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Research Article

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Photoprotective and Therapeutic Effects of Apamin, Melittin and Phospholipase A2 on Human Keratinocyte Cell Line

İnsan Keratinosit Hücre Hattında Apamin, Melittin ve Fosfalipaz A2'nin Fotokoruma ve Fototerapik Etkisi

Esin Akbay*, Alp Yiğit Özdemir, Şebnem Erfen and Mehmet Ali Onur

Department of Biology, Faculty of Science, Hacettepe University, Ankara, Turkey.

ABSTRACT

The aim of this study was to determine the protective and therapeutic properties of bee venom components Apamin, Melittin and Phospholipase A2 against UV damage in human keratinocyte cell line. The cosmetic and therapeutic effects of bee venom have been reported in previous studies, but it is not known which components of the venom are most effective. For this purpose, an in vitro UV damage model was first designed. Components of bee venom were tested at different concentrations. Cell viability, cytotoxicity and apoptotic processes were analysed on the designed model. As a result, Apamin was observed to exert a significant protective effect against UV-induced cell death at all concentrations. The photoprotective effects of Melittin were observed at all concentration times, and it was found that the photoprotective effects were increased at high concentrations. Our results showed that Phospholipase A2 can be used as a photoprotective and phototherapy agent. This study is the first to demonstrate the protective and therapeutic properties of bee venom components against UV damage. As a result, it has been shown that these components can be successful photoprotective and phototherapeutic agents against UV-induced damage, but more detailed studies should be done to minimize their toxic effects on cells.

Key Words

Apamin, melittin, phospholipase A2, photoprotection, phototherapy, HaCaT cell line.

ÖZ

Bu çalışmanın amacı, arı zehiri bileşenleri olan Apamin, Melittin ve Fosfolipaz A2'nin insan keratinosit hücre hattındaki UV hasarına karşı koruyucu ve tedavi edici özelliklerini belirlemektir. Arı zehirinin kozmetik ve tedavi edici etkileri önceki çalışmalarda bildirilmiştir, ancak zehirin hangi bileşenlerinin en fazla etkiye sahip olduğu bilinmemektedir. Bu amaçla ilk olarak bir in vitro UV hasar modeli tasarlandı. Arı zehirinin bileşenleri farklı konsantrasyonlarda test edildi. Hücre canlılığı, sitotoksisite ve apoptotik süreçler tasarlanan model üzerinde analiz edildi. Sonuç olarak, Apamin'in tüm konsantrasyonlarda UV ile indüklenen hücre ölümüne karşı önemli bir koruyucu etki gösterdiği gözlendi. Melittin'in ışık koruyucu etkileri tüm konsantrasyon sürelerinde gözlendi ve yüksek konsantrasyonda ışık koruyucu etkilerinin arttığı tespit edildi. Sonuçlarımız, Fosfolipaz A2'nin bir fotokoruyucu ve fototerapi ajanı olarak kullanılabileceğini gösterdi. Bu çalışma, arı zehiri bileşenlerinin UV hasarına karşı koruyucu ve tedavi edici özelliklerini gösteren ilk çalışmadır. Sonuç olarak, bu bileşenlerin UV kaynaklı hasara karşı başarılı fotokoruyucu ve fototerapötik ajanlar olabileceği gösterilmiştir, ancak hücreler üzerindeki toksik etkilerini en aza indirmek için daha detaylı çalışmalar yapılmalıdır.

Anahtar Kelimeler

Apamin, melittin, fosfalipaz A2, fotokoruma, fototerapi, HaCaT hücre hattı.

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Correspondence to: E. Akbay, Department of Biology, Hacettepe University, Ankara, Turkey.

E-Mail: akbayesin@gmail.com

INTRODUCTION

The long-term exposure to the ultraviolet (UV) rays emitted from the sun causes different kinds of reactions by bringing along cell responses. Problems such as sensitivity, inflammation, premature skin aging, cell death and cancer are at the top of these reactions. Amount of damage incurred at the cells increases by accumulation of deoxyribonucleic acid (DNA) damages due to insufficient DNA repair mechanisms with increasing the exposure times to the UV rays [1]. There are three types of UV lights according to UV light's wavelength; short-wave (100-280 nm) UVC, medium-wave (280-320 nm) UVB and long-wave (320-400 nm) UVA. UVC is usually absorbed by the ozone layer of the atmosphere. The skin-damage that occurred via UV rays depends on the type, wave-length, energy and exposure time of light. Usually there are two types of UV-dependent damages which are UVA- and UVB-dependent damage [2]. UVA-dependent damages mostly cause hyperkeratosis and epidermal hyperplasia by increasing the expression of metalloproteinases which becomes grounds for abnormal overgrowth of elastic fibers and decrease in collagen [3]. However, UVB-dependent damage effects are generally the direct changes on biomolecules which is causing sunburn and tanning conditions. In the literature it could be seen that UVB rays target the DNA and create dimeric products between pyrimidine bases called photolesions which is causing skin cancer [4]. Previous studies suggested that, while UVB is causing melanoma and non-melanoma skin cancer, UVA leads carcinogenic and mutagenic actions by generation of reactive oxygen species which damages the DNA [5].

Bee venom is a mixture of diverse chemicals. It consists of different types of peptides and enzymes such as Apamin (APA), Melittin (MEL), hyaluronidase, Phospholipase A2 (PLA A2) and lysophospholipase. Bee venom reveals anti-inflammatory and anti-bacterial effects. In this context, it has been used as both therapeutic and cosmetic agent such as anti-acne products, and it continues to be used today [6]. The antibacterial feature of bee venom comes from the MEL component which is composed from 26 amino acids. It is a toxic agent that is destroying the bacteria's cell wall. Besides, MEL blocks expression of genes that cause inflammation thus creating an anti-inflammatory effect [7]. APA has been used as a therapeutic agent in the central nervous system for many years with its canal blocking properties [8,9]. Recently, some studies have shown that APA has other beneficial effects on human health too. APA provides anti-inflammatory effects by blocking the activations of some inflammatory chemokines and cytokines and shows therapeutic effects on keratinocytes [10]. PLA A2 is an important enzyme-based molecule in the structure of bee venom. It is highly influential on cell responses, phospholipid mechanisms and regulation of the immune and inflammatory systems [11]. PLA A2 increases the pigmentation by up-regulating the activity of tyrosinase in melanocytes [12].

