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Abstract

Anti-Inflammatory and Antioxidant Activity of Aloe Vera Extract in Immortalized Human Keratinocytes HaCaT †

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Aloe vera leaf pulp has been utilized for over 5000 years as a medicinal plant by various civilizations, including the Egyptians, Romans, and Indigenous peoples of Africa, Asia, and the Americas [1]. In contemporary times, it is employed for the treatment of skin infections and burns, digestive disorders, and immune system enhancement [2]. The objective of this study was to investigate the anti-inflammatory and antioxidant activity of an aqueous extract of Aloe vera leaf pulp in immortalized human keratinocytes HaCaT to evaluate its biomedical potential for wound care management. Aloe vera gel powder obtained by leaf pulp freeze-drying was extracted in distilled water and then centrifuged. Preliminary compositional characterization consisted of the determination of total phenolic content by the Folin–Ciocalteu method, protein content using Bradford assay, and ascorbic acid content by the 2,6-dichlorophenolindophenol method. The assessment of in vitro cytocompatibility was performed in a stabilized cell line of human HaCaT keratinocytes treated with different concentrations of Aloe vera. Based on the results of the neutral red assay, the cytocompatible extract concentrations were selected and used in further in vitro tests. An experimental model in vitro mimicking the inflammatory and pro-oxidant milieu specific for skin wounds was developed using HaCaT cells cultivated in stress conditions by treatment with t-butyl hydroperoxide (t-BHP). After 24 h of cultivation in the presence of different concentrations of Aloe vera, the concentrations of the pro-inflammatory cytokine interleukin 8 (IL-8) secreted in the culture media were determined using a specific ELISA kit. In addition, the intracellular reactive oxygen species (ROS) production was quantified using a diacetyldichlorofluorescein assay, flow cytometry analysis, and histogram processing using FlowJo software v10.10. Statistical analysis was performed on control–sample pairs of interest. The following results were obtained in regard to chemical composition: total phenolic content of 3 mg gallic acid equivalents/g dry weight, 0.13 mg protein/g dry weight, and 0.39 mg ascorbic acid/g dry weight. Cell culture testing revealed that Aloe vera extract was cytocompatible within a wide range of concentrations between 0.1 and 1 mg/mL. In the wounded milieu model, the extract showed the capacity to inhibit IL-8 secretion at concentrations of 0.1 and 0.25 mg/mL (Figure 1). Moreover, the extract inhibited the production of intracellular ROS at a concentration of 0.25 mg/mL as a result of high phenolic and ascorbic acid content. In summary, the findings of this study provided further evidence supporting the antioxidant and anti-inflammatory properties associated with Aloe vera gel in stressed HaCaT cell culture. As a result, this research demonstrated

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that Aloe vera gel could be used in the development of composite biomaterials that can be effectively applied in the treatment of skin lesions.

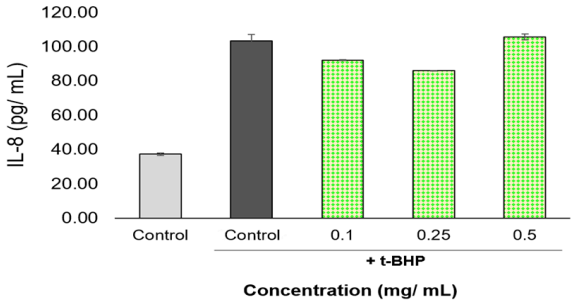

Figure 1. The effect of 24 h pre-treatment with Aloe vera extract on IL-8 production in t-BHP stimulated HaCaT skin cells (light gray—untreated and unstimulated cells; dark gray—untreated, stimulated cells; green—treated and stimulated cells).

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