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Ex vivo UV-C Protective Effect of *Aloe vera*

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Abstract

Chronic exposure to UV-C can cause sunburn, skin cancer, oxidative stress as well as photo-aging. Many herbs and plant extracts have recently been recognized as a potential source of sunscreen due to their UV-absorbing properties. *Aloe vera* L., which has been used for therapeutic purposes by humans for centuries, is also among these plants. In this study, it was aimed to reveal the sunscreen properties of the latex and gel parts of *A. vera* and the effect of these properties on cells exposed to UV rays. In this study, primarily, the lethal effect of UV-C rays on healthy cells was observed over time. Then, the latex and gel parts of the obtained *A. vera* plant were separated and spread on the surface of petri dishes as a single and double layer. By adding a determined number of cells of the petri dishes, the protection of *A. vera* against the lethal effect of 1, 1.5 and 2 hours of UV-C exposure was investigated. According to the study findings, in all cells in the control group, an increasing number of deaths occurred as the UV-C exposure time was prolonged and no viable cells remained at the 2nd hour. The double-layered *A. vera* latex and gel groups exhibited less cell death than the control and still had viable cells at 2 hours. From this point of view, it has been shown in this study that a protective product that can be made using *A. vera* can prevent the damages that may develop due to UV-C exposure.

1. Introduction

The World Health Organization defines UV-C rays as the most damaging ray to the skin when compared to other components of sun rays [1]. Even only a small fraction of UV-C rays can penetrate the deep layer of the skin [2]. In addition, due to its UV-C ionization feature, it acts as a strong mutagen and can cause immune-mediated disease and cancer in adverse situations [3]. Given the increasing dangers associated with UV exposure, the use of sunscreen agents in various formulations has increased. Products developed as sunscreens often contain components that can absorb or disperse UV effectively [4]. In protection from the sun and the ultraviolet rays it causes, plants and plant extracts are generally accepted as a potential source of UV protection due to their UV absorbing and antioxidant properties [5-7]. *Aloe vera*, known as one of these plants, is a member of the Liliaceae (Lily) family [8, 9]. There is information on the ancient Egyptian papyrus and Mesopotamian clay tablets that *A. vera* was used in the treatment of infections, solving skin problems, and also as a laxative [10]. At the same time, it is among the data in the literature that *A. vera* suppresses neuroblastoma [11] and glioblastoma/astrocytoma [12] cells by showing anti-inflammatory activity and has anticarcinogenic potential. *A. vera* leaves have two different parts, latex and gel, with different chemical compositions. The latex part is obtained from the pericyclic cells and the gel part is obtained from the parenchyma cells [13].

The outermost large leaves are cut 2-3 times a year for medicinal purposes [10]. The active ingredients in the gel are anti-inflammatory, antioxidant [14], immunoregulatory [15], antidiabetic [16], antiproliferative [17], wound healing [18] and antimicrobial effects [19] were determined. The antioxidant property of *A. vera* is due to the abundant amounts of vitamin A, vitamin C, E, B12, choline and

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folic acid in its structure. In addition, in an in vitro study using veracylglucan B isolated from the gel, it was demonstrated that it had a significant antiproliferative (cytotoxic) effect on fibroblast culture [17]. *A. vera* is used in medicine, cosmetics, food industry, in many fields as capsules and creams [20]. It is especially preferred in the field of dermatology and it prevents wrinkles by accelerating the formation of collagen and elastin in its content and is used in the treatment of sun-induced burns, especially secondary-degree burns [21, 22].
Within the scope of this study, it was aimed to observe the protective effect of the latex and gel obtained from the *A. vera* plant, which is used as a pharmaceutical raw material in the cosmetic industry and dermatology, against UV-C, which is one of the harmful rays from the sun and kills cells.

2. Material and Method

2.1. Plant Material and Preparation Extract

Healthy and fresh *A. vera* leaves having a length of 30–45 cm were collected from Gaziantep University in Gaziantep/Turkey. The plant material was identified by Dr. H. Tekin, Botanist, University of Gaziantep. Leaves were washed with distilled water to remove dirt. After removing the spikes, the leaves were cut transversely into pieces and the thick epidermis was carefully separated from the parenchyma (Fig 1). The resulting latex and gel parts were homogenized and freeze-dried.

Figure 1. *A. vera* latex and gel parts

2.2. Preparation of *A. vera* Coated Petri dishes

A. vera latex and gel parts were spread as a thin layer on the top cover of the petri dishes, both on one and both sides (Fig 2). Petri dishes, which were left to dry at room temperature without sunlight, were then stored at room temperature.