Keratinocytes are the common cell type in the epidermis layer of human skin and they are one of the most important cells in the pathogenesis of various skin diseases. HaCaT cells are a spontaneously transformed immortal aneuploidy cell line which is derived from human keratinocyte cells [10]. Besides that, HaCaT cell line is commonly used as a substitute for keratinocytes to study UV-dependent cell damage [13].

Bee venom is a promising component in pharmacology and cosmetics due to its anti-inflammatory, anti-bacterial, anti-acne, anti-cancer, anti-fungal, antiviral effects and therapeutic properties against skin diseases. Studies show that bee venom is effective in treating UV-dependent photoaging in human skin [14]. However, the lack of scientific studies has made it difficult to understand which bee venom component is more effective on skin disease and conditions such as photodamage. Here, we investigated the photoprotective and phototherapeutic properties of APA, MEL and PLA A2, which are three components of bee venom, on HaCaT via using an in vitro model. To understand which bee venom component is more effective on phototherapy and photoprotection, we measured cell viability, cytotoxicity, apoptosis and necrosis then compared the results with each other.

MATERIALS and METHODS

Materials

Penicillin-streptomycin (P/S) (CAS Number: 3810-74-0), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (CAS Number: 298-93-1), acridine orange (AO) solution (CAS Number: 65-61-2), and propidium iodide (PI) solution (CAS Number: 25535-16-4) were obtained from SigmaAldrich (Germany). Dulbecco's modified Eagle's medium (DMEM) (Cat Number: FG 0415) and fetal bovine serum (FBS) (Cat Number: S 0415) were purchased from Biochrom AG, Germany. All chemicals

and solvents were obtained as cell culture grade. Human keratinocyte cell line (HaCaT) was purchased from the American Type Culture Collection (ATCC).

Cell culture

HaCaT cells were cultured with high-glucose DMEM containing 10% FBS, 1% P/S. After sufficient cell number was reached, the cells were seeded at 10^4 cells/well in 96-well culture dishes to establish an in vitro model. Experimental groups were formed at the 24th hour following the culturing.

Concentration trials

Trials have been carried out to determine the working concentrations of APA, MEL and PLA A2 that do not have a lethal effect on HaCaT. All substances are applied to the cells for 24, 48 and 72 hours in serum-free medium at three different concentrations which are D1 = 0.1 µg/ml, D2 = 0.5 µg/ml and D3 = 1 µg/ml. The concentrations planned to be tested were determined by reviewing the literature [10,15,16].

Establishment of in vitro phototherapeutic and photoprotection model

To investigate the photoprotective and phototherapeutic effects of APA, MEL and PLA A2 we generated a photodamage model with UVA light [17,18].

To create the phototherapeutic model, cells were irradiated (40 J/cm2) with a UVA bulb (Philips PL-S 9W / 10 UVA Bulb). Before irradiation processes, the medium on the cells was replaced with phosphate buffered saline (PBS) and the lids of the cell culture dishes were left open in laminar flow during the irradiation period. After irradiation, bee venom components prepared in different concentrations in PBS were applied to the cells for 24, 48 and 72 hours.

For the establishment of the photoprotection model, the selected bee venom components were applied to the cells transferred to the 96-well culture dish at three different concentrations for 24, 48 and 72 hours. At the specified time intervals, the media on the cells were replaced with PBS solution in order to create an accurate photodamage model, and the cells were irradiated at 40 J/cm2 energy with a UVA bulb (Philips PL-S 9W / 10 UVA Bulb). The percentage of photoprotection was calculated by the equation 1.

$$\%Photoprotection = 100 - \left(100x\frac{Positive\,Control - Experimental\,Group}{Positive\,Control - Negative\,Control}\right) \quad (1)$$

The "positive control" group represents any material and group of cells that were not subjected to irradiation. The "negative control" group constitutes the cell group that has not been subjected to any material application but only irradiated. "Experimental groups" are groups of cells that are exposed to bee venom products and irradiated.

Measuring cell viability

MTT assay is a standardized calorimetric cell viability test. It reflects the number of viable cells by determining the ability of living cells to form MTT formazan with the activity of succinate dehydrogenase enzyme [19].

Cell viability was measured in designed incubation periods (24, 48 and 72 hours) via MTT assay. The culture medium was replaced with a serum-free medium containing 10% MTT solution and incubated for 4 hours at 37°C. After formazan crystals were formed MTT solution was replaced with isopropyl alcohol to solve the crystals. The absorbance was measured at wavelength 570 nm in the ELISA microplate reader (µQuantTM, BiotekW Instruments Inc, USA). The ratio of cell viability was obtained by calculating the percentages of the experimental groups compared to the positive control (Equation 2).

$$\%Cellular\,Viability = \frac{Treated\,Group\,Absorbance}{Control\,Group\,Absorbance}x100 \quad (2)$$

Cytotoxicity assay

As a result of the cell viability analysis, it was observed that the most effective application time for APA, MEL and PLA A2 in terms of photoprotective and phototherapeutic effects was 24 hours. Based on this result, the cytotoxicity test was performed only for 24 hours.

