REGENERATIVE PROPERTIES OF ALOE VERA JUICE ON HUMAN KERATINOCYTE CELL CULTURE

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Abstract

Aloe Vera, a medicinal plant widely used in food supplements, beverages, pharmaceuticals and cosmetics, was used as an effective natural remedy for its healing properties and positive influence in different inflammatory skin disorders. In particular, regenerative properties of Aloe Vera juice are investigated on human keratinocyte cells that are used as an in vitro skin model system. The therapeutic efficacy of the plant extract was evaluated using trypan blue assay and the counting cell determination. The obtained results showed that Aloe Vera juice could represent a natural therapeutic strategy through the topical route.

Keywords: Aloe Vera; viability activity; human keratinocyte cells

Introduction

Skin health is increasingly becoming an important aspect of primary health care among many communities. Many preventive and
treatment options are used according to the various international guide lines and therapeutic and cosmetic approaches to achieve varying degrees of success, especially in the case of patients having some degree of skin damage [9, 16]. A novel and alternative approach for possible benefits concerning skin damage is represented by various plant-based treatments [13, 14, 19]. In fact, plant constituents such as carotenoids and flavonoids are involved in protection against excess light and contribute to the prevention of UV damage in humans [15]. At the same time, a wide range of chemical components isolated from the *Fraxinus* species, such as coumarins, secoiridoids, phenylethanoids, flavonoids and lignans are recently being investigated for their anti-inflammatory activity and skin-regenerating effects [8, 18]. Moreover, *Prunella vulgaris* L. (*Labiatae*) and its main phenolic acid component, rosmarinic acid (RA) have shown a remarkable efficacy as photoprotectants against harmful effects of UV radiation [12]. Among various natural therapeutic remedies, *Aloe Vera*, a medical plant widely used in food supplements, beverages, pharmaceutical and cosmetics [4], has been recognized as an effective natural remedy for its healing properties and positive effects in various inflammatory skin disorders [7, 20, 21]. *Aloe Vera* contains 75 potentially active constituents such as vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids and carries on biological and toxicological activities [1]. Aloenin, magnesium lactate, aloe-emodin, barbaloin, cinnamic and succinic acids, mannose-6-phosphate, polysaccharides and flavonoids were found in the *Aloe Vera* juice and play an important role in the anti-inflammatory and antioxidant activity of the plant [5, 6], and they are also responsible of its pharmacological properties [2, 10, 11, 17].

The aim of this study was the evaluation of the regenerative properties of *Aloe Vera* juice on human keratinocyte cell culture, namely NCTC2544 human cell line. It was used as *in vitro* skin based-model system and regenerative properties of plant extracts have been evaluated using trypan blue assay. A suitable effect on cutis regeneration is carried out and curative activities of plant components have been examined as a function of viability percentages of juice.

**Materials and methods**

**Materials**

Human keratinocyte cells (NCTC2544) are obtained from Istituto Zooprofilattico della Lombardia e dell’Emilio Romagna (Brescia, Italy). DMEM (Dulbeco’s Modified Eagle Medium) culture medium, fetal bovine serum (FBS), penicillin-streptomycin solution and Trypsin/EDTA (1x)
solution are purchased from GIBCO (Invitrogen Corporation, Giuliano Milanese (Mi), Italy). Amphotericin B solution (250 μg/ml), trypan blue solution (0.4 % v/v), phosphate buffer solution (PBS) are obtained from Sigma Chemicals Co. (St. Louis, USA). *Aloe Vera* is provided from the botanical garden of University “Magna Græcia” of Catanzaro (Italy). All other materials and organic solvents are of analytical grade (Carlo Erba, Milan, Italy).

**Extraction of juice**

*Aloe Vera* juice is mechanically extracted from plant leaves. They are cut from the trunk and roots of the plant and the terminal tip and lateral thorns are eliminated. The gelatinous substance inside the leaves is separated from the protective external envelop in a dark room to prevent the oxidation of the plant extract. The obtained substance is then divided into different pieces and homogenized (15000 rpm) for 1 min by means of an Ultra-Turrax T 25 equipped with an S25N-8G homogenizing probe (IKAWERKE) under continuous cooling. The triturated mixture is filtered in the dark through 0.45 μm polystyrene filter, divided into 2 ml volume tubes and stored for 3 days at -20°C before being used for the *in vitro* experiments. Every tube used in the experiment was completely wrapped in alluminium foil to prevent any possible degradation of the juice.

**NCTC 2544 cell cultures**

Human keratinocyte cells (NCTC 2544) were transferred from cryovial tubes to plastic culture dishes (100 mm diameter) and seeded at 5 % CO₂ for three days at 37°C by using DMEM supplemented with penicillin (100 UI/ml), streptomycin (100 μg/ml), amphotericin B solution (1 % v/v) and FBS solution (10 % v/v) as culture medium throughout all *in vitro* experiments. After the incubation time, NCTC 2544 cells (80 % of confluence) were harvested with 2 ml of trypsin solution, washed with a phosphate buffer solution (2 ml) and transferred into 10 ml plastic centrifuge tubes. Then, culture medium (4 ml) was added to human keratinocytes, thus achieving a final volume of 8 ml. Cells were centrifuged using a Megafuge 1.0 (Heraeus Sepatech) at 1200 rpm for 10 min at room temperature. Pellets were separated from the supernatant and re-suspended with 6 ml of culture medium to obtain 1×10⁶ cells/ml. The cell suspension was diluted up to a final concentration of 5×10⁴ cells/ml and 1 ml of diluted cellular suspension was seeded into 12 well plastic culture dishes before the *in vitro* investigations.
Viability study

