

RESEARCH ARTICLE

Immunomodulatory effects of *Aloe vera* and its fractions on response of macrophages against *Candida albicans*

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Abstract

Natural products are important resources in traditional medicine and have been long used for prevention and treatment of many diseases. Medicinal plants have immunomodulatory properties. Aloe is one of the herbal medicines widely used in natural treatment and alternative therapy for various types of diseases. *Aloe vera* has been shown to modulate the immune response. Macrophages have been shown to play an essential role as the first line of defense against invading pathogen. *Candida albicans* is a communal and opportunistic pathogen in humans. In this study, we investigated the effect of *A. vera* extract and its fractions on infected macrophages with *C. albicans*. Viability of intraperitoneal macrophages was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test. Cell viability of infected macrophages was increased by the extract and dose of some isolated fractions dependently. The extract as well as R100, R50, R30, and R10 fractions of *A. vera* significantly increased cell viability of macrophages in most doses. R5 and F5 fractions showed no significant difference in comparison with control group. Further studies in animal models and human are necessary to clarify the modulatory effects of *A. vera* on macrophage function. Isolation and purification of *A. vera* components are also needed to find out the effective molecules.

Keywords: *Aloe vera*, immunomodulatory, cell viability, macrophages, *Candida albicans*

Introduction

Aloe vera (*barbadensis* Mill, family Liliaceae), known as “with yellow,” goat horn,” or “Sgh flower,” in local, is native to Iran and grows in Persian Gulf regions such as Bushehr and Hormozgan provinces. In traditional Iranian medicine (TIM), Aloe was mentioned as a medical plant for treatment of different diseases. Recently, many studies have also shown the efficiency of this herb in a variety of diseases and for validity test of its traditional uses. It has been reported that aloe has different applications as for its effect against viral diseases,⁽¹⁻³⁾ antibacterial activity,⁽⁴⁾ anti-inflammatory activity,⁽⁵⁻⁷⁾ and immunomodulatory action.⁽⁸⁾

The dimorphic fungus *Candida albicans* is both a communal and an opportunistic pathogen in humans. Depending on the underlying host defect, this microorganism is able to cause a variety of infections that range from mucosal candidiasis to life-threatening invasive

infections. The frequency of the latter has increased in recent years as a result of an expanding immunocompromised population.^(9,10) Unfortunately, in patients with immunocompromised diseases (e.g. AIDS) and who use corticosteroids and antibiotic, infections with *Candida* occur,⁽¹¹⁻¹⁴⁾ because innate immunity including phagocytic cells and polymorphonuclear leukocytes (PMN) cannot become active against *C. albicans*.⁽¹⁵⁻¹⁷⁾ These studies indicate that the macrophage activity regulates the control of and resistance to *C. albicans* infections.

Many studies show that plants of genus *Aloe* have immunomodulatory activity including both immunostimulatory and anti-inflammatory effects on macrophages.⁽¹⁷⁻²⁰⁾

A few mechanisms have been suggested for antimicrobial effect of Aloe. Therefore in this study, we determined the effect of *A. vera* gel extract and its isolated fractions

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on macrophages response of Balb/C mice against *C. albicans*.

Materials and methods

Microorganism and culture condition

C. albicans Persian Type Culture Collection (PTCC, 50-27) is used in this study. This isolate was obtained from our laboratory collection. The yeast was cultured on Sabouraud's dextrose agar (SDA) for 24–48 h at 30°C and suspended in culture medium GEYP containing 2% glucose, 0.3% yeast extract, and 0.1% peptone (supplemented with penicillin 100 IU/mL and streptomycin 100 µg/mL) to give a concentration of 10^4 cells/mL. The cells were collected by centrifugation at 1200 g and 5°C for 10 min and washed three times with 10 mM phosphate-buffered saline (PBS, pH 7.4) (Darmstadt, Germany).