To determine cytotoxicity, lactate dehydrogenase (LDH) release was measured using the protocol defined by the manufacturer (Pierce LDH cytotoxicity assay kit, Thermo Scientific, Waltham, MA, USA) over a 24 hours period. The absorbance was measured at wavelength 490 nm in the ELISA microplate reader (µQuantTM, BiotekW Instruments Inc, USA). Percentage of cytotoxicity was calculated by using the manufacturer's protocols (Equation 3).

$$\%Cytotoxicity = \frac{Compound\,treated\,LDH\,activity - Spontaneous\,LDH\,activity}{Maximum\,LDH\,activity - Spontaneous\,LDH\,activity}x100 \quad (3)$$

Apoptotic cell staining

AO and PI are nucleic acid specific fluorochromes. When these bind to DNA, they emit green and orange fluorescence respectively. While AO can cross the membrane of living and early apoptotic cells, this transition is not the case for PI.

For this reason, viable cells are seen with green nuclei and intact structure, while apoptotic cells exhibit a bright green nucleus due to chromatin condensation. Both late apoptotic cells and necrotic cells can be stained with AO and PI. Chromatins condensed in late apoptotic cells due to the high intensity emission produced by PI causes an orange nucleus to be observed. However, necrotic cells show an intact orange core. In the framework of these explanations, AO/PI staining is an important quantitative evaluation [20].

After photoprotection and phototherapeutic models were applied to cells in a 96-well culture dish, the media or PBS on the cells was removed. After washing the cells with PBS, they were stained with AO/PI mixture. After the dye was removed, cells were rinsed twice with PBS. After the rinsing process was completed, imaging was performed with fluorescence microscopy (Olympus IX70 Inverted Microscope, Japan). From the photographs obtained, cells were counted based on the method described in previous studies to determine the potential of different groups to induce apoptosis and necrosis in cells [20,21]. Counting was done according to previous studies. Uniform green core cells with organized structure, intact plasma membrane and orange or green cytoplasm, living cell (1); cells with bright green areas of chromatin condensation in the nucleus, early apoptotic cell (2); cells with areas of dense orange chromatin condensation, late apoptotic cell (3); and cells with orange intact nuclei were counted as necrotic cells (4). For each test group, three different images were analyzed by manually examining and counting cells. The quantification of apoptotic and necrotic cells was calculated according to the equation 4 and equation 5.

$$\%Apoptotic\ Cells = \frac{Total\ number\ of\ apoptotic\ cells\ (early\ or\ late)}{Total\ count\ cell} x100 \quad (4)$$

$$\%Necrotic\ Cells = \frac{Total\ number\ of\ necrotic\ cells}{Total\ count\ cell} x100 \quad (5)$$

Statistical analyses

All the experimental data were represented as the mean ± standard deviation (SD). Data were compared with two-way analysis of variance (two-way ANOVA) using GraphPad Prism version 5.0 (GraphPad Software Inc., San Diego, CA, USA), which is a statistical software. $p < 0.05$ and $p < 0.01$ indicate statistical significance. Experiments were performed at least in triplicates.

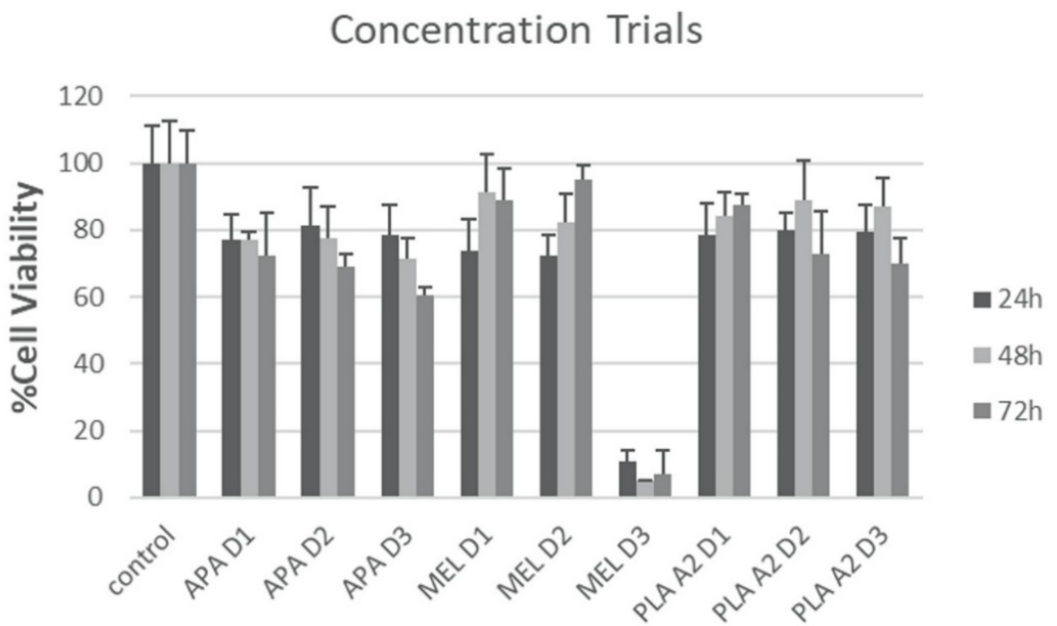

Figure 1. Different concentrations of APA, MEL and PLA A2 were applied on the HaCaT cell line. The percentage of living cells after an incubation time of 24, 48 and 72 h were measured by MTT assay and calculated in terms of relative values. h: hours.