The regenerative property of Aloe Vera extract was evaluated on human keratinocyte cells (NCTC2544) by determining the viability activity of its juice using trypan blue dye exclusion assay. NCTC 2544 cells were seeded at a density of 5×10⁴ cells/ml into 12-well plastic culture dishes. After 48 h of incubation, the culture medium was replaced for each well with a solution of fresh culture medium and Aloe Vera juice was added (1 ml) at final scalar dilutions (10, 30 and 50 % v/v of plant extract). Viability experiments were carried out at different incubation times, i.e. 3, 6, 12, 24, 48 h. Untreated NCTC2544 cells were used as control in the various experiments. To perform the trypan blue dye exclusion assay, NCTC2544 were harvested using trypsin/EDTA (1×) solution (2 ml), washed twice with phosphate buffer solution (2 ml) and transferred into 5 ml plastic centrifuge tubes. Culture medium (4 ml) was added to plastic centrifuge tubes up to a final volume of 8 ml. Samples were then centrifuged with a Megafuge 1.0 (Heraeus Sepatech) at 1200 rpm at room temperature for 10 min. The supernatant was withdrawn and the pellet suspended in 100 μl of trypan blue buffer. The amount of viable cells was observed with a hematocytometer chamber using an optical microscope (Labophot-2, Nikon, Japan). The percentage of cell proliferation was calculated using the following equation:

\[
\text{% Viability} = \left( \frac{CV}{CT} \right) \times 100
\]

where \( CV \) is the number of viable cells treated with different Aloe Vera juice concentrations and \( CT \) the total number of viable control cells. Values of cell viability are expressed as the mean of six different experiments ± standard deviation.

NCTC2544 cell growth determination

NCTC 2544 cells were seeded at a density of 5×10⁴ cells/ml into 12-well plastic culture dishes. After 24 h the cell culture medium was removed and cells were washed with PBS buffer solution (1 ml) and replaced with fresh culture cell medium containing Aloe Vera juice at different concentrations (10 %, 30 % and 50 % v/v). In all experiments, untreated NCTC2544 cells were used as control. The growth of human keratinocyte cells was determined at different incubation times, i.e. 3, 6, 12, 24 and 48 h. The improvement of cell number was carried out by counting the viable cells. NCTC2544 cells were harvested using trypsin/EDTA (1×) solution (2 ml), washed twice with PBS (2 ml) and transferred into 5 ml plastic centrifuge tubes. Culture medium (4 ml) was added to the cells to achieve a final volume of 8 ml of cell suspension. The obtained samples were centrifuged with a Megafuge 1.0 (Heraeus Sepatech) at 1200 rpm at
room temperature for 10 min. The supernatant was withdrawn and the pellet suspended into 100 μl of cell culture medium. The number of NCTC2544 cells was counted with a hematocytometer chamber using an optical microscope (Labophot-2, Nikon, Japan). Each determination represented the mean value of six different experiments ± standard deviation.

**Statistical analysis**

The statistical analysis of the data was carried out using ANOVA t-test. A p value <0.05 was considered statistically significant. All values are reported as the average ± standard deviation.

**Results and discussion**

The therapeutic properties of the various components of *Aloe Vera* have gained a certain amount of attention during the last decades as potential natural remedies in the pharmacological treatment of cutaneous diseases based on the skin degeneration, thus using the regenerative properties of this extract [16].

The regenerative properties of *Aloe Vera* juice were tested on NCTC2544 cells as a function of both the drug concentration (dose-dependent proliferate effect) and the incubation time (plant extract exposition effect). To achieve a clear picture of the real curative effect of these plant components, the cellular proliferation, induced by *Aloe Vera* juice, was evaluated. As shown in Fig. 1, NCTC2544 cells treated with *Aloe Vera* juice showed a percentage of proliferation superior to that of untreated cells (control) for the different concentrations tested (10, 30 and 50% v/v), during an incubation time of 48 hours. The improvement of cell viability percentages, in the NCTC2544 cells, was confirmed by counting the number of cells for the different *Aloe Vera* juice concentrations (Fig. 2). In particular, the cellular growth was directly proportional to the concentration of plant juice used (Fig. 2). This effect is probably correlated to a gradual increase of type I/type III collagen matrix occurring in the case of stimulation of NCTC2544 cells with a concentrated solution of *Aloe Vera* juice, showing how the different concentrations of plant extract were able to improve the amount of collagen in the connective matrix [3]. The obtained results are in agreement with those achieved in the case of NCTC2544 cells stimulated for 48 h. In this case, it was noteworthy that a gradual increase of cell number was obtained for the different concentrations when compared to untreated cells and other incubation times (Fig. 2).
Figure 1
Viability percentages of NCTC2544 human keratinocyte cells treated with Aloe Vera juice at different concentrations: (■) 10% v/v; (○) 30% v/v; (□) 50% v/v. Untreated cells are used as control (●). The cellular viability has been evaluated as a dose-dependent effect for different exposition times: 3 h; 6 h; 12 h; 24 h; 48 h. Error bars if not visible are within the symbols. Each point represents the mean value of six different experiments ± standard deviation.

Figure 2
Improvement of NCTC2544 cell number after stimulation with different concentrations of Aloe Vera juice (10, 30 and 50 % v/v) for various exposition times (3, 6, 12, 24, 48 h). Untreated cells are used as control. Error bars if not visible are within the symbols. Each value represents the mean of at least five different experiments ± standard deviation.
Conclusions

The experimental investigations have shown that plant extracts may be used as natural agents for the treatment of different skin disorders or damage. This effect is probably correlated to the different components present in Aloe Vera juice that are able to promote the regenerative process of the skin as a function of the dose-dependent concentrations and the incubation time.

The obtained results show that Aloe Vera juice offers various opportunities for the development of natural therapeutic strategies through the topical route.

References

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