IN VITRO EVALUATION OF THE BIOLOGICAL ACTIVITY POTENTIAL OF ALOE VERA GEL: ANTIOXIDANT ACTIVITY AND CYTOTOXIC EFFECTS IN HEPG2 AND L929

Zeynep TAŞTAN1, Gulcin ALP AVCI2, Tugba UYSAL KILIC3, Emre AVCI3

Address(es):
1 Hitt University, Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Corum, Türkiye.
2 University of Health Sciences, Department of Basic Medical Sciences, Gülhane Faculty of Dentistry, Ankara, Türkiye.
3 University of Health Sciences, Department of Biochemistry, Gülhane Faculty of Pharmacy, Ankara, Türkiye.

*Corresponding author: gulcin.alpavci@sdu.edu.tr, alp.gulcin@yahoo.com

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ABSTRACT

Background: The place of natural and herbal products in alternative therapy is very important. A. vera is also an important plant that can be used in alternative therapy. Our study aimed to determine the antioxidant activity and cytotoxic effects of Aloe vera gel on HepG2 and L929 cell lines. Methods: The effects of A. vera on HepG2 and L929 were evaluated via the MTT analysis. The total antioxidant status and DPPH methods were used to determine the antioxidant capacity. Results: In our study, the mean cytotoxic effect on HepG2 of A. vera was determined to be 60.47 mg/mL at 24 hours and 44.64 mg/mL at 48 hours. In a healthy L929 fibroblast cell line, the best stimulating effect of A. vera was in 48 hours, and the EC50 value was calculated as 21.98±18.87 µg/mL. TAS was determined to be 0.2 mmol/L in A. vera. Free radical scavenging activity was determined at 21.58 mg/mL. Conclusions: According to the obtained data, it was determined that A. vera gel has a concentration- and time-dependent cytotoxic effect on HepG2, while it has a proliferative effect on healthy cells. The fact that A. vera gel has antioxidant capacity, together with its positive effects on cells, shows that it can be an important support in treatments.

Keywords: Aloe vera, Cytotoxicity, Antioxidant activity, Hep-G2, L929

INTRODUCTION

Located on the African continent (Sudan, Somalia, Socotra Island, and the Arabian Peninsula), Aloe spp. is suitable for cultivation in various regions with warm subtropical and tropical climates (Siri et al., 2020). Aloe vera is a plant that dates to the years before B.C. and is used in various fields. A. vera is used in various skincare, burn and wound treatment, food supports, immune system activating agents, medicine, pharmacology, textiles, and many industrial areas (Bjora et al., 2015; Chen et al., 2023). A. vera leaf gel is used in various skin conditions such as arthritis, conjunctivitis, venereal wounds, bruises, acne, blisters, ringworms, boils, burns, wounds, eczema, and ringworms (Laryea and Boruayne, 2019; Ghuman et al., 2016). Extracts from A. vera gel have been shown to have beneficial heterogeneous properties such as increasing blood flow in scar tissue, penetrating tissue, tissue numbing, preventing bacterial and fungal and viral growth, and expanding capillaries (Waylon et al., 2020). This gel is taken from compounds such as superoxide dismutase, carboxypeptidase, mannose-6-phosphate, and glutathione peroxidase, which function in the biological mechanism when they contain antibacterial, antioxidant, and anti-inflammatory properties (Eid et al., 2019). Thanks to these features, it helps regulate the immune system and heal wounds and burns (Maharjan et al., 2015; Eid et al., 2019). Various toxicity studies on A. vera extracts and gels are available (Matsuda et al., 2008; Maharjan et al., 2015). It has been argued that the content of Aloe vera gel has immune potential, is antimutagenic, antiproliferative, induces apoptosis of tumor cells, and has an anti-metastasis effect (Hussain et al., 2015; Atik et al., 2019). Our study aimed to determine antimicrobial and antioxidant activity and cytotoxic effects on the hepatocellular carcinoma (HepG2) and fibroblast (L929) cell lines of Aloe Vera gel. The aim of our study is to evaluate the biological activity potential of Aloe Vera Gel in Vitro.

MATERIAL AND METHODS

Preparation of A. vera

A. vera (L.) Burm. F. plant is commercially available. Firstly, fresh A. vera leaves were washed and dried at room temperature. In the upright position, the leaf was cut in half, and the gel part was carefully separated with the help of a spatula. The separated gel was homogenized and filtered.

Cell Culture Procedure

The Hepatocellular Carcinoma (HepG2) cell line and the Mouse Fibroblast (L929) cell line were used in our study. Cells were cultured in Dulbecco’s Modified Eagles Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 20 µg/mL penicillin/streptomycin (100 UI), and 1% L-glutamine at 37°C in 5% CO2.

Cytotoxicity Test

3-(4,5 dimethyl-tetrazolium-2-yl) 2, 5-diphenyl tetrazolium bromide (MTT) colorimetric analysis was used to scan cytotoxic activity. For this purpose, it was used in plates with 96 wells, and the experiment was carried out in a total volume of 200 µL. 5x104 cells were cultured in each well of the 96 plates. DMEM and cells were used as positive controls, while only DMEM was used for the negative control. A. vera gel, extract, and milk concentrations were added as 500-250-125-62.5-31.125-15.62-7.81 mg/mL. It was then incubated for 24 to 48 hours. The liquid in the incubation wells was aspirated (50 µL/well), followed by the cells being measured at 570 nm using an ELISA microbe reader. The percentage of living cells determined by the equation % viability = (absorbing processed cells/absorbing control cells) x 100 was determined. IC50 cell growth, the sample amount that provides 50% inhibition, was calculated from a dose-response curve. The cytotoxic effect of A. vera gel and controls was evaluated by comparing the IC50 values of samples for all cell lines.