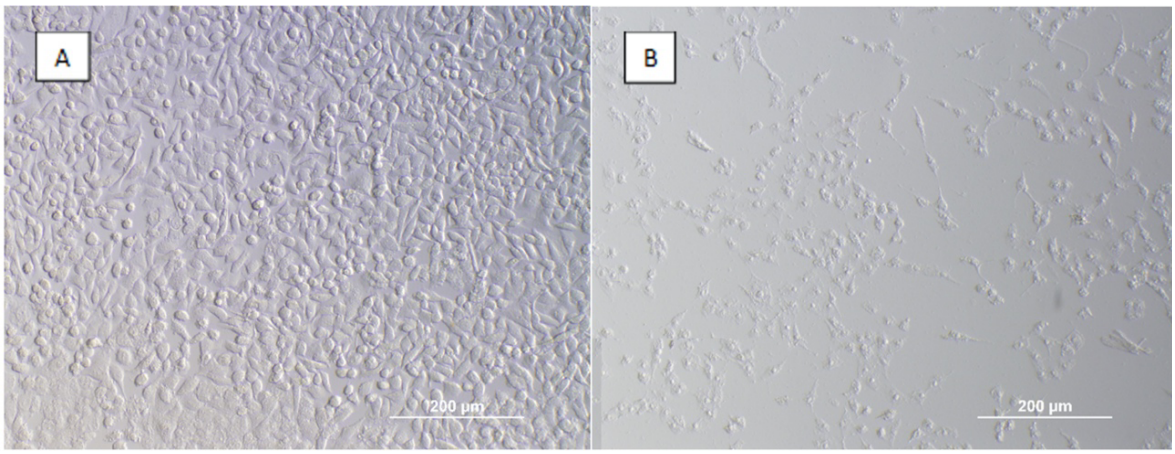

Figure 2. Morphological image of healthy HaCaT cells (A), HaCaT cells with the treatment of 1 µg/ml of MEL for 24 h (B). The images of cells are x10 magnification.

RESULTS

Cell line study

HaCaT cell lines were routinely cultured on high-glucose DMEM containing 10% FBS and 1% P/S. The cells were maintained in a humidified atmosphere containing 5% CO_2 at 37°C. The medium was changed every two days. No contamination was observed during bee venom components treatment throughout the experiments. After this stage, analysis was started.

Concentration trials

In the concentration trial experiment, it was determined that APA, MEL and PLA A2 have no lethal effect on HaCaT, except for MEL D3 (Figure 1). According to this result, MEL D3 was removed from the experimental groups in the remaining experiments. Also, cell images supporting the concentration assay plot are shown in Figure 2.

Measuring cell viability

MTT tests were performed to identify the viable cell. The results of the cell viability assay analysis performed to determine both photoprotective (Figure 3) and phototherapy (Figure 5) effects of APA, MEL and PLA A2 on the HaCaT cell line. Cell viability of less than 20% was obtained in the group not treated with APA, MEL and PLA A2 and exposed to UVA light (negative control). The negative control group was an important group in terms of showing the effect of UVA on cells.

The higher cell viability of APA, MEL and PLA A2 treatments compared to the negative control group is important in terms of demonstrating the photoprotective potential. It is observed that cell viability does not decrease below 60% for 24, 48 and 72 hours in APA treatment groups. The maximum protective effect was determined at 48 hours as cell viabilities 87.55% for 0.1 µg/ml, 84.67% for 0.5 µg/ml, and 79.82% for 1 µg/ml concentration. The maximum photoprotective effect was seen with 91.81% cell viability for the 0.1 µg/ml concentration of MEL in 48 hours. Similarly, at a concentration of 0.5 µg/ml of MEL, maximum protection was observed at 48 hours. The highest photoprotective effect for PLA A2 was observed at concentrations of 0.5 µg/ml with 93.09% cell viability and 85.37% cell viability at 72 hours, while at the 48th hour it was observed at 1 µg / ml concentration with 73.93% cell viability. Although cell viability was observed to be higher than negative control at D3 concentration application of PLA A2, it was observed that cell morphology was severely altered (data not shown). According to the statistical analysis results, a significant difference was found in all concentrations of APA and at the concentration of MEL at 0.1 µg/ml at 72 hours compared to the control values.

The % photoprotective effect calculated using the % vitality values obtained from the MTT results is graphically given in Figure 4. When the graph was examined, it was observed that APA at a concentration of 0.1 µg/ml and MEL at a concentration of 0.5 µg/ml at the 48th hour and PLA A2 at a concentration of 0.1 µg/ml at the 72nd hour had maximum protection.