Preparation of *A. vera* gel and extraction

A. vera gel obtained from new leaf was mixed with distilled and deionized water with ratio of 1:1 and centrifuged (14,000 g/15 min) to remove insoluble particles. Aloe gel extract in concentration of 1 g/mL was fractionated by using Amicon Ultra-4 Centrifugal Filter Devices (ACFD, Germany). By this method, the extract was fractionated to six fractions based on their molecular weights; R100 (>100 kDa), R50 (100 kDa > R50 > 50 kDa), R30 (50 kDa > R30 > 30 kDa), R10 (30 kDa > R10 > 10 kDa), R5 (10 kDa > R5 > 5 kDa), and F5 (<5 kDa). Extract and fractions were used in different dilutions according to previously published method.⁽²¹⁾

Macrophages cell culture

Peritoneal macrophages were obtained from 8- to 10-week female Balb/C mice (purchased from Razi Research Institute, Tehran, Iran) using 5-mL cold normal saline (injectable grade). Cells were washed twice, resuspended

in RPMI medium (Sigma, St. Louis, MO), and supplemented with 10% fetal calf serum (FCS). Subsequently, the suspensions were centrifuged at 1500 g and 6°C for 10 min. The supernatant was then discarded and RPMI with 10% FCS was added. The number of macrophages was calculated by Neobar cytometer. The 4×10^5 cells per well were added to 96-well tissue culture microtiter plates (Falcon BD Labware, Franklin Lakes, NJ). To remove nonadherent cells, cultures were washed with warm normal saline (injectable grade at 37°C) 2 h after incubation (37°C and 5% CO₂). Macrophages were treated with 2×10^5 *C. albicans* per well after washing, and afterward the RPMI with 10% FCS containing various doses of the extract and its fractions was added to the wells (five wells for each dose). The positive control cultures were five *C. albicans*-infected wells. Also, five wells were induced with mitogen in the presence of *C. albicans* as mitogen-induced control, and five uninfected and untreated wells were used as negative control.

Macrophages cell viability

Macrophages cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. This assay was performed 24 h after incubation of macrophages with various doses of extract and its fractions. The 5 mg/mL MTT (Darmstadt, Germany) was prepared in PBS, filtered, and stored at -20°C and then added to each well as one-tenth of its volume. The supernatants were gently removed 4 h after MTT supplement and the formazan crystals were resolved in acidic isopropanol (0.04 M HCl in isopropanol) and the absorbance values were read at 540 nm with a plate reader (Stat-Fax 2100; Palm USA).

Results

As shown in Figure 1, macrophages viability significantly increased in the presence of mitogen phorbol

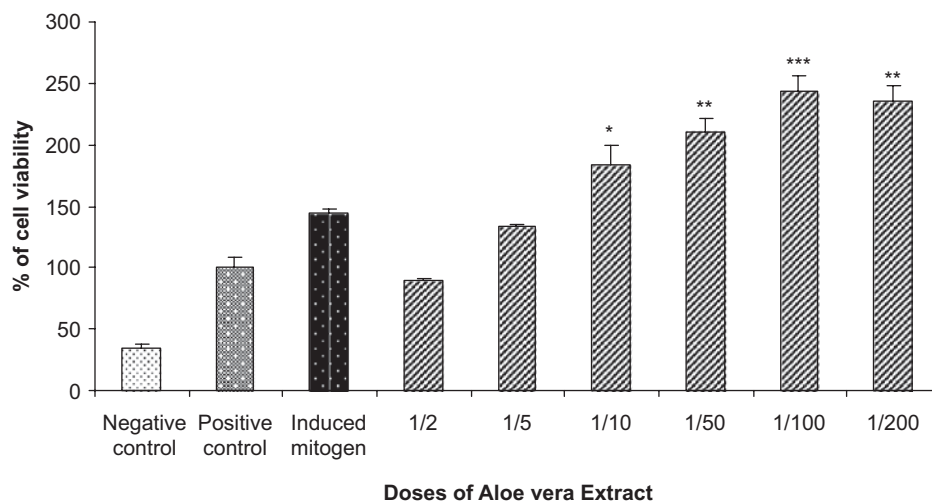


Figure 1. The effect of different doses of *A. vera* gel extract on macrophage viability against *C. albicans* infection. *Denotes significant differences. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared with *C. albicans*-infected positive control. Negative control: uninfected-untreated control group. Positive control: *C. albicans*-infected control group. Induced mitogen: *C. albicans*-infected mitogen-induced group. Mitogen: phorbol 12-myristate 13-acetate (PMA).

