

Research Article



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Cytotoxic and apoptotic effectiveness of Cypriot honeybee (*Apis mellifera cypria*) venom on various cancer cells

<https://doi.org/10.1515/tjb-2023-0109>

Received July 13, 2023; accepted February 9, 2024;

published online April 11, 2024

Abstract

Objectives: The bee stinger is the defense organ of honeybees. The venom sac of a worker bee is connected to its stinger, which is used as a defense mechanism, and it has a potent and complex combination of substances that is unique in the animal kingdom. Many immune-related illnesses have been successfully treated with bee venom and recent evidence on the efficacy of applications targeting malignancies has attracted considerable attention.

Methods: The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) test was used to determine the cytotoxicity of the crude venom, and the flow cytometric analysis was used to determine the apoptotic potential. The cytotoxic activity of *Apis mellifera cypria* venom collected from two different apiaries in Cyprus was evaluated for the first time against breast (MDA-MB-231), colon (Caco-2), cervix (HeLa), prostate (PC-3), pancreas (Panc-1), lung (A549), glioblastoma (U-87MG) human cancerous and healthy lung fibroblast (CCD-34Lu) cells.

Results: The venom concentration that killed 50 % of the cells (inhibitory concentration, IC_{50}) is expressed as venom cytotoxicity. The IC_{50} values of *A. m. cypria* crude venom on cultured cells varied from 4.18 ± 0.75 to 22.00 ± 1.71 $\mu\text{g/mL}$ after treatment with crude venom for 48 h, with the most potent activities against PC-3, Panc-1, and HeLa cells. Analysis of apoptotic cells by flow cytometry of both venom samples

showed that bee venom slightly induced early apoptosis on A549 and Panc-1 cells.

Conclusions: The venom of the *A. m. cypria* is discussed in this article, displaying promising results as a potential source for an alternative treatment method because of its cytotoxic effect.

Keywords: *Apis mellifera cypria*; cytotoxicity; apoptosis; Cypriot honeybee; venom

Introduction

Cancer can affect any part of the body and is caused by genetic mutations that disrupt the normal regulation of cell growth. It is a complex disease with various types and subtypes, each requiring different treatment approaches. According to projections, there will be roughly 1,918,030 new cancer diagnoses in the USA in 2020, or 5,250 cases a day [1]. Cancer detection and therapy have been negatively impacted by the emergence of coronaviruses in 2019. Traditional anticancer therapies include chemotherapy to trigger the apoptosis of cancer cells as well as surgical therapy to remove tumors; however, the efficacy, safety, and side effects of these treatments remain problematic. For the development of novel medicines, natural compounds are a promising source because they exhibit a broad range of biological activities and have anticancer effects through interaction with different signaling pathways to inhibit the growth of tumors. As a result, it is crucial to develop new cancer treatment approaches [2]. This growing interest in natural products is primarily due to their potential in developing new drugs and therapies. By studying these compounds with few negative side effects, scientists hope to discover novel treatments for various diseases and conditions [3]. Natural products are considered a rich source of bioactive compounds with therapeutic potentials due to the vast chemical diversity in nature [4]. Because they have a higher safety margin, are less expensive, and have a wider range of bioactivity than synthetic medicines, natural therapies like apitherapy are currently given favor [5].

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Apitherapy is a complementary therapy that uses honeybee products, most significantly bee venom, to treat a variety of human illnesses [6]. Due to its abundance of bioactive compounds including melittin and phospholipase A2 (PLA2), bee venom (BV) is one of the most extensively investigated natural extracts in recent years due to its broad range of biological activities that may serve as a foundation for the development of novel therapies [3].

Melittin is derived from bee venom and is known for its potent antimicrobial and anticancer properties. Its amphipathic nature enables it to insert into the lipid bilayer of cell membranes, disrupting their integrity and leading to cell lysis. With greater anti-tumor activity in MCF-7 cells, honeybee venom is effective against HT-29 colon and MCF-7 breast cancer cells. Both on its own and in conjunction with chemotherapy or other medications that have been repurposed, honeybee venom has been shown to have considerable bioactive potential for the treatment of colon and breast cancer [7]. Wang et al. examined the mechanism behind melittin's cytotoxic effect and showed that melittin can cause hepatocellular carcinoma cells (HCC) to go through apoptosis by activating the Ca^{2+} /calmodulin-dependent protein kinase-II, transforming growth factor-beta-activated kinase-1 and c-Jun N-terminal kinase (CAMKII-TAK1-JNK/p38) signaling pathway. Additionally, melittin is capable of rendering tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-resistant HCC cells sensitive to TRAIL-induced apoptosis, most likely via activating the CAMKII-TAK1-JNK/p38 pathway and blocking the I κ B α kinase-NF κ B pathway [8].