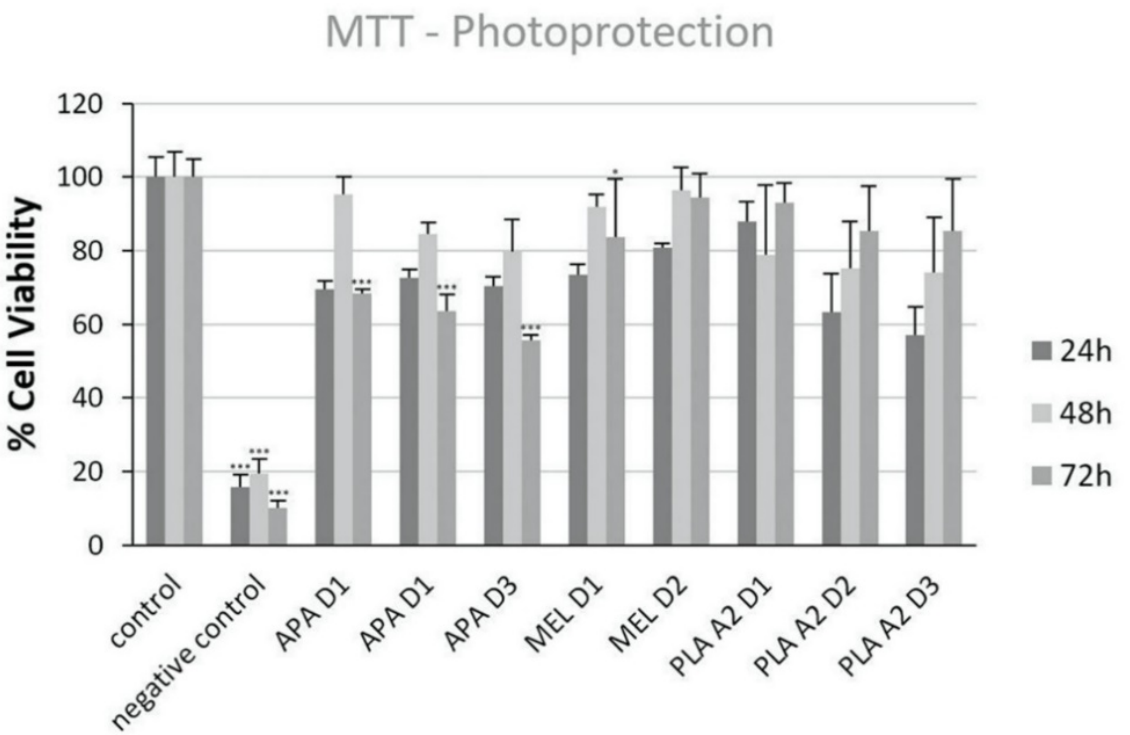

Figure 3. The percentage of living cells after an incubation time of 24, 48 and 72 h were measured by MTT assay and calculated in terms of relative values. h: hours; *: $p < 0.05$; ***: $p < 0.001$.

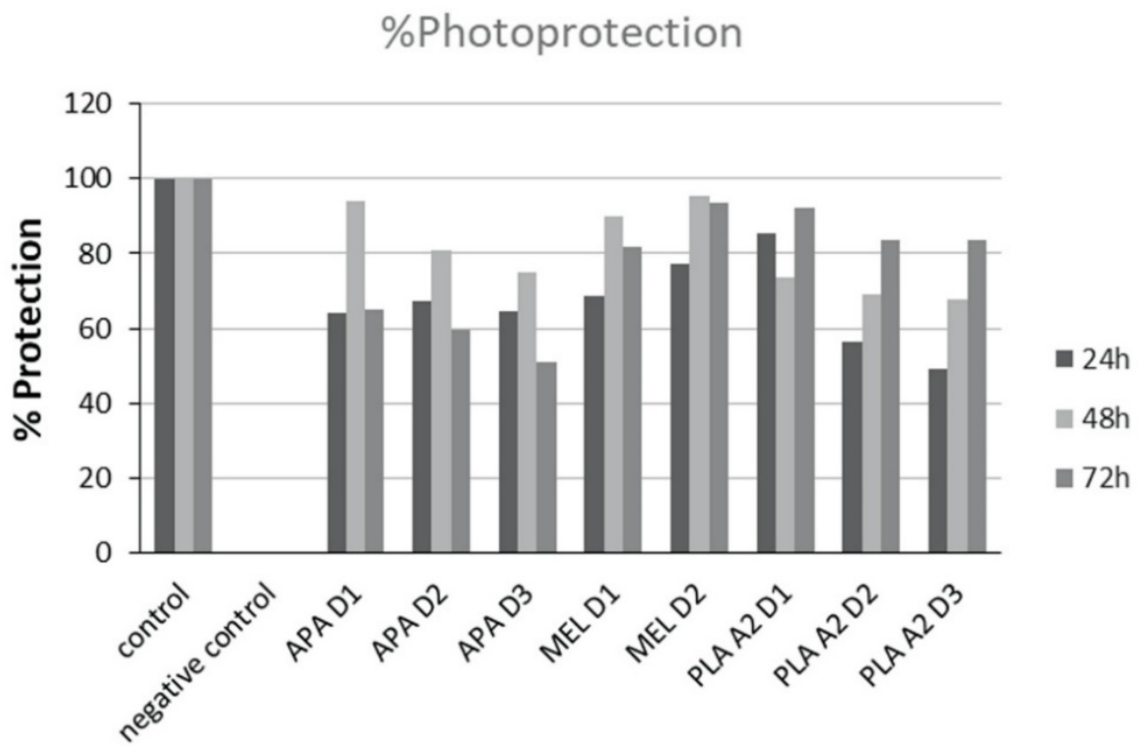

Figure 4. The % photoprotection values were calculated according to the equation with the data obtained from MTT analysis. h: hours.