12-myristate 13-acetate (PMA) compared with *C. albicans*-infected positive control ($P=0.044$). *A. vera* gel extract in various dilutions including 1:10, 1:50, 1:100, and 1:200 caused significant elevation in macrophages viability in comparison with *C. albicans*-infected positive control ($P=0.025, 0.004, 0.001, \text{ and } 0.002$, respectively). Figure 1 also reveals that some dilutions of *A. vera* gel extract including 1/50, 1/100, and 1/200 caused significant elevation in macrophages viability in comparison with mitogen-induced control.

Data presented in Figure 2 shows that R100 fraction of *A. vera* gel extract in dilutions of 1/5, 1/10, 1/50, 1/100, and 1/200 significantly increase macrophage viability in compared with *C. albicans*-infected positive control ($P=0.024, 0.010, 0.005, 0.005, \text{ and } 0.001$, respectively). Figure 2 also reveals that some dilutions of R100 fraction

including 1/10, 1/50, 1/100, and 1/200 caused significant elevation in macrophages viability in comparison with mitogen-activated positive control ($P=0.003, 0.046, 0.027, \text{ and } 0.000$, respectively).

Data presented in Figure 3 shows that R50 fraction of *A. vera* gel extract in dilutions of 1/5, 1/10, 1/50, 1/100, and 1/200 significantly increases macrophage viability compared with *C. albicans*-infected positive control ($P=0.017, 0.022, 0.005, 0.007, \text{ and } 0.002$, respectively). Figure 3 also indicates that some dilutions of R50 fraction including 1/50 and 1/200 caused significant increase in macrophage viability compared with *C. albicans*-infected positive control ($P=0.013 \text{ and } 0.018$, respectively), and 1/2 dilution caused a significant decrease in macrophages viability in comparison with mitogen-activated control.

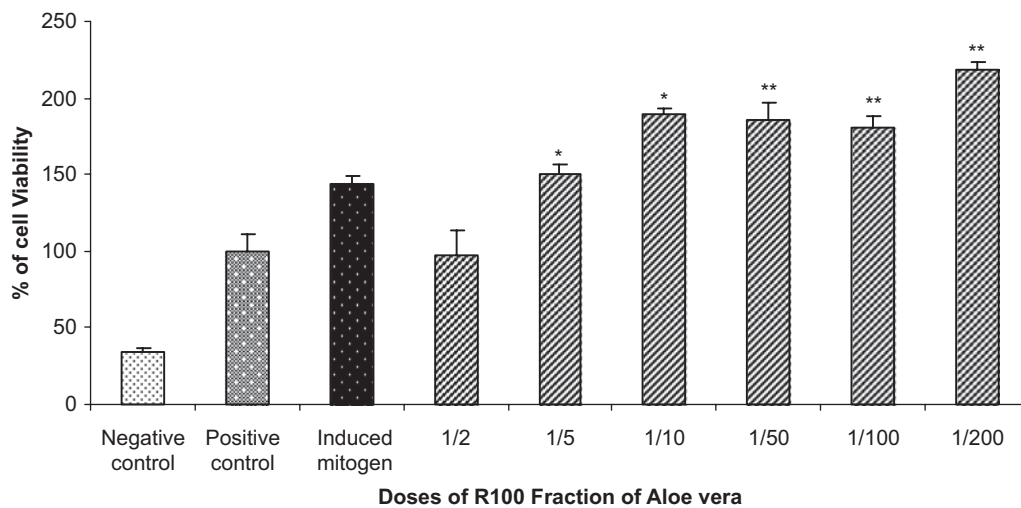


Figure 2. The effect of different doses of R100 fraction of *A. vera* extract on macrophage viability against *C. albicans* infection. *Denotes significant differences. $*P < 0.05$, and $**P < 0.01$ compared with *C. albicans*-infected positive control. Negative control: uninfected-untreated control group. Positive control: *C. albicans*-infected control group. Induced mitogen: *C. albicans*-infected mitogen-induced group. Mitogen: phorbol 12-myristate 13-acetate (PMA).