Figure 2. Spread of *A. vera* parts on the petri dish

2.3. Cell Culture

2.3.1. Production of Cells

In cell culture studies, human umbilical vein endothelial cells (HUVEC) were used because it is a healthy cell line. The HUVEC cell line obtained from the American Cell Culture Collection (ATCC) cell bank was used. Dulbecco's Modified Eagle Medium (DMEM) medium containing inactivated 10% Fetal Bovine Serum (FBS), L-glutamine, penicillin/streptomycin was used for growth and growth of cells. Cells were produced in flasks in this medium and kept in a 37 ℃ incubator containing 5%

CO_2. The cells produced were then used for UV studies.

2.3.2. Observation of Morphological Effects of UV-C on Cells

The cells produced and covering the flask base were treated with UV-C for 1, 2 and 3 hours. A UV transluminator (DNR-IS) device, which produces light with a wavelength of 254 nm and an intensity of 8000 µW/cm at room temperature, was used as a UV-C light source (Fig 3). At the end of each hour, cells were observed morphologically under an invert microscope.

Figure 3. UV-C application to cells

2.3.3. Determination of Protective Efficacy of *A. vera* Against UV-C Exposure

For the study, the medium of the cells growing in sufficient quantity was aspirated and washed with PBS (phosphate buffered saline). Afterwards, it was treated with Tris/EDTA and incubated in an environment containing 5% CO_2 at 37 °C, and the cells were separated from the surface. Then, it was centrifuged at 800 rpm for 5 minutes and the pellet part was homogenized with the medium. Viable cells were counted on Thoma slide using Trypan Blue dye.

In order to evaluate the UV-C protective effect of *A. vera* on cells, HUVEC cells were seeded in petri dishes with 500,000 cells. The previously prepared *A. vera* latex and gel-coated petri dishes were closed on the cells and exposed to UV-C at 254 nm for 1 hour. After counting the viable cells at the end of 1 hour, the cells were exposed to UV-C for 1.5 hours, 2 hours and 3 hours and counting was repeated (Fig 4).

Trial groups are as follows;

1. Control Group: 500,000 cells in the medium (petri dish untreated)
2. Latex One Side: 500,000 cells in the medium + the outer surface of the petri dish is covered with shell
3. Gel One Side: 500,000 cells in the medium + the outer surface of the petri dish is coated with gel
4. Latex Double Side: 500,000 cells in the medium + the inner and outer surfaces of the petri dish are covered with shell
5. Gel Double Side: 500,000 cells in the medium + the inner and outer surfaces of the petri dish are coated with gel

Figure 4. UV-C application to *A. vera* coated cell plates

2.4. Statistical Analysis

Each experiment was performed at least in triplicate. Average values are given with standard error of the mean (SEM). Differences between average values were tested for significance using 2way ANOVA and considered as significant for $P < 0.05$. All statistical tests were carried out utilizing the GraphPad Prism program for Windows version 8.4.2

Table 1. Cell counts in groups exposed to UV-C

UV application time	Control	Latex one-side	Gel one-side	Latex double-side	Gel double-side
Number of Cells Before Application (0th hour)	500000	500000	500000	500000	500000
Viable Cell Count After 1 h UV-C	140000	210000	280000	210000	280000
Viable Cell Count After 1.5 h UV-C	70000	90000	140000	100000	140000
Viable Cell Count After 2 h UV-C	0	0	0	25000***	35000***

3. Results and Discussion

3.1. Morphological Observation of UV-C Damage

The morphological structure of the cells that covered the surface of the flask and did not have any UV-C exposure is shown in Figure 5. Here the cells are spread out and appear to be together. After 1 hour of UV-C application, it is seen that the cells start to die and separate from each other as in Figure 6. After the 2nd hour UV-C exposure, the cells were seen to die completely (Fig 7), while at the 3rd hour, it was observed that the cell nuclei were fragmented (Fig 8).