Recently, medical practitioners and certified apitherapists have started using honeybee venom to treat patients with autoimmune and chronic disorders. Lab research and clinical trials have proved the effectiveness of honeybee venom as a biotherapy [6]. Since ancient times, BV has been used to treat autoimmune illnesses [9], Lyme disease [10], and chronic fatigue syndrome in addition to treating back pain, rheumatoid arthritis [11], and skin ailments. In some studies, honeybees have also been proven to be effective in treating multiple sclerosis [12], influenza [13], fibromyalgia, and postherpetic neuralgia [14]. Recently, bee venom has been investigated as a potential cancer treatment [3, 15–17]. In recent studies, it has been reported that bee venom and its components have anti-neurodegenerative, anti-inflammatory, anti-nociceptive, anti-cancer, anti-bacterial, anti-parasitic and immunotherapeutic effects [18]. The literature suggests that, in addition to its curative properties, bee venom may lessen the negative effects of other types of pharmaceuticals and conventional drugs [6]. Honeybees are social insects that spend their entire life completely dependent on the environment. Similar to other bee products like honey, pollen, propolis, bee bread, apilarnil, royal

jelly, and bee wax, the quality of bee venom depends on environmental conditions. The amount and content of bee venom may vary depending on the variety of pollen sources, seasonal differences, collecting season, bee races, and method of collection. Pollen sources are directly affected by the protein components of bee venom. Additionally, environmental temperature affects the protein quality profile of bee venom. The ideal temperature for bee venom's high protein diversity ranges from 33 °C to 36 °C [19].

Because of its isolated region, Cyprus is considered an important island for beekeeping as it is carried out in areas far from industrial pollution. Besides, it is also home to the Cyprus honeybee (*Apis mellifera cypria*), whose homeland is Cyprus. According to Pollman [20], the honeybees of Cyprus belong to a distinct subspecies called *A. m. cypria*. Although there is a limited number of studies on the genetic characterization of *A. m. cypria*, according to morphological analysis it is observed that *A. m. cypria* is more similar to *Apis mellifera syriaca* and *Apis mellifera anatoliaca* than *Apis mellifera adami* and *Apis mellifera meda* [21]. *A. m. cypria* is a small and dark-colored bee breed known for its aggressive character, Supplement Figure 1A and B. It is especially important to protect the pure breed of *A. m. cypria* which is exposed to crossbreeding due to its aggressive nature. Aggressive bees produce more protein in their venom, therefore, they are extremely important in medical fields for apitherapeutic purposes.

Considering the great potential of bee venom, the main goal of this research was to evaluate the cytotoxic and apoptosis-inducing effects of *A. m. cypria* venom on a variety of cancer cells and to assess its potential as a therapeutic agent.

Materials and methods

Bee venom

All tests were performed with the collected venom of *A. m. cypria* from two different apiaries in Cyprus (Mallidag (Magosa) Village and Lefkosa Center). Venom samples were collected using the electroshock method as primarily described by Markovic & Mollnar [22]. The electroshock method is considered the safest bee venom collection method [23]. The electroshock machine consists of a wire grid placed in the hive, a glass surface fixed to the bottom of the grid, and a compartment where the venom will be collected. In this system, electric current supplies the hive at intervals as an external threat, the honeybee injects its venom by injecting its stinger into the glass surface when it comes into contact with the wire grid. The collection process with this method lasts a maximum of 30 min per hive and does not harm the bees [23]. Venom is then collected on a glass surface and scraped off the surface.

The collected samples are lyophilized and kept dry at +4 °C in amber bottles. For the cytotoxicity study, the lyophilized venom was dissolved in sterile physiological saline as a stock solution (2 mg/mL).

Morphometric analysis

For the morphometric analysis, 15–20 individuals from each hive were measured in this study. Twenty landmarks on the right-side forewings were digitized, Supplement Figure 2. The forewings of worker bees were dissected and prepared on slides and their photos were taken under a BAB-STR45 microscope (BAB Ltd., Ankara, Turkey) for geometric morphometric analysis. Tps data files were prepared using tpsUtil64 and landmarks were digitized on the images using tpsDig264. Canonical variate analysis (CVA), Procrustes ANOVA test, and discriminant function analysis (DFA) were performed with the MorphoJ program.