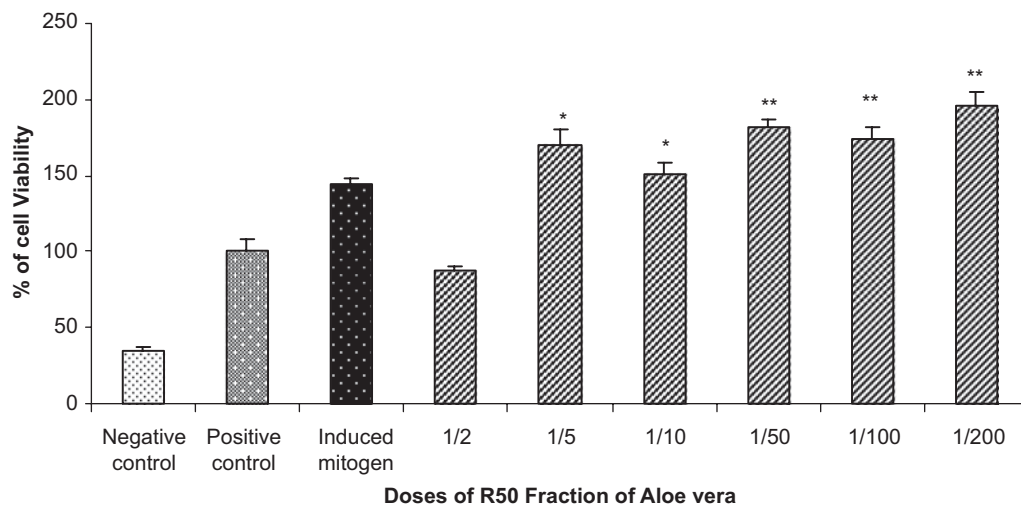


Figure 3. The effect of different doses of R50 fraction of *A. vera* extract on macrophage viability against *C. albicans* infection. *Denotes significant differences. $*P < 0.05$, and $**P < 0.01$ compared with *C. albicans*-infected positive control. Negative control: uninfected-untreated control group. Positive control: *C. albicans*-infected control group. Induced mitogen: *C. albicans*-infected mitogen-induced group. Mitogen: phorbol 12-myristate 13-acetate (PMA).

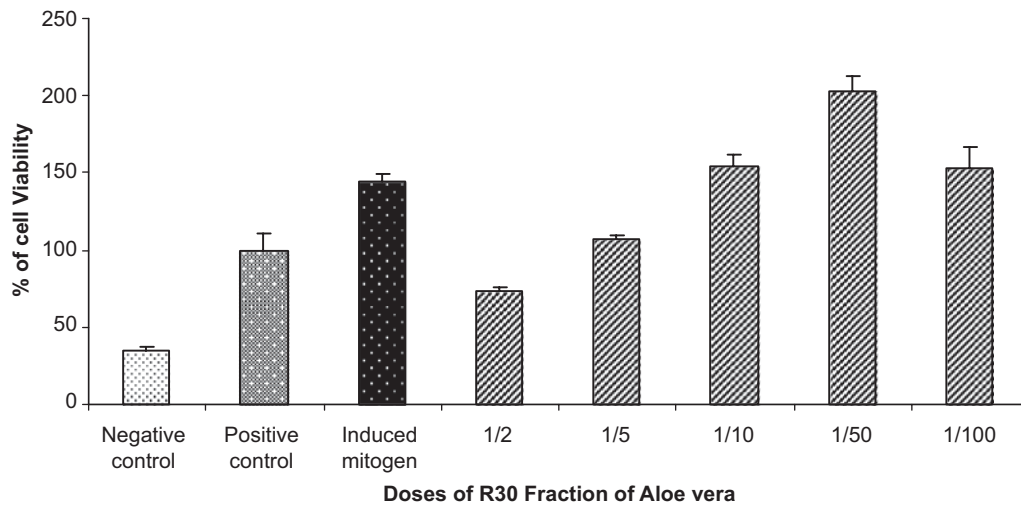


Figure 4. The effect of different doses of R30 fraction of *A. vera* extract on macrophage viability against *C. albicans* infection. Negative control: uninfected-untreated control group. Positive control: *C. albicans*-infected control group. Induced mitogen: *C. albicans*-infected mitogen-induced group. Mitogen: phorbol 12-myristate 13-acetate (PMA).

