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

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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; Chelu *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 Borquaye, 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), 20µg.ml^{-1} penicillin/streptomycin (100 UI), and 1% L-glutamine at 37ºC in 5% CO_2.

Cytotoxicity Test

3-(4,5 dimethyltiyazole 2-il) 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. 5x10^3 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 500-250-125-62.5-31.25-15.62-7.81 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 processed for 3 hours at 37°C with MTT (Sigma Aldrich) solution (10 µL5 mg/mL PBS). Finally, the cells were broken down with 100 µl of DMSO. Absorbance was 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) × 100 was determined. IC_{50} 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 IC_{50} 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 ºC. It was applied to samples at twice the rate, and the sample was incubated in the dark for 30 minutes at 37 ºC and measured at 517 nm. After the first measurement, it was incubated in the dark at 37 ºC 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 IC_{50} and EC_{50} (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^{-1} evaluated in our study, the best percentage of viability on the HepG2 cell line was determined as 7.81 mg.ml^{-1} at 24 hours and 62.5 mg.ml^{-1} at 48th hours, as shown in Figure 1.

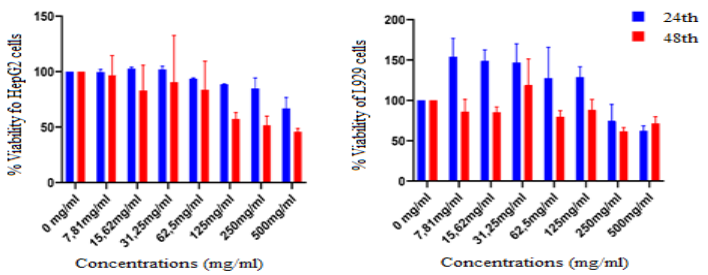

Figure 1 Results of % viability of Hep-G2 cells and L929 fibroblast cells of *Aloe vera* gel

The mean cytotoxic effect on the HepG2 cell line was determined to be 60.47 mg. mL^{-1} at the 24th hour and 44.64 mg. mL^{-1} at the 48th hour. Also, the IC_{50} (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 Hep-G2 and L929 cells

	24th hours	**48 th hours**
IC_{50} ± SD (µg.ml^{-1})	153.0 ± 12.73	79.92 ± 34.68
EC_{50} ±SD(µg.ml^{-1})	141.4 ± 19.38	21.98 ± 18.87

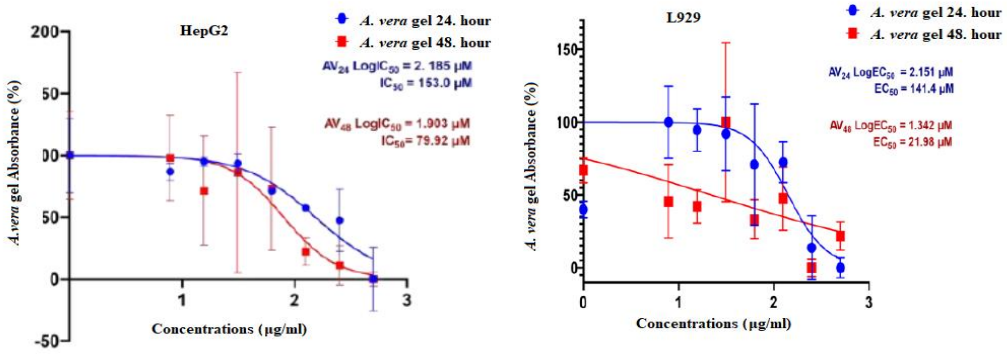

Figure 2 The IC_{50} and EC_{50} data graph in HepG2 and L929 of *Aloe vera* gel, respectively

IC_{50} was 153.0±12.73 and 79.92±34.68, respectively, in the case of exposure for 24 and 48 hours. In parallel with the studies in the literature, it did not damage the cells except for certain doses in the control cells and did not show a toxic effect. The cause of death in healthy cells occurring at certain doses was thought to be due to high doses and insufficient nutrients and oxygen for the cells to use. In the healthy L929 fibroblast cell *line, A. vera was* expected to stimulate, not inhibit. In some wells, the reverse effect was observed depending on the density rate of the gel, and it was determined to inhibit it. The average concentration values were 11.86 mg/ml and 86.45 mg/ml in 24 and 48 hours, respectively. EC_{50} (half-maximal effective concentration) values were calculated (Table 1 and Figure 2). The effect of *A. vera* gel is inversely proportional to the EC_{50} value. The best stimulating effect is *A. vera* in 48 hours, and the EC_{50} value was calculated as 21.98±18.873 µg/ml. As a result of the 24-hour *A. vera* treatment in the L929 cell line, the highest 500 mg/mL concentration was observed, while the 48-hour treatment showed the highest 125 mg/mL concentration.

The percentages of the vitality of *Aloe vera* in living cells are also given in Figure 1. The results determined according to the total antioxidant status measurement kit procedure were interpreted according to the manufacturer's reference values. Antioxidant data, which was determined as 0.2 mmol. L^{-1} in *A. vera* gel, were evaluated as normal according to the references. Also, the DPPH (1,1-diphenyl 2-picrylhydrazil) free radical scavenging activity of aloe vera gel was determined to be 21.58 mg. mL^{-1}.

A. vera is a plant that has been used in various studies in different fields such as food, cosmetics, and health since 1981 **(Tan *et al*., 2013; Sehitoglu *et al*., 2018; Erdem *et al*.,2020; Karatoprak *et al*., 2022)**. In some studies, it has been reported that *A. vera* can prevent hepatocyte damage, induce cell death through apoptosis, decrease cyclin D1 expression, and increase p21 expression. Our study aimed to determine the antioxidant status, cytotoxic effects on HepG2, and proliferation effects on L929 cell lines of A. vera gel. In a study using *A. vera* gel, it was shown that the viability of human breast (MCF-7) and cervical (HeLa) cancer cells decreased according to the concentration and the time they interacted with the substance **(Hussain *et al*., 2015; Atik *et al*., 2019).** In this study, viability was not significantly reduced in healthy cells treated with *A. vera* gel, unlike cancerous cells. The results of this study showed that aloe vera gel significantly reduced the proliferation of cancer cells but was not toxic to normal cells. In a study on hepatocellular carcinoma cells, *A. vera* was examined at concentrations of 62.5–125–250 µg.ml^{-1}, and it has been found to have a cytotoxic effect on HepG2 cells. In the Hormozi et al. (2017) study, mouse embryonic fibroblast cells were exposed to 50–100–150 µg. mL^{-1} *A. vera* 12–24–72 times, and it was reported that cell viability increased depending on the concentration **(Hormozi *et al*., 2020).**

The effects of *A. vera* on the human stomach (AGS), colon (HT-29, HCT116), and hepatocellular (HEP-G2) cells were investigated **(Maharjan *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 Hep-G2 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, chromone, and anthraquinone 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 **(Haghani *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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