 µg/mL, or 5

µg/mL groups. Between the groups exposed to 7.5 µg/mL and higher concentrations of BV and the 1.25 µg/mL group, there is a statistically significant difference. In addition, cell cytotoxicity considerably rises when BV concentrations reach 12 µg/mL and above.

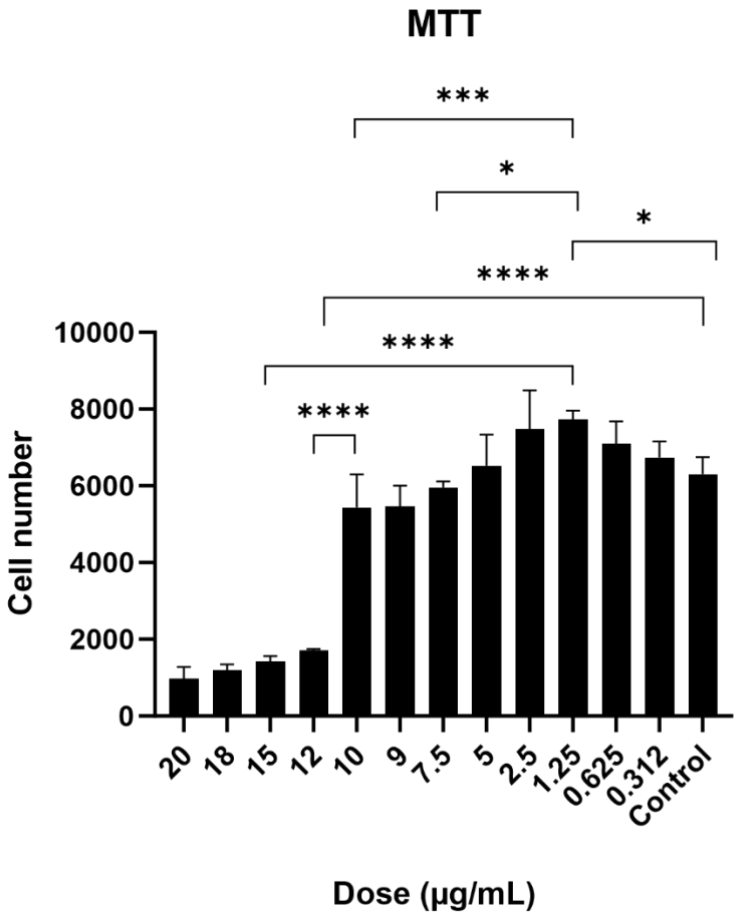

Figure 1: Calu-3 cells numbers exposed to 20 µg/mL, 18 µg/mL, 15 µg/mL, 12 µg/mL, 10 µg/mL, 9 µg/mL, 7.5 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.625 µg/mL and 0.312 µg/mL concentrations of BV obtained from the absorbance values of the MTT test (* P < 0.05, *** P < 0.001, **** P < 0.0001).

Şekil 1: *MTT testinin absorbans değerlerinden elde edilen arı zehirinin 20 µg/mL, 18 µg/mL, 15 µg/mL, 12 µg/mL, 10 µg/mL, 9 µg/mL, 7.5 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.625 µg/mL and 0.312 µg/mL konsantrasyonlarına maruz kalan Calu-3 hücreleri sayıları (* P < 0,05, *** P < 0,001, **** P < 0,0001).*

Scratch assay

Scratch assay was performed on Calu-3 cells exposed to 15 µg/mL, 10 µg/mL, 7.5 µg/mL, 1.25 µg/mL and 0.312µg/mL concentrations of BV. At the end of 24 hours, the wound line was not completely closed in any group, including the control group. In the group exposed to 1.25 µg/mL BV, the wound line closed relatively more than the other groups. In the 0.312 µg/mL, 7.5 µg/mL, and 10 µg/mL groups, the wound line closure levels were near to one

another. In the 15 µg/mL group, it was observed that at the end of the 2nd hour, the cells were observed to lose their cytoplasmic extensions and were thought to be progressing towards apoptosis.