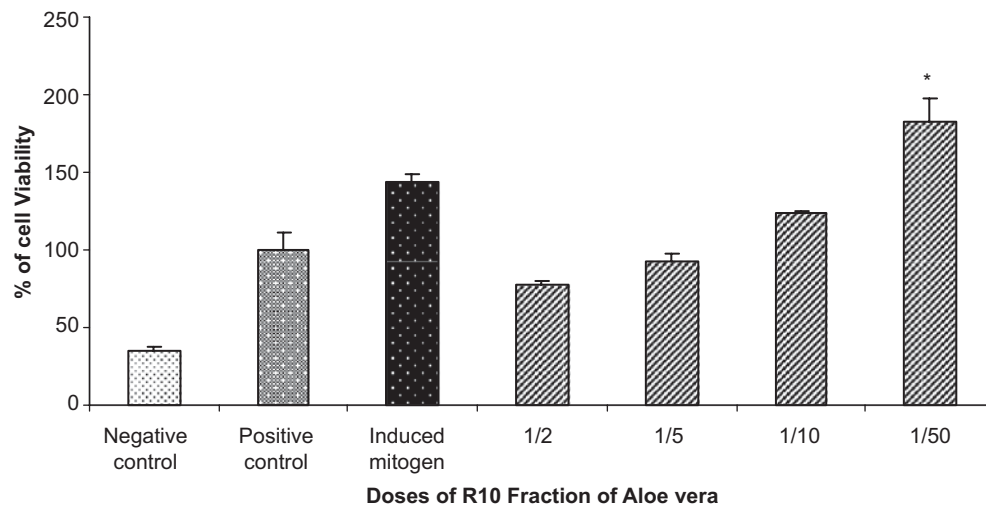


Figure 5. The effect of different doses of R10 fraction of *A. vera* extract on macrophage viability against *C. albicans* infection. Negative control: uninfected-untreated control group. Positive control: *C. albicans*-infected control group. Induced mitogen: *C. albicans*-infected mitogen-induced group. Mitogen: phorbol 12-myristate 13-acetate (PMA).

Data provided in Figure 4 demonstrate that R30 fraction of *A. vera* gel extract in dilutions of 1/10, 1/50, and 1/100 significantly increases macrophage viability in comparison with *C. albicans*-infected positive control ($P=0.018$, 0.002 , and 0.039 , respectively). The figure also shows that 1/50 dilution of R30 fraction caused significant elevation ($P=0.001$), and 1/2 and 1/5 dilutions caused a significant decrease in macrophages viability in comparison with mitogen-activated control ($P=0.001$ and 0.008 , respectively).

Data presented in Figure 5 show that R10 fraction of *A. vera* gel extract in dilution of 1/50 significantly increases macrophage viability in comparison with *C. albicans*-infected positive control ($P=0.013$). Furthermore, the figure indicates that some dilutions of R10 fraction including 1/2, 1/5, and 1/10 caused a significant decrease in

macrophages viability compared with mitogen-activated control ($P=0.002$, 0.001 , and 0.046 , respectively). R5 and F5 fractions showed no significant effects on macrophages viability in all applied doses (Figures 6 and 7).

All of the *C. albicans*-infected *A. vera*-treated or mitogen-induced cultures showed significant differences in macrophages viability compared with negative control cultures.