Protein content determination

Protein content was determined using the bicinchoninic acid (BCA) assay (Pierce Kits, ThermoScientific, Bremen, Germany). Protein content was determined for each diluted venom sample (1 mg/mL) using the Smith method at a wavelength of $\lambda=562$ nm via an ultraviolet (UV) – visible spectrophotometer (Thermo, Bremen, Germany) [24]. The assay relies on the color changing of the sample from green to purple. This change occurs proportionally with the total protein concentration, so the amount of protein in a solution is measured using colorimetric techniques.

Cell culture

The cytotoxic potential of *A. m. cypria* venom collected from two different apiaries in Cyprus was demonstrated against human cancerous cells; colon adenocarcinoma (Caco-2), lung adenocarcinoma (A549), breast adenocarcinoma (MDA-MB-231), cervix adenocarcinoma (HeLa), glioblastoma-astrocytoma (U87-MG), prostate adenocarcinoma (PC-3), pancreas epithelioid carcinoma (Panc-1) and healthy lung fibroblast (CCD-34Lu) cells. The cell lines used for the experiment are purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). The cells are maintained in Dulbecco's modified Eagle's medium F12, containing 10 % heat-inactivated fetal bovine serum, 0.1 % penicillin/streptomycin, and incubated at 37 °C in a humidified atmosphere of 5 % CO₂. The cells were subcultured twice a week.

In vitro cytotoxicity assay

The colorimetric 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay is used to assess the cytotoxicity of the crude venom [25]. The purpose of the MTT assay is to measure viable cells. The MTT assay is aimed to determine the number of viable cells by measuring mitochondrial reductase activity. The mitochondrial activity is determined by the conversion of tetrazolium salt (MTT) into formazan crystals, and then dimethyl sulfoxide is added to the wells to resolve the crystals. Thus, by measuring the optical density (OD) of the wells in the 96-well plates, the increase or decrease of the viable cells is detected [26].

In 96 well microplates, each cell line is grown for 24 h at a starting density of 10⁵ cells/mL. Then, each cell was treated with three different

concentrations of venom (50, 5, 0.5 µg/mL) and incubated for 48 h at 37 °C. After the incubation period, the culture medium is removed and 20 µL of MTT (from 2.5 mg/mL stock solution) is added to the wells and the plates are incubated for 4 h at 37 °C. After 4 h, 150 µL of a solubilizing solution, dimethyl sulfoxide is added to the wells and OD is measured at $\lambda=570$ nm by UV visible spectrophotometry (Thermo, Bremen, Germany). The cell viability (%) is determined using the below formula, Eq. (1):

Viability %

$$= \frac{(\text{absorbance of treated cells}) - (\text{absorbance of untreated cells})}{(\text{absorbance of control}) - (\text{absorbance of untreated cells})} \times 100 \quad (1)$$

Morphological studies

The morphological examinations of the cells are carried out using an inverted microscope following 48 h of treatment (Zeiss, Germany).

Determination of inhibitory concentration (IC₅₀) and statistics

Cytotoxicity is measured as an increase in the mean percentage of cytotoxicity in comparison to the unexposed control $\pm$ SD. Cytotoxicity control levels are set at zero percent. A four-parameter logistic model is used to fit the data to a sigmoidal curve and determine the concentration of the venom that inhibits 50 % of cell growth (IC₅₀). The result is displayed as the average of three different measurements. Calculations were performed using GraphPad prism 8.4.2 software (GraphPad 8.4.2, San Diego, CA, USA) and the IC₅₀ values were reported at a 95 % confidence interval.

Flow cytometry analysis for apoptosis

The apoptotic cells are measured by a flow cytometer using the FITC Annexin V – PI Apoptosis Detection kit (BD, SanJose, CA, USA). The presence of apoptotic cells was assessed based on the exposure of phosphatidylserine residues. Since lung and pancreas cancers are among the most common and most lethal cancers in the world, A549 and Panc-1 cell lines were selected. Cell lines are seeded in a 6-well plate at a density of 5×10^4 cells/well and cultured for 24 h at 37 °C with 5 % CO₂. Cells are then treated with 50 % IC₅₀ and IC₅₀/2 for 48 h. After 48 h, cells are observed with an inverted microscope (Zeiss, Germany). The cells are then harvested with 1 $\times$ trypsin, collected in 2 mL Eppendorf tubes, and washed twice with cold PBS using centrifugation for 5 min at 1,000 rpm at 4 °C. Carefully resuspended cells are placed in 100 µL of 1 $\times$ Annexin V binding buffer. Samples are then analyzed using a flow cytometer (BD Accuri™ C5, CA, USA).