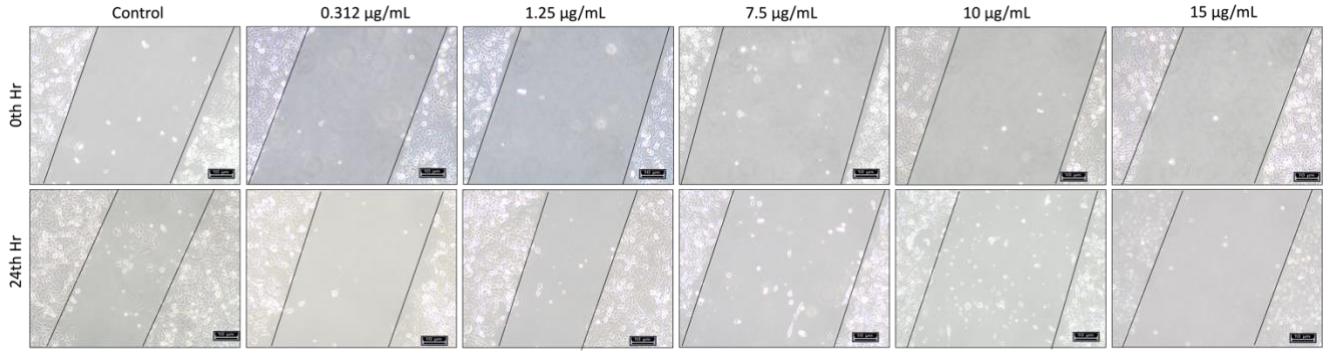

Figure 2: Scratch assay results of 15 µg/mL, 10 µg/mL, 7.5 µg/mL, 1.25 µg/mL and 0.312 µg/mL and control groups.
Şekil 2: *15 µg/mL, 10 µg/mL, 7,5 µg/mL, 1,25 µg/mL ve 0,312 µg/mL ve kontrol gruplarının yara iyileşmesi analizi sonuçları.*

4. Discussion and Conclusion

Due to its anticancer effects and also anti-inflammatory, antioxidant, antifungal, antiviral, antibacterial, and analgesic qualities BV is the most investigated venom among other arthropod venoms (7,9). Numerous research conducted both in vitro and in vivo have reported that BV affects the cell cycle, angiogenesis, apoptosis, cytotoxicity, and metastasis of cancerous cells (3, 16, 17, 18, 19, 20). In this study, we investigated the anticancer effect of BV on the Calu-3 cells. We especially focused on which doses of BV are more effective in damaging Calu-3 cells. The following doses were chosen for this purpose: 20 µg/mL, 18 µg/mL, 15 µg/mL, 12 µg/mL, 10 µg/mL, 9 µg/mL, 7.5 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.625 µg/mL, and 0.312 µg/mL (13).

Cell proliferation and scratch assay results of our study have shown that BV has both proliferative and cytotoxic effects on Calu-3 cells. It is found that these effects depend on the range of the doses. BV had a statistically significant proliferative effect on Calu-3 cells when applied at 1.25 µg/mL however, it showed a certain cytotoxic effect at doses of 12 µg/mL and above. Between the control group and the remaining doses, there is no noticeable difference. In a study investigating the effect of BV treatment on A549 human lung cancer cells, viability tests were performed using BV doses of 0.5 µg/mL, 1 µg/mL, 5 µg/mL, 10 µg/mL and 20 µg/mL. A comparable explanation for our findings was provided by stating that BV had a dose-dependent effect and that A549 cells were not toxically affected by BV at a concentration of 1 µg/mL (21). Zhang et al. showed that BV inhibits blood vessel formation to reduce tumor invasion. However, they showed that this effect would be significantly effective after the 4mg/ml dose and above. It has been indicated that the use of BV in lower doses is no different from the control group in the treatment of small-cell lung cancer, similar to our study (22). Research on cancers other than lung cancer, including pancreatic, colon, and hepatocellular carcinoma, reported no significant difference in response to a 1 µg/mL BV dose when compared to control groups (23,24,25). In the study conducted on lung cancer cells, the effects of BV were investigated and indicated that BV increases the production of reactive oxygen species. In the study where doses ranging from 0.5 µg/mL to 8 µg/mL were selected, it was shown that BV causes mitochondrial damage and apoptosis known as ferroptosis, in every dose. They demonstrated that the wound line closed over time in the BV-exposed cells (1 µg/mL) group than in the

control group, indicating that BV inhibited the migration of lung cancer cells (26). In addition to all these, there are also some studies conducted on healthy cells such as mesenchymal stem cells, showing that the effect of BV on cells is dose-dependent (27). Other studies examining the effects of BV on different cancer cell lines such as lung, breast, leukemia and cervical cancer have also revealed similar findings that BV is cytotoxic on cancer cell lines even at 0,5-1 µg/mL doses (28,29,30,31,32,33). This is believed to be caused by variations in the BV isolation technique or the lung cell line that was employed.

According to the results of our study's cell proliferation and scratch assays, BV had a cytotoxic and proliferative effect on Calu-3 cells which is capable of varying based on dosages. The study's outcomes how crucial dosage adjustment is when employing BV due to its cytotoxic effect in lung cancer studies. Even though we have achieved a better understanding of how BV components work, our knowledge might still be improved by looking at how BV affects Calu-3 cells when combined with other substances or by developing the purification method for BV.

Conflict of Interest

The author declared that there is no conflict of interest

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Authors' Contributions

Motivation / Concept: Özge ÖZGENÇ ÇINAR
Design: Özge ÖZGENÇ ÇINAR
Control/Supervision: Özge ÖZGENÇ ÇINAR
Data Collection and / or Processing: Özge ÖZGENÇ ÇINAR
Analysis and / or Interpretation: Özge ÖZGENÇ ÇINAR
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Writing the Article: Özge ÖZGENÇ ÇINAR
Critical Review: Özge ÖZGENÇ ÇINAR

Ethical Statement

An ethical statement was received from the authors that the data, information, and documents presented in this article were obtained within the framework of academic and ethical rules and that all information, documents, evaluations and results were presented in accordance with scientific ethics and moral rules

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