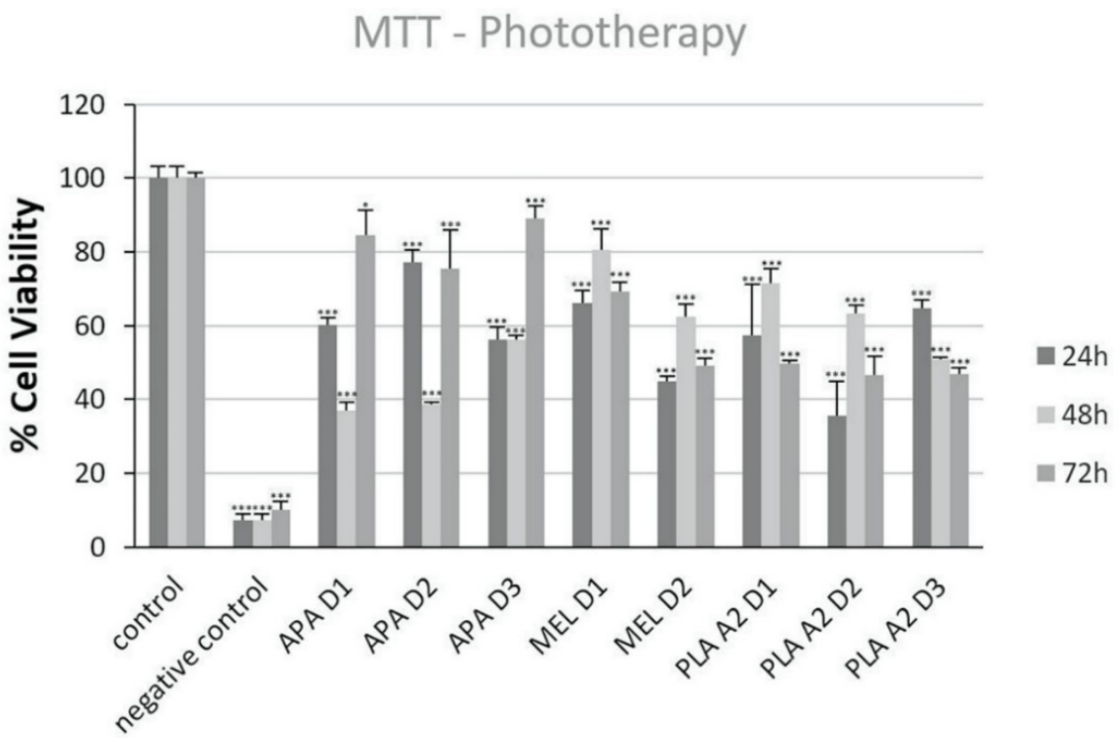

Figure 5. APA, MEL and PLA A2 were treated at 0.1, 0.5 and 1 µg/ml concentrations on the UVA irritated HaCaT and MTT analysis was performed at 24, 48 and 72 h. The percentage of living cells were measured after an incubation times by MTT assay and calculated in terms of relative values. h: hours. *: $p < 0.05$; ***: $p < 0.001$.

Cell viability of less than 10% observed in negative control in phototherapy groups indicates that UV irradiation was performed correctly. High cell viability was determined at all concentrations of phototherapy groups compared to negative control.

In APA, one of the phototherapy groups, the lowest phototherapy effect was observed at a concentration of 0.1 µg/ml at the 48th hour, while the highest phototherapy effect was observed at a concentration of 0.1 µg/ml at the 72nd hour. In MEL, although the highest phototherapy effect was observed at 48th hour, cell viability was observed to decrease below 50% at the 72nd hour with 0.5 µg/ml concentration application. The highest phototherapy effect of PLA A2 was seen at a concentration of 1 µg/ml in 24 hours. It was observed that the same product had the highest phototherapy effect at concentrations of 0.1 and 0.5 µg/ml at 48 hours. According to the results of statistical analysis, a significant difference was found in all groups compared to the control, except for the 1 µg/ml concentration of APA administered at the 72nd hour (Figure 5).

Cytotoxicity assay

The comparative results of the MTT and LDH analyzes are given in Figure 6. LDH analysis was performed at 24 hours and compared to 24 h MTT values. Cell cytotoxicity was calculated according to the % cytotoxicity equation given previously. As seen in Figure 7A and B, when cell viability and cytotoxicity values are compared, cytotoxicity values confirm the cell viability values. According to the result of LDH analysis for phototherapy, the most cytotoxic value was seen in MEL 0.1 µg/ml concentration. Cell cytotoxicity is below 22% in other bee venom products and concentrations. It is seen that % cytotoxicity values are below 20% in photoprotection groups.

Apoptotic staining

As a result of applications morphological changes due to apoptosis and necrosis observed in the cell nucleus were examined with AO/PI. Nuclear fragmentation and membrane shrinkage in cells continue to be hallmarks of apoptotic cells and distinguish between apoptotic (early and late) and necrotic cells. This separation is commonly used by the AO/PI double staining method. With this staining method, AO dye is taken up by both