SDS-PAGE analysis

SDS-PAGE was performed in order to separate the complex mixture of bee venom proteins by their molecular weight and to control their purity. 4 % stacking gel for SDS-PAGE gel (15.1 g Tris/HCl pH 6.8, 0.34 mL PAA, 25 µL SDS, 1.50 mL ultrapure water, 12.5 µL ammonium persulfate, 10 µL TEMED) and 15 % resolving gel (250 mM Tris/HCl pH 6.8, 2.50 mL PAA, 50 µL SDS, 1.17 mL ultrapure water, 25 µL ammonium persulfate,

10 μ L TEMED) were used. Samples were loaded in the presence of Laemmli buffer and visualized by staining the separated proteins in the gel with Coomassie blue.

Statistical analysis

Statistical analyses were performed by using SPSS for Windows 10.0 and GraphPad Prism 8.4.2 statistical analysis programs. The results were compared according to the control group using the one-way ANOVA test and adjusted using Dunnett's analysis. Values were expressed as mean $\pm$ SD. A value of $p < 0.05$ was considered statistically significant.

Results

Morphometric analysis

Samples taken from Cyprus Mallidag Village and Lefkosa Center were compared to samples taken from 43 provinces from all over Türkiye (unpublished data). According to the results, comparisons of Lefkosa – İzmir, Lefkosa – Aydın, Lefkosa – Rize, Lefkosa – Şanlıurfa were not significant for Procrustes distances ($p > 0.0001$) which is used to determine absolute shape differences. Comparisons Mallidag – Aydın, Mallidag – Balıkesir, Mallidag – Bursa, Mallidag – Düzce were not significant for Procrustes distances ($p > 0.0001$). Comparisons Lefkosa – Mallidag were significant for Procrustes distances ($p < 0.0001$). Results showed that Lefkosa and Mallidag are two completely different populations in Cyprus. Morphometric analysis showed that honeybee samples taken from two different locations in Cyprus could be influenced by other subspecies like *A. m. anatolia* as stated by Kandemir et al. [21]. Besides, it is important to determine different morphometric characters in the samples taken from these isolated regions and to support them with molecular genetic methods in order to determine *A. m. cypria*.

Protein content determination

The protein concentration of *A. m. cypria* lyophilized crude venom (1 mg/mL) determined by the Smith (BCA) assay was 577.6 and 670.8 μ g/mL for Lefkosa Center and Mallidag Village samples, respectively. These results were similar to the study conducted by Şirin et al. [27].

In vitro cytotoxic activity

Cytotoxicity assays are performed on various human cancerous cells (A549, Caco-2, HeLa, MDA-MB-231, U-87MG,

Panc-1, and PC-3) and one healthy cell line (CCD-34Lu) with crude venoms of *A. m. cypria* collected from two different apiaries in Cyprus.

The effect of the venom on cells is evaluated via the MTT assay after treatment with different concentrations of crude venom for 48 h. IC_{50} values of the cells treated with Lefkosa Center venom varied between 8.69 ± 0.09 and 18.81 ± 3.09 μ g/mL, while the IC_{50} values of the cells treated with Mallidag Village venom were between 4.18 ± 0.75 and 22.00 ± 1.71 μ g/mL (Table 1, Figures 1 and 2). The crude venom from Lefkosa Center exhibited the most potent activity against PC-3 and Panc-1 cells with IC_{50} values of 8.69 ± 0.09 and 10.79 ± 1.97 μ g/mL respectively. Against PC-3 and HeLa cells, the most powerful activity was observed with the IC_{50} values of 4.18 ± 0.75 and 6.57 ± 1.27 μ g/mL with the venom sample collected from Mallidag Village.

Results indicated that the most resistant cells among the tested cell lines were Caco-2 cells, with IC_{50} values of 18.81 ± 3.09 μ g/mL and 22.00 ± 1.71 μ g/mL for Lefkosa Center and Mallidag Village samples, respectively. The venom also caused a decrease in the cell viability in direct proportion to the increase in concentration, which was consistent with the morphological changes observed within the cells by the inverted microscope.

Apoptosis assay

An apoptosis detection kit is used to evaluate the apoptosis rates of cells by combined staining of Annexin V-FITC and PI. Cells with no venom treatment were considered as the control group. Results showed that, with the specific concentrations, venoms had a significant effect on the induction of apoptosis for all tested cell lines, both for early apoptosis and late apoptosis, Figures 3 and 4.

Table 1: IC_{50} values (μ g/mL) for *Apis mellifera cypria* venom on cell lines following crude venom exposure.