Determination of Antioxidant Activity

Total antioxidant status measurement

The Total Antioxidant Status Kit (TAS, Rel Assay Diagnostic, Baran Medical, Turkey) was used, and the kit protocol was implemented. Data were evaluated at 600 nm via spectrophotometry according to the manufacturer’s instructions.
DPPH (1,1-diphenyl 2-picrylhydrazyl) free radical sweeper activity determination

DPPH free radical removal activity was carried out according to a variant of the Blois method (1958). DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was calculated and weighed to be 0.1 mM. The weighed amount was dissolved in ethanol and stored in the dark at +4 °C. It was applied to samples at twice the rate, and the sample was incubated in the dark for 30 minutes at 37 °C and measured at 517 nm. After the first measurement, it was incubated in the dark at 37 °C for another 30 minutes and measured at 517 nm.

Statistical analysis

All experiments were designed and performed in repeated and controlled sets. The data obtained in our study were given as the mean value ± standard deviation. In addition, cell analysis data were calculated with the Excel (2016) program, and statistical calculations were made by determining the IC₅₀ and EC₅₀ (half the highest inhibitory concentration and half the maximally effective concentration) values with the GraphPad Prism (9.0) program.

RESULTS AND DISCUSSION

While investigating the cytotoxic effect of the gel content of the A. vera plant on the HepG2 cell line in our studies, the healthy fibroblast cell line L929 was used as the control group. The cytotoxic and proliferative activities of A. vera gel were determined at different rates according to the concentrations. At concentrations ranging from 500 mg/mL to 7.81 mg/mL evaluated in our study, the best percentage of viability on the HepG2 cell line was determined as 7.81 mg/mL at 24 hours and 62.5 mg/mL at 48 hours, as shown in Figure 1.

Figure 2 IC₀ and EC₀ data graph in HepG2 and L929 of Aloe vera gel, respectively

The mean cytotoxic effect on the HepG2 cell line was determined to be 60.47 mg/mL at the 24th hour and 44.64 mg/mL at the 48th hour. Also, the IC₀ (half of the highest inhibitory concentration) values of A. vera gel in the HepG2 cell line are given in Table 1 and Figure 2.

Table 1 Inhibitory and effective concentrations of Aloe vera gel on HepG2 and L929 cells

IC₀ ± SD (µg/mL) | 24th hours | 48th hours
--- | --- | ---
153.0 ± 12.73 | 79.92 ± 34.68
141.4 ± 19.38 | 21.98 ± 18.87

Figure 1 Results of % viability of HepG2 cells and L929 fibroblast cells of Aloe vera gel

The effects of A. vera on the human stomach (AGS), colon (HT-29, HCT116), and hepatocellular (HepG2) cells were investigated (Majarahan et al., 2015). In conclusion, the antitumor effects of A. vera were determined, and it did this by stimulating apoptosis. It did not show any toxic effect on fibroblast cells selected as control cells, and it was emphasized that it did not inhibit their growth (Akey et al., 2015). In our study, at concentrations varying between 500 mg/mL and 7.81 mg/mL, the mean cytotoxic effect was 60.47 mg/mL in 24-hour treatment and 44.64 mg/mL in 48-hour administration. The study that supports these results is the one conducted in our study, it was determined that the viability of the HepG2 cell line, which was applied to A. vera gel, decreased, especially after 48 hours of application. It killed cancerous cells and proliferated healthy L929 cells. In the research, it was seen that our study data were compatible with the current literature. Antioxidants are compounds that prevent, or slow down biomolecule oxidative damage caused by ROS through free radical scavenging and enzyme regulation (Sanchez et al., 2020). Aloe vera has antioxidant properties thanks to its α-tocopherol, carotenoids, ascorbic acid, flavonoids, tannins, vitamin C, vitamin E, chrome, and antraquinome components (Benson et al., 2015). Many studies have shown the antioxidant effect of A. vera. In studies, mice with Type II diabetes mellitus were diagnosed in lymph, kidney, liver, heart, and skin tissues (Haghighi et al., 2020; Cruz et al., 2022). It is stated that lipid peroxidation is inhibited by the effect of A. vera, and it has a protective effect against lipid peroxidation. In our study, unlike other studies, the total antioxidant content of raw A. vera gel Antioxidant levels were measured using the total antioxidant (TAS) and DPPH methods. As a result of the TAS, the data result for A. vera gel raw is 0.2 mmol/L. The antioxidant content is at a normal level, according to TAS kit reference values. When evaluated with the DPPH method, it was found to have an antioxidant capacity of 21.58 mg/mL. Although there are differences in value between the values in the use of A. vera extracted with ethanol and used in its raw
form in the studies, the A. vera plant has antioxidant capacity. It has been reported in studies that the gel has no antioxidant effect when it is used raw, but when it is extracted with water, it has an antioxidant effect with phenols, β-carotene, flavonoids, ascorbic acid, and α-tocopherol content (Haghani et al., 2020). In the study we conducted, A. vera gel was used in its raw form, and it was observed that it could show antioxidant effects with two different detection methods. This result is inconsistent with Ozsoy et al.’s (2009) study (Ozsoy et al., 2009). However, the antioxidant effect was determined in A. vera raw extract.

CONCLUSION

In conclusion, according to the obtained data, it was determined that A. vera gel has a concentration- and time-dependent cytotoxic effect on hepatocellular carcinoma cells, while it has a proliferative effect on healthy cells. The fact that A. vera gel has antioxidant capacity, together with its positive effects on cells, shows that it can be an important support in treatments.

DECLARATIONS

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