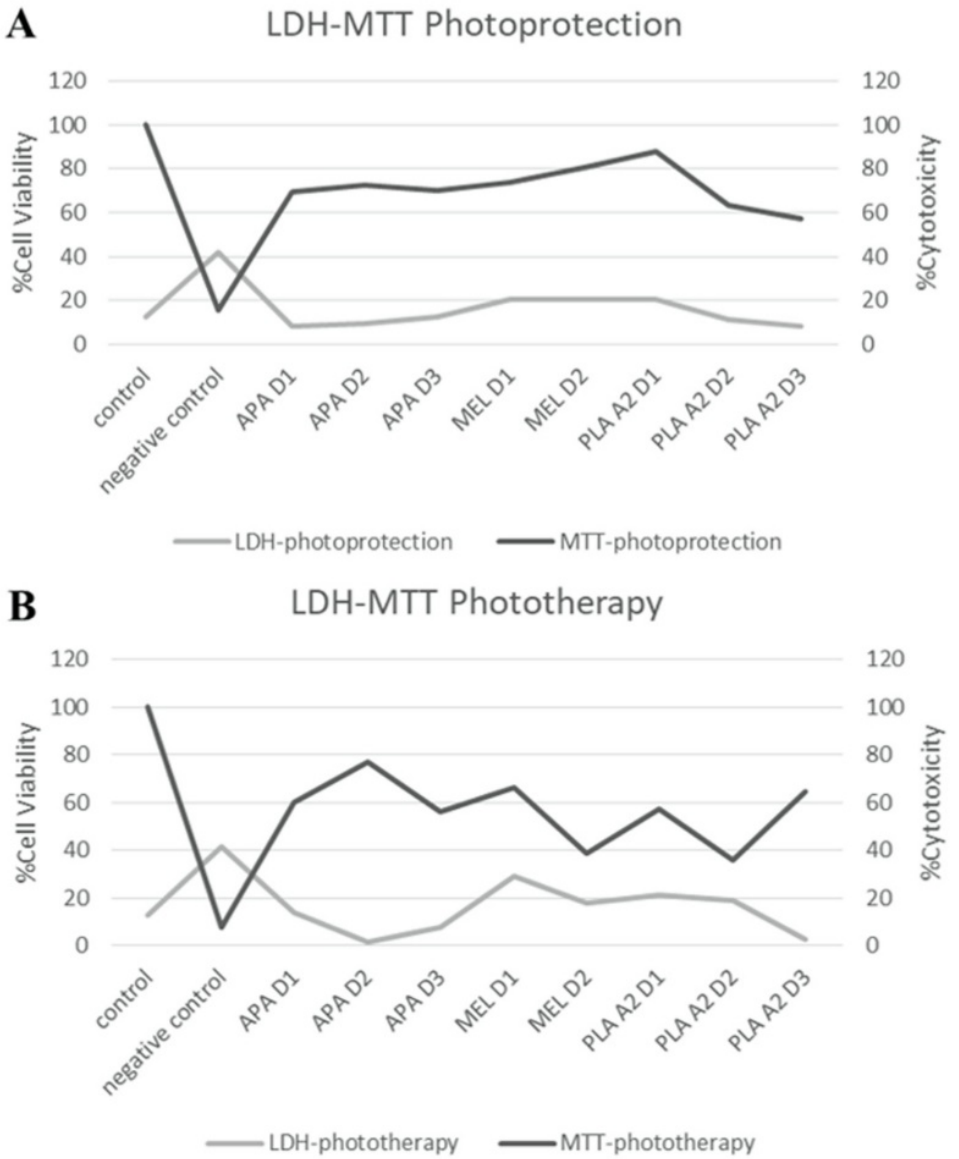

Figure 6. APA, MEL and PLA A2 were treated at 0.1, 0.5 and 1 µg/ml concentrations on the UVA irritated HaCaT and MTT analysis was performed at 24, 48 and 72 h. The percentage of living cells were measured after an incubation times by MTT assay and calculated in terms of relative values. h: hours. *: $p < 0.05$; ***: $p < 0.001$.

living and non-living cells and emits green fluorescence, while PI is taken up only by inanimate cells and emits orange or red fluorescence.

The results obtained from fluorescence images of HaCaT cells after AO/PI dual staining are represented in Table 1. UVA irradiation predominantly triggers late apoptosis and necrosis in HaCaT cells. Bee venom components caused early apoptosis in HaCaT cells in photoprotection groups but did not cause late apoptosis and necrosis. However, in phototherapy groups late apoptosis and necrosis was observed. In the 48 hours application of APA D2, 72 hours application of MEL D2 and 24- and 72 hours applications of PLA A2 D2 necrosis was observed above 60%.

Table 1. % apoptosis and necrosis of HaCaT cells after photoprotective and phototherapeutic applications with APA, MEL and PLA A2.

Groups		Photoprotection			Phototherapy		
		24h	48h	72h	24h	48h	72h
control	%apoptosis	19.19	13.21	9.17	19.19	13.21	9.17
	%necrosis	0	0	0	0	0	0
negative control	%apoptosis	98.39	85.04	81.89	98.39	85.04	81.89
	%necrosis	9.68	7.09	3.94	9.68	7.09	3.94
APA D1	%apoptosis	55.66	9.90	25.78	41.99	41.88	7.34
	%necrosis	6.60	2.61	27.01	0	3.25	27.03
APA D2	%apoptosis	18.03	12.72	26.65	55.73	7.51	54.57
	%necrosis	7.32	10.76	18.06	0	66.25	4.88
APA D3	%apoptosis	21.47	23.58	59.38	54.64	61.46	24.76
	%necrosis	7.07	0.44	15.34	0.52	0	1.43
MEL D1	%apoptosis	25.32	7.94	15.66	46.79	54.75	46.79
	%necrosis	2.66	11.30	29.27	0.32	0.90	23.80
MEL D2	%apoptosis	11.96	26.98	12.95	77.35	65.13	22.15
	%necrosis	6.62	9.77	6.70	0.35	3.17	67.11
PLA A2 D1	%apoptosis	14.22	16.95	18.84	57.87	46.59	30.62
	%necrosis	4.90	6.50	1.75	0	0.77	14.98
PLA A2 D2	%apoptosis	24.93	14.84	20.00	64.61	40.39	67.28
	%necrosis	4.20	0.71	1.70	0	1.48	11.98
PLA A2 D3	%apoptosis	2.87	0.65	10.43	45.50	43.14	36.36
	%necrosis	22.99	30.65	13.48	5.50	11.11	19.25

DISCUSSION

Bee venom consists of many different components which are mixtures of peptides, lipids, carbohydrates, amino acids and enzymes like APA, MEL and PLA A2. APA and MEL, which are among the most common components of bee venom, have peptide structures and show antimicrobial, anti-viral, anti-inflammatory and anti-fungal properties [6]. It is a known fact that by taking large amounts of bee venom inside the body, an allergic effect may occur in the body. However, studies show that bee venom can be used cosmetically and therapeutically on the body [22,23]. Bee venom can introduce skin photoprotection against UVB-rays and significantly inhibits the production of matrix metalloproteinase-1 and stromelysin-1 which increases after photo irradiation. Due to that bee venom could be a valuable agent against photoaging [14]. Also, bee venom exhibited a photoprotective effect against photoaging by reducing the level of matrix metalloproteinases proteins in the skin [24]. However, it has not been fully investigated which component of bee venom is more effective against UV-induced cell damage. In this study, we showed and compared the protective and therapeutic properties of bee venom components APA, MEL and PLA A2 against UV damage.

APA is a bee venom component which is a Ca^{2+}-activated K^+ channel blocker. It is acting like an allosteric inhibitor on these channels. Due to that, studies usually focus on channel blocking properties in the central nervous system of APA [25]. Apart from this, a study showed that APA can be used as a therapeutic agent against skin diseases such as atopic dermatitis. APA creates an anti-inflammatory effect on tumour necrosis factor-α (TNF-α) and interferon-γ proinflammatory cytokines via preventing the activation of nuclear factor kappa B (NF-κB) and Janus kinase/signal transducer (JAK/STAT) and activator of transcription (STAT) pathway. It was also observed that 0.1, 0.5, and 1 µg/ml concentrations of APA did not adversely affect the viability of HaCaT cells [10]. Similarly, APA did not show a highly toxic effect on HaCaT at any concentration used. In our study, we showed that APA can also be used as a photoprotective

and phototherapeutic agent. APA demonstrated a significant protective effect against UV-induced cell death at all concentrations used. However, as its concentration increased, a decrease in its photoprotective effect was observed. Regardless of concentration, the photoprotective properties of APA against UVA-induced damage were revealed at the 48th hour of administration and its phototherapeutic properties at the 72nd hour of administration.