Cell lines	Lefkosa center	Mallidag village	Doxorubicin ^a
A549, lung	16.79 ± 0.34	11.70 ± 1.01	4.81 ± 4.79
Caco-2, colon	18.81 ± 3.09	22.00 ± 1.71	19.2 ± 2.52
HeLa, cervix	15.30 ± 0.93	6.57 ± 1.27	2.99 ± 0.90
U-87MG, glioblastoma	17.65 ± 2.64	16.15 ± 1.48	7.41 ± 3.86
Panc-1, pancreas	10.79 ± 1.97	15.38 ± 2.65	7.89 ± 5.69
PC-3, prostate	8.69 ± 0.09	4.18 ± 0.75	3.88 ± 1.05
MDA-MB-231, breast	18.09 ± 1.22	16.62 ± 0.42	14.32 ± 2.33
CCD-34Lu, healthy lung fibroblast	14.34 ± 0.69	11.80 ± 1.31	6.47 ± 0.89

^aCytotoxic agent as a positive control. All data are expressed as the mean $\pm$ SD.

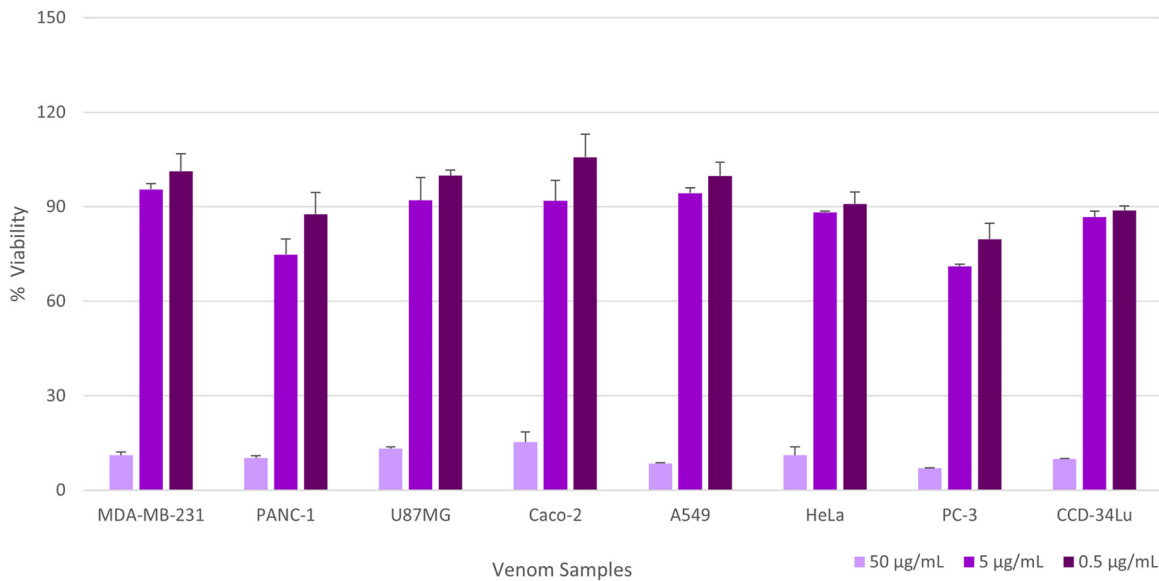


Figure 1: Cytotoxic effect of *Apis mellifera cypria* venom from Lefkosa center on human different cancer and healthy cells after 48-h exposure to different venom concentrations (50, 5, and 0.5 µg/mL). Cell viability is determined by MTT assay. Control is exposed to vehicle only, which is taken as 100 % viability. Data are expressed as mean±SD.