Discussion

This study was carried out to determine the effect of *A. vera* gel extract and its isolated fractions on peritoneal macrophages against *C. albicans* infection. The results indicated that *A. vera* gel extract induces the macrophage cell viability against *C. albicans*. The optimum effect of *A. vera* gel extract

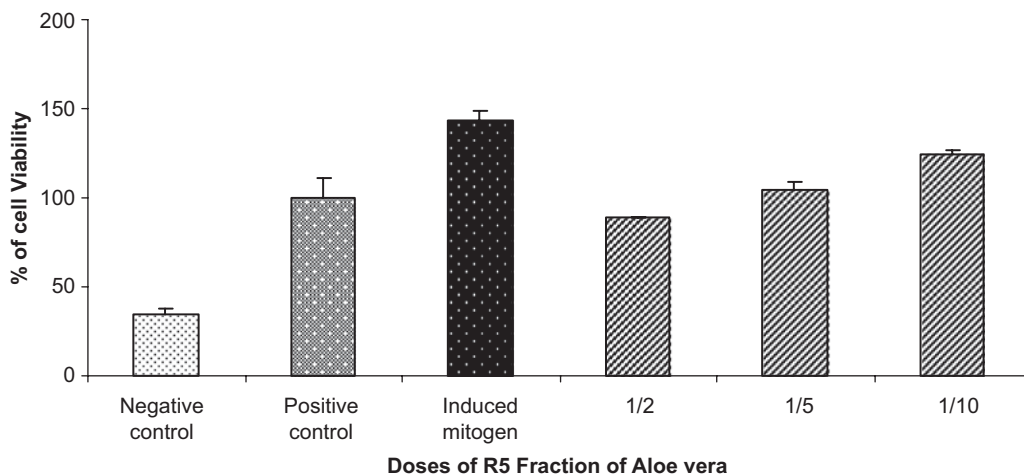


Figure 6. The effect of different doses of R5 fraction of *A. vera* extract on macrophage viability against *C. albicans* infection. Negative control: uninfected-untreated control group. Positive control: *C. albicans*-infected control group. Induced mitogen: *C. albicans*-infected mitogen-induced group. Mitogen: phorbol 12-myristate 13-acetate (PMA).

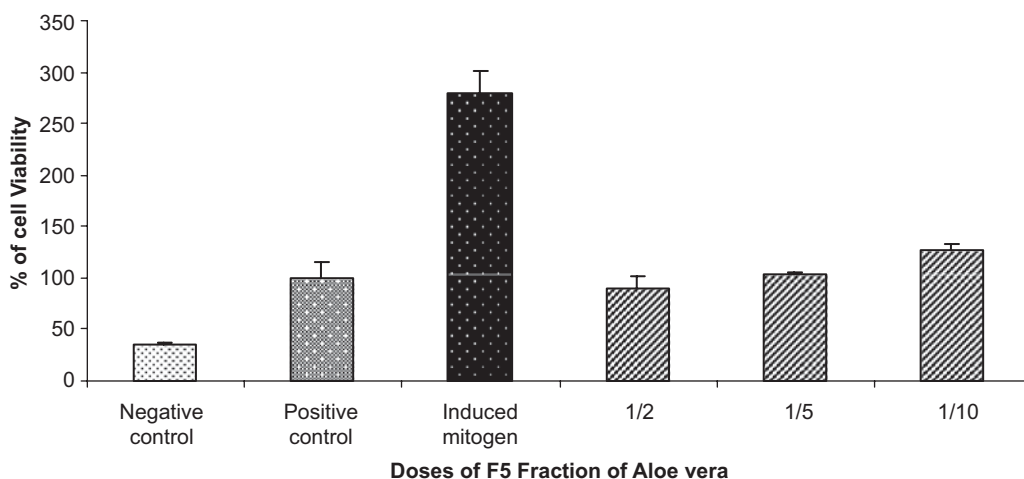


Figure 7. The effect of different doses of F5 fraction of *A. vera* extract on macrophage viability against *C. albicans* infection. Negative control: uninfected-untreated control group. Positive control: *C. albicans*-infected control group. Induced mitogen: *C. albicans*-infected mitogen-induced group. Mitogen: phorbol 12-myristate 13-acetate (PMA).

on cell viability of macrophages is achieved in dilution of 1/100 of *A. vera* gel extract (243% or 2.4-folds of positive control group). In addition, we have performed fractionation of *A. vera* gel extract and isolated six fractions from *A. vera* based on molecular weight ranges. Among these fractions, R100 showed most increasing effect on macrophages cell viability (217% or 2.1-folds of positive control group). Other fractions including R50, R30, R10, R5, and F5 in optimal conditions caused 196%, 203%, 182%, 124%, and 127% effect on macrophage cell viability, respectively.