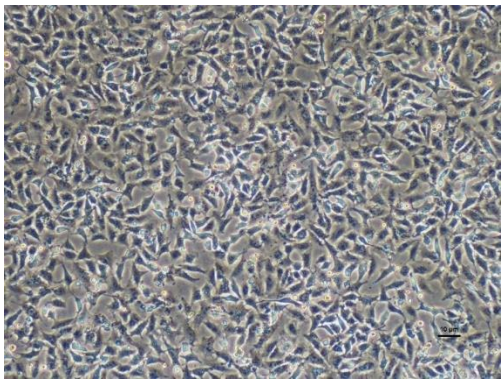

Figure 5. Control group cell image

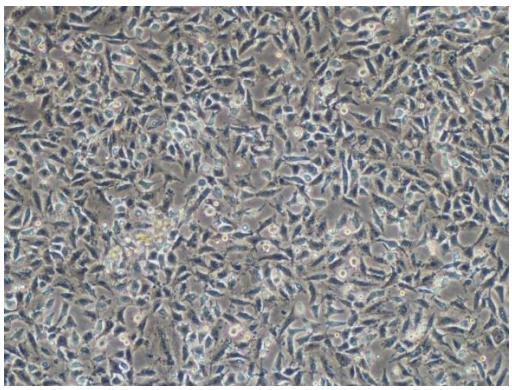

Figure 6. Cell Image Exposed to UV-C for 1 hour

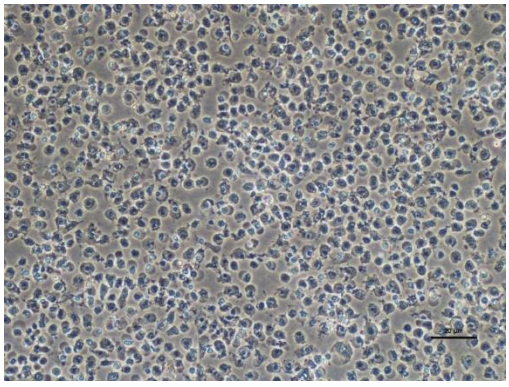

Figure 7. Cell Image Exposed to UV-C for 2 hours

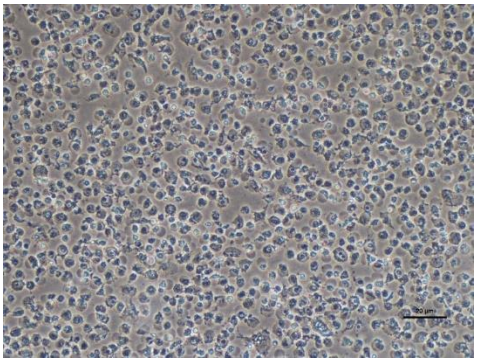

Figure 8. Cell Image Exposed to UV-C for 3 hours

3.2. Determination of Protective Efficacy of *A. vera* Against UV-C Exposure

The numerical differences of the cells in the experimental and control groups as a result of UV-C application are shown in Table 1. When *A. vera* latex and gel layer is added between the cells and the UV-C beam, the change in the situation is remarkable. In the experimental setups formed as a single or double layer with gel, after 1.5 hours of UV application, it was determined that the viability was 2 times higher than the control group, and 1.28 and 1.42 times more viability in the latex single-sided and latex double-sided layer groups, respectively. After 2 hours of UV-C exposure, it was observed that all cells in the control, latex single side and gel single side groups died, while 25000 viable cells were observed in the latex double side group and 35000 live cells on the gel double side.

UV is absorbed by nucleic acids, proteins and cellular membranes. UV absorption leads to the destruction of chemical bonds and free radical formation, initiating a series of oxidative free radical-driven reactions in the presence of oxygen. These reactions cause necrotic and apoptotic cell death [23-24]. It has been found that many plants show protective effects by absorbing UV light in the wavelength range of 300 - 400 nm and activating the antioxidant defense system thanks to their polyphenol content [25-27]. The fact that *A. vera* has a high polyphenol content [28] and has significant antioxidant activity [29] can be considered as the reason why the number of deaths in the *A. vera* containing groups in this study was less than the control. Many similar studies with *A. vera* have also yielded results to support this. When the literature data in which the latex and gel of *A. vera* are evaluated together, it is seen that there are studies that contain the information that UV rays absorption is quite high [30] and that it also has protective properties against UV-A and UV-B damage [31, 32]. The studies on the *A. vera* gel part were examined, it was found that

studies showing the decrease in skin elasticity caused by UV-B [33] and the sterols in the gel prevent skin photo-aging [34]. In addition, when studied in rats and guinea pigs exposed to UV and gamma radiation, it was determined that *A. vera* gel showed wound healing activity in conditions such as cuts, burns and eczema [35-38].

4. Conclusion and Suggestions

According to the findings, it is seen that the leaf and especially the gel parts of *A. vera* are quite effective in protecting healthy cells against UV-C damage. It is thought that this effect may have the potential to be used in many areas. First of all, it is seen that it has the potential to be used as a topical UV-C protective product in the cosmetics industry. In addition, it comes to mind that UV-C protective glasses can be created with *A. vera* leaf and gel to protect people from damage to their eyes. In conclusion, we think that *A. vera* can be used as a natural source of protection against UV-C and the findings obtained should be supported by further studies on the way to product transformation.

Contributions of the authors

The authors' contributions to the paper are equal.**Conflict of Interest Statement**

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics

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