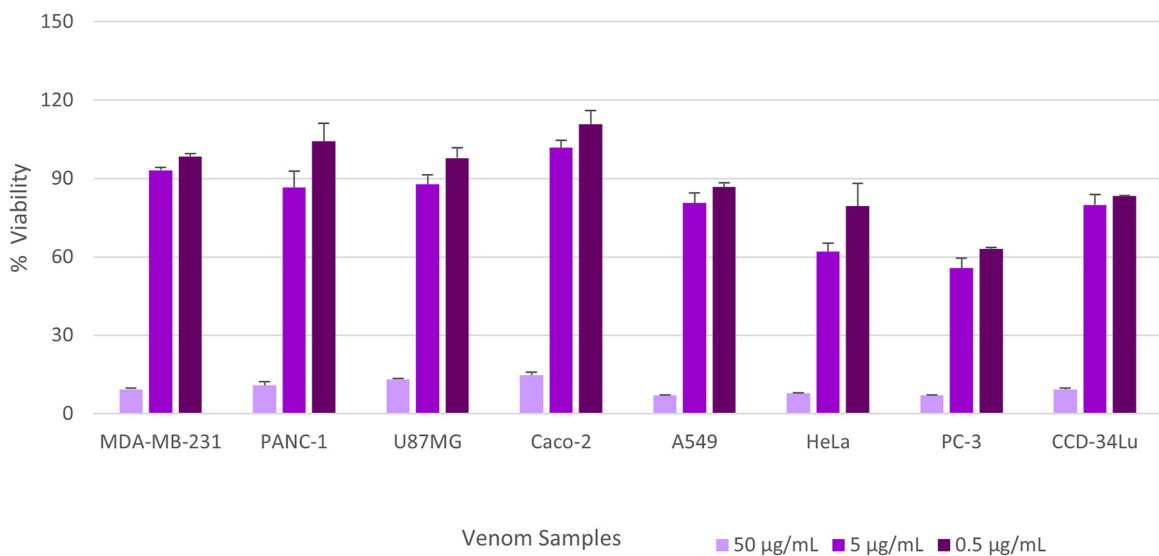


Figure 2: Cytotoxic effect of *Apis mellifera cypria* venom from Mallidag village on human different cancer and healthy cells after 48-h exposure to different venom concentrations (50, 5, and 0.5 µg/mL). Cell viability is determined by MTT assay. Control is exposed to vehicle only, which is taken as 100 % viability. Data are expressed as mean±SD.

SDS-PAGE analysis

As a result of SDS-PAGE analysis, seven protein bands for Lefkosa and nine protein bands for Mallidag were displayed on the gel, respectively. A dense protein band with a molecular weight of less than 5 kDa representing the main

protein mellitin was visualized in both gels. Besides, it was determined that two other important protein bands displayed in the 20–40 kDa range were hyaluronidase and phospholipase A2 enzymes, respectively, Figure 5. These results are consistent with those of Şirin et al. with three clearly observed protein bands [27].

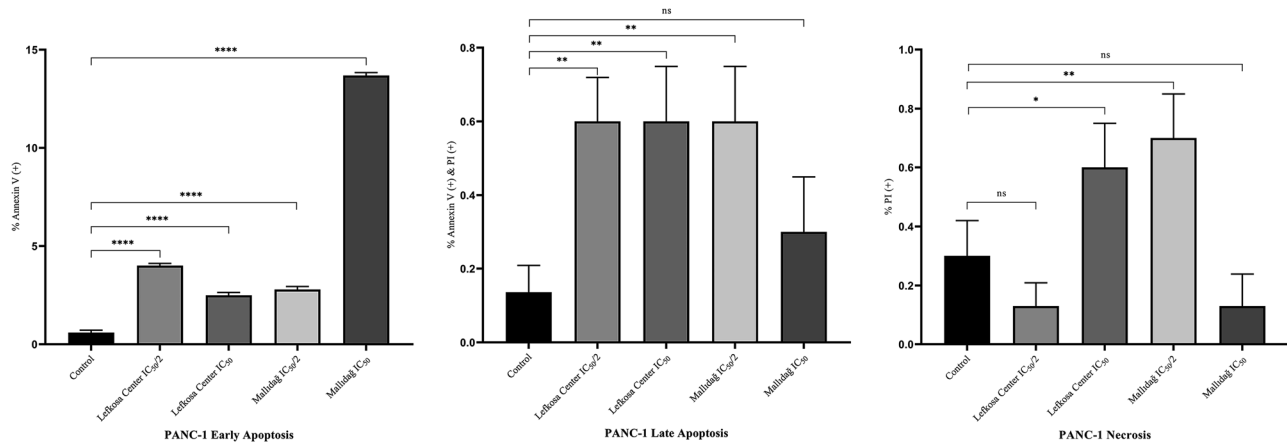


Figure 3: Flow cytometric analyses of Lefkosa center and Mallidag village venom on PANC-1 (pancreas) cells with Annexin V-FITC. All data are expressed as the mean±SD of three independent experiments. n.s., non-significant; *p<0.05; **p<0.01; ***p<0.001.