C. albicans is one of the most frequent pathogens among the medically important *Candida* species, causing severe candidiasis in immunocompromised patients.^(9,10) Recently, there have been many reports on immunomodulatory effects of *A. vera* gel including macrophage activation. Different reports describe a wide variety of both immunostimulating and immunosuppressive effects of *A. vera* gel.⁽¹⁷⁻²⁰⁾ In the present study, we demonstrated

that aqueous extract of *A. vera* in almost all applied concentrations causes significant elevations in macrophages cell viability against *C. albicans*.

Our finding, which indicates that the high-molecular-weight components of R100 fraction of *A. vera* extract induce more macrophage viability in comparison with other fractions, is in agreement with the report by Djeraba et al.⁽¹⁸⁾ They have shown that acemannan, a beta-(1,4)-acetylated mannan isolated from *A. vera* with 274–375 molecular weight, causes an increase in macrophage activity⁽¹⁸⁾; this molecule is isolated in R100 fraction in our study. Although there are some differences between the study of Djeraba et al. and the present research, such as the model to be examined, as well as applied materials and conditions, these findings reveal that the immunomodulatory effect of *A. vera* gel is related to high-molecular-weight components. Our finding also revealed that the immunomodulatory effect of *A. vera* is dose-dependent and lower doses show significant effects.

The results of the present study also designate R50, another fraction that contains components between 50 and 100kDa molecular weights showed immunomodulatory activity by inducing macrophage cell viability. This finding indicates the presence of other active component(s) (50–100kDa) having immunomodulatory properties in *A. vera*. However, other fractions containing lower molecular weights did not show any alteration in the macrophage cell viability with all applied doses. Recently, Das et al. isolated a 14-kDa protein from aloe leaf gel extract with antifungal and anti-inflammatory activity.⁽⁵⁾

In our study, R10 fraction contains molecules between 10 and 30kDa including the 14-kDa isolated protein, as the results showed we did not find any alteration in macrophages cell viability by using R10 fraction.

In conclusion, we proposed the principal immunomodulatory effect of *A. vera* gel extract on macrophage viability against *C. albicans*. Our findings also indicated that R100 fraction that contains high-molecular-weight components (MW > 100kDa) is the most effective fraction of *A. vera*. Further *in vitro* investigations, as well as studies in animal models and human, are needed to clarify the exact mechanisms of immunomodulatory effects of *A. vera* against *C. albicans* infection. Also, biochemical studies are necessary to find out the exact dose and component responsible for this effect.

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Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article.