MEL is a linear peptide composed from 26 amino acids that makes up 50% of the dry weight of bee venom. It shows anti-inflammatory, anticancer and antibacterial effects on cells [26]. It was demonstrated that in a previous study, MEL significantly reduced TNF-α, Interleukin (IL) 1-β, Toll Like Receptor (TLR) 2 and Cluster of differentiation 14 expression on keratinocytes, thereby preventing Propionibacterium acnes-induced skin inflammation [27]. MEL also inhibits the production of chemokines by reducing NF-κB, JAK/STAT, STAT1 and STAT3 in HaCaT cells and it can be used as a therapeutic agent against atopic dermatitis [15]. In another study it was demonstrated that the MEL exerted anti-inflammatory effects by blocking the primer signalling pathways of inflammatory cytokines against Porphyromonas Gingivalis LPS-mediated keratinocyte damage. This study also showed that 0.1, 0.5 and 1 µg/ml of MEL did not reduce the cell viability of HaCaT cells in 24 hours of application [28]. However, we demonstrated that 1 µg/ml application of MEL decreases cell viability dramatically. After the application of 24 hours it disrupted the structure of the cell and caused cell death. On the other hand, it was demonstrated that other concentrations of MEL did not have a lethal effect on HaCaT cells. We showed that MEL could be used as a photoprotective and phototherapeutic agent against UV-induced cell damage at certain time periods. Photoprotective effects of MEL were observed in all concentration time periods and its photoprotective effects were increased as the concentration increased. Furthermore, MEL was seen as the most successful protective agent against the UVA-induced photo damage among other bee venom components. Besides, in this study it was demonstrated that MEL was not a successful phototherapy agent as it was as a photoprotective agent. The most successful phototherapeutic effects of MEL were observed at 48 hours treatment in D2 concentration and its therapeutic effects were decreased as the concentration increased. Considering all these results, it turned out that the MEL compound of bee venom can be used as a photoprotective agent but for using it as a phototherapy agent much more study is needed.

PLA A2 is the enzyme compound of bee venom and it forms a family of structurally related proteins that hydrolyze both fatty acids and phospholipids [29]. In a previous study it was demonstrated that PLA A2 enhances TLR ligand dependent activation in keratinocytes by increasing the phosphorylation of NF-κB and mitogen-activated protein kinases pathways. With this process, PLA A2 creates a membrane modification and might increase the therapeutic effect for skin-wound healing [16]. However, PLA A2 is known as the major allergen component of bee venom [30]. PLA A2 induces the release of IL-33 and inducts the cell death by hydrolyzing the phospholipids to generate lysophospholipids [31]. In our study we tried three different concentrations of PLA A2 on HaCaT in three different time periods to observe its cytotoxic effects on cells. Based on our results, cell viability was not decreased under 70% in all concentrations. Even though PLA A2 showed a protective effect against the UVA irradiation, photoprotective properties were decreased as the concentration increased. The highest photoprotective effects were observed at 72 hours of application. The phototherapy effect was not as high as the other bee venom components in the PLA A2 groups. The most successful phototherapeutic effect was seen in 48 hours application of D1 concentration. For this time period, cell viability was observed at 70%. Although PLA A2 showed a good photoprotective effect and partially phototherapeutic effect in terms of cell viability at D1 concentration, as a result of the concentration trials, it was found that the morphology of the HaCaT cells treated with higher doses of PLA A2 was deteriorated. After treatment with PLA A2 for 1 µg/ml concentration, the spindle shape of the cells was disrupted and the cells took a round form. It makes sense for this component of bee venom, which shows enzymatic reactions to break down phospholipids on the cell surface, to exert such an effect. Our results were indicated that PLA A2 could be used as a photoprotective and phototherapy agent. However higher concentrations of it could be lethal for the cells due to its effects that disrupt the cell morphology. More detailed studies are required on this subject in order to determine the most accurate concentration for using the PLA A2 as a photoprotective or phototherapy agent.

Studies have shown that bee venom, which has been used as a natural ingredient in the pharmaceutical and

cosmetic industry, can be used as a protective agent against skin phototoxicity, photosensitivity and UV-induced damage [14,24]. It is also used as a therapeutic agent against skin problems such as atopic dermatitis and acne [10,27]. However, protective or therapeutic effects of individual components of bee venom against UV-induced skin problems have not been studied in any study in the literature until now. We demonstrated that APA and MEL did not show lethal effects on the HaCaT cell for 0.1 and 0.5 µg/ml concentrations. However, MEL showed highly cytotoxic effects at the concentration 1 µg/ml. Even though APA did not show such cytotoxic effects on HaCaT cells at this concentration, it dropped cell viability more than its other concentrations. It has also been observed that PLA A2 may exhibit allergic effects as a result of the deterioration of cell morphology at its increasing concentrations. It needs more studies for understanding its use as photoprotective and examining its allergic nature in detail. In their viable concentrations, APA, MEL and PLA A2 showed photoprotective effects against UVA-induced damage on HaCaT cells. In between these three components MEL was observed as the most successful agent against UVA-damage. Also, APA, MEL and PLA A2 increased cell viability after the photodamage treatment on cells yet their phototherapeutic properties were less successful compared to their photoprotective properties.

CONCLUSION

In conclusion, APA, MEL and PLA A2 could be successful photoprotective and phototherapeutic agents against UV-induced damage on skin cells but more detailed studies are required to minimize their toxic effects on cells. Also, further research is needed to evaluate their molecular pathways of photoprotection and phototherapy.

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