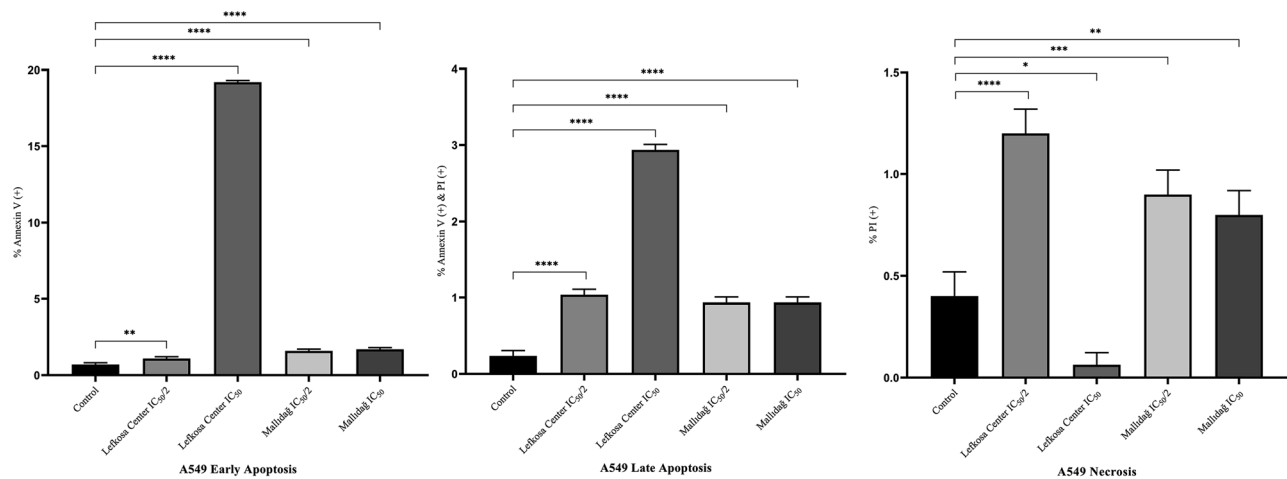


Figure 4: Flow cytometric analyses of Lefkosa center and Mallidag village venom on A549 (lung) cells with Annexin V-FITC. All data are expressed as the mean±SD of three independent experiments. n.s., non-significant; *p<0.05; **p<0.01; ***p<0.001.

Discussion

Honeybee venom with its bioactive compounds plays important roles in the effects of bee venom on the human body. For example, melittin is known for its antimicrobial and anti-inflammatory properties, while apamin has been found to have neuroprotective effects. Additionally, the presence of enzymes like phospholipase A2 and hyaluronidase contribute to the venom's ability to break down cell membranes and facilitate the spread of venom in the body [6]. Bee venom is frequently used in traditional medicine to treat inflammatory illnesses and reduce pain. Currently, researchers are looking at further bee venom-related therapies, as bee venom is a recent therapy option for several tumors [15]. In this regard, we investigated the

cytotoxic effects of *A. m. cyprina* crude venom on cancer and non-cancerous cells to assess its potential for further bioactivity-guided study.

The necessity for novel chemotherapeutic drugs has proven to be critical since cancer is one of the major health concerns among those diagnosed [28]. The anticancer properties of natural extracts, such as some venoms and toxins derived from snakes, scorpions, bees, and other venomous animals, have been demonstrated in numerous studies [3]. Nowadays, BV is used frequently to cure different cancers. Various functional protein molecules are found in this venom. The main peptide in bee venom, melittin, has potent membrane-perturbing properties that are the basis for its antimicrobial, antiviral, antifungal, and anticancer properties. Multiple melittin molecules enter the cell

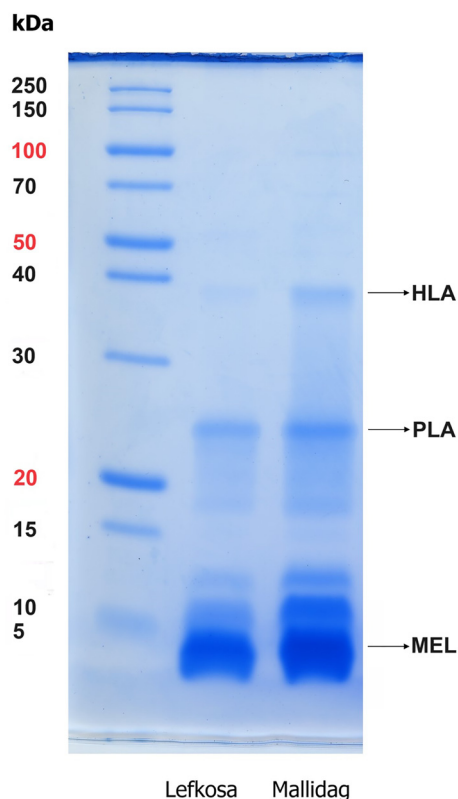


Figure 5: SDS-PAGE gel imaging of proteins in bee venom. HYA, hyaluronidase; PLA, phospholipase; MEL, mellitin. Reference bands at 20, 50, and 100 kDa are indicated in red.

membrane and degrade the phospholipids, causing cell lysis. Cancer cells undergo apoptosis when exposed to antitumor drugs, and this process is crucial in halting the growth of tumors. Consequently, by causing apoptosis in some cell types, BV can contribute to the treatment of cancer [15]. To fully comprehend the specific mechanisms by which bee venom and its components exert their anticancer effects on human breast cancer cells, additional research is required. This knowledge will enable the development of more targeted and effective treatment strategies, potentially leading to a significant reduction in side effects associated with current breast cancer therapies [29].