References

- Lee, J.K., Lee, M.K., Yun, Y.P., Kim, Y., Kim, J.S., Kim, Y.S., Kim, K., Han, S.S., Lee, C.K. Acemannan purified from *Aloe vera* induces phenotypic and functional maturation of immature dendritic cells. *Int Immunopharmacol* 2001, 1, 1275–1284.
- Singh, B., Sood, N. Significance of explant preparation and sizing in *Aloe vera* L. A highly efficient method for *in vitro* multiple shoot induction. *Sci Horticul* 2009, 122, 146–151.
- Gulia, A., Sharma, H.K., Sarkar, B.C., Upadhyay, A., Shitandi, A. Changes in physico-chemical and functional properties during convective drying of *Aloe vera* (*Aloe barbadensis*) leaves. *Food Bioprod Proc* 2010, 88, 161–164.
- Bergamante, V., Ceschel, G.C., Marazzita, S., Ronchi, C., Fini, A. Effect of vehicles on topical application of *Aloe vera* and Arnica Montana components. *Drug Deliv* 2007, 14, 427–432.
- Das, S., Mishra, B., Gill, K., Ashraf, M.S., Singh, A.K., Sinha, M., Sharma, S., Xess, I., Dalal, K., Singh, T.P., Dey, S. Isolation and characterization of novel protein with anti-fungal and anti-inflammatory properties from *Aloe vera* leaf gel. *Int J Biol Macromol* 2011, 48, 38–43.
- Reuter, J., Jocher, A., Stump, J., Grossjohann, B., Franke, G., Schempp, C.M. Investigation of the anti-inflammatory potential of *Aloe vera* gel (97.5%) in the ultraviolet erythema test. *Skin Pharmacol Physiol* 2008, 21, 106–110.
- Vázquez, B., Avila, G., Segura, D., Escalante, B. Antiinflammatory activity of extracts from *Aloe vera* gel. *J Ethnopharmacol* 1996, 55, 69–75.
- Qiu, Z., Jones, K., Wylie, M., Jia, Q., Orndorff, S. Modified *Aloe barbadensis* polysaccharide with immunoregulatory activity. *Planta Med* 2000, 66, 152–156.
- Calderone, R.A. *Candida* and Candidiasis. ASM Press, New York, 2001.
- Garber, G. An overview of fungal infections. *Drugs* 2001, 61 (Suppl 1), 1–12.
- Garbino, J., Kolarova, L., Rohner, P., Lew, D., Pichna, P., Pittet, D. Secular trends of candidemia over 12 years in adult patients at a tertiary care hospital. *Medicine (Baltimore)* 2002, 81, 425–433.
- Krcmery, V., Krupova, I., Denning, D.W. Invasive yeast infections other than *Candida* spp. in acute leukaemia. *J Hosp Infect* 1999, 41, 181–194.
- Mahfouz, T., Anaissie, E. Prevention of fungal infections in the immunocompromised host. *Curr Opin Investig Drugs* 2003, 4, 974–990.
- Tharayil John, G., Shankar, V., Talaulikar, G., Mathews, M.S., Abraham Abraham, M., Punnakuzhathil Thomas, P., Korula Jacob, C. Epidemiology of systemic mycoses among renal-transplant recipients in India. *Transplantation* 2003, 75, 1544–1551.
- Cohen, M.S., Isturiz, R.E., Malech, H.L., Root, R.K., Wilfert, C.M., Gutman, L., Buckley, R.H. Fungal infection in chronic granulomatous disease. The importance of the phagocyte in defense against fungi. *Am J Med* 1981, 71, 59–66.
- Jensen, J., Warner, T., Balish, E. Resistance of SCID mice to *Candida albicans* administered intravenously or colonizing the gut: role of polymorphonuclear leukocytes and macrophages. *J Infect Dis* 1993, 167, 912–919.
- Im, S.A., Oh, S.T., Song, S., Kim, M.R., Kim, D.S., Woo, S.S., Jo, T.H., Park, Y.I., Lee, C.K. Identification of optimal molecular size of modified aloe polysaccharides with maximum immunomodulatory activity. *Int Immunopharmacol* 2005, 5, 271–279.
- Djeraba, A., Quere, P. *In vivo* macrophage activation in chickens with acemannan, a complex carbohydrate extracted from *Aloe vera*. *Int J Immunopharmacol* 2000, 22, 365–372.
- Karaca, K., Sharma, J.M., Nordgren, R. Nitric oxide production by chicken macrophages activated by acemannan, a complex carbohydrate extracted from *Aloe vera*. *Int J Immunopharmacol* 1995, 17, 183–188.
- Zhang, L., Tizard, I.R. Activation of a mouse macrophage cell line by acemannan: the major carbohydrate fraction from *Aloe vera* gel. *Immunopharmacology* 1996, 35, 119–128.
- Ghazanfari, T., Yaraee, R., Farahnejad, Z., Rahmati, B., Hakimzadeh, H. Macrophages activation and nitric oxide alterations in mice treated with *Pleurotus florida*. *Immunopharmacol Immunotoxicol* 2010, 32, 47–50.