In this study, the MTT test was used to examine cancer cell growth following *A. m. cypria* venom treatment. These findings suggest that the cytotoxicity of *A. m. cypria* venom may have potential therapeutic applications in cancer treatment. Further research is needed to explore the specific mechanisms underlying its cytotoxic effects and to evaluate its efficacy and safety in clinical settings [3, 15, 30]. The results suggested that the *A. m. cypria* crude venom collected from Lefkosa Center has a significant potent cytotoxic effect against PC-3 and Panc-1 cells with IC_{50} values of 8.69 ± 0.09 and $10.79 \pm 1.97 \mu\text{g/mL}$ while venom from Mallidag Village

had the most potent activity on PC-3 and HeLa cells with IC_{50} values of 4.18 ± 0.75 and 6.57 ± 1.27 respectively. When compared to the positive control drug agent doxorubicin, Lefkosa Center venom exhibited higher cytotoxicity against Caco-2 cells than the control, also Panc-1 IC_{50} results were similar. Mallidag village venom had comparable IC_{50} results with doxorubicin on PC-3, Caco-2, and HeLa cells. The cytotoxicity against MDA-MB-231 was comparable with doxorubicin for both venom samples. Although the venoms also showed a cytotoxic effect on the healthy cell line CCD-34Lu, IC_{50} values showed that venoms were less cytotoxic compared to the positive control agent doxorubicin (Table 1). Additional research with isolated proteins and peptides should be carried out in order to clarify the mechanism of action and specificity of the venom. Badawi reported a similar result, stating that the anti-prostate cancer effects induced by toxic agents in bee venom were mediated through multiple mechanisms [31].

Apoptosis is a form of programmed cell death that is brought on by regular physiological processes and results in cell shrinkage and membrane permeability loss [32]. It is well established that the ideal approach for an anti-cancer agent to function is to induce the target cancer cells to undergo apoptosis. Apoptosis is essential for the growth and balance of multicellular organisms. Apoptosis is an orderly, well-regulated dying process that occurs in both normal and pathological situations [33]. Recent studies have reported that bee venom has the ability to induce apoptosis [33–36]. In light of this, Annexin V-FITC/PI analysis was carried out to identify the apoptotic stage, and the results showed a significant, early apoptosis effect induced in A549 and Panc-1 cells treated with 50 % inhibitory concentration and half 50 % inhibitory concentration of the venoms. Venom collected from the Lefkosa Center exhibited a notable dose-dependent early apoptotic effect on A549 cells in IC_{50} concentration. Venom sample from Mallidag Village also showed a dose-dependent early apoptosis effect on A549 and Panc-1 cells (Figures 3–5).

Conclusions

The study's findings gave context for and proof that *Apis mellifera* venom from *A. m. cypria* demonstrates potent cytotoxic activity against a variety of human cancer cells and is a natural source with significant promise for treating cancer. More research will aid in a better understanding of the venom's action mechanism. Additionally, the discovery and alteration of the venom's active ingredients that induce cytotoxicity can yield information for studies on the treatment of cancer. By using the venoms that are freely

accessible across the nation, the usage of bee venom in apitherapeutic applications can be increased. The results of this study are also important for the protection of the Cyprus bee as a pure breed, which is in danger of extinction through crossbreeding. The purity of Cypriot bees should be protected by detecting pure lines and applying proper breeding techniques to them. More multidisciplinary research must be done to achieve high medical-grade evaluations of the venom of specific breeds of bees.

Acknowledgments: We would like to thank Kırat Kasapoğlu, President of the North Cyprus Nicosia Beekeepers Foundation, for enabling us to communicate with beekeepers, and Hasan-Çigdem Bodur from the village of Mallidag for allowing us to collect bee venom from their apiaries. Also, we would like to thank Benjamin F. Hempel for his support, especially in SDS-PAGE studies. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Research ethics: Not applicable.

Informed consent: Not applicable.

Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The authors state no conflict of interest.

Research funding: None declared.

Data availability: Not applicable.

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Supplementary Material: This article contains supplementary material (<https://doi.org/10.1515/tjb-2023-0109>).