

Research Article:

Effect of Aloe Vera Herbal Extract on Innate Immunity in Balb/c Mice Against *Candida Albicans*



Zohre Farrahnejad^{1*}, Ali Mohammad Mohseni Majd²

1. Department of Medical Mycology, School of Medical Sciences, AJA University of Medical Sciences, Tehran, Iran.

2. Immunoregulation Research Center, Shahed University, Tehran, Iran.



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ABSTRACT

Background: Based on the reports, Aloe vera extract, a herbal medicine, has antimicrobial and anti-inflammatory effects. Also, this medicinal plant has considerable effects on innate immunity through cytokine secretion which resist fungal infections such as *Candida albicans*. Moreover, Aloe vera is considered as immunomodulatory agent and regulate inflammatory responses.

Materials and Methods: In this study, twenty Balb/c mice were divided into five groups: four groups of infected mice with *Candida albicans* treated with different doses of Aloe vera extract and one healthy control group. Then, macrophage vital activity was measured by NBT assay. Nitric Oxide (NO) production as representative of macrophage activity was assayed by MTT test. Tumor Necrosis Factor alpha (TNF- α) and Interleukin-12 (IL-12) cytokines were measured by ELISA assay.

Results: Based on our study results, macrophage vital activity and NO production decreased significantly. However, TNF- α and IL-12 cytokines increased significantly in mice model infected by *Candida albicans*.

Conclusion: Aloe vera extract could enhance macrophage activity through increase in TNF- α and IL-12 cytokines in *Candida albicans* infections. Therefore, Aloe vera could stimulate innate immune responses to eliminate *Candida albicans*.

Introduction

Aloe vera possesses some unique and beneficial properties in the field of medicinal plants. In traditional Iranian medicine, Aloe vera was considered as a medicinal plant for the treatment of different diseases [1]. In today's modern medicine, there are no consensus over any disease that can be cured by medicinal

plants or their effective therapeutic ingredients. Several studies demonstrated that Aloe vera has antimicrobial, immunomodulatory, and anti-inflammatory activities against pathogens [2, 3].

Some studies have conducted on the use of mixture of chemical ingredients that makes their statistical interpretation difficult. Limiting spectrum of effects have been studied on immune system that known effects of Aloe

* Corresponding Author:

Zohre Farrahnejad, PhD

Address: Department of Medical Mycology, School of Medical Sciences, AJA University of Medical Sciences, Tehran, Iran.

Phone: +98 (912) 2773149

E-mail: z.farahnejad@yahoo.com

vera extracts often be able to effect on innate immunity insignificantly such as inflammation as compared to adaptive immune responses. However, Aloe vera is considered an immunomodulatory agent in inflammatory conditions [4]. Although, more than 400 species of Aloe vera have been identified so far, Aloe vera (L) Brum. F. is usually utilized in research studies [5]. Also, Tumor Necrosis Factor alpha (TNF- α) and Interleukin-12 (IL-12) cytokines have a prominent role in innate immune responses to eliminate infections. TNF- α production is up-regulated when exposed to infections. Also, IL-12 could help produce other cytokines by immune system components, especially macrophages [6-8].

Some studies indicate that Aloe vera hydroalcoholic extract has antimicrobial activity on a variety fungal species. In this regard, innate immune responses could eliminate the pathogenic fungal organism but Aloe vera could facilitate and potentiate this performance via stimulating macrophage function as innate immune component [9, 10]. According to previous studies, Aloe vera can increase cell viability in macrophage. On the other hand, people who suffer from immunodeficiency diseases can be infected by opportunistic pathogens such as *Candida albicans* [4, 11]. Therefore, we evaluated the effect of Aloe vera extract on innate immunity in Balb/C mice model against *Candida albicans*.

Materials and Methods

Animal preparation

Twenty heads of Balb/c mice were purchased from Pasture institute. Animals in this study were female and healthy and 8-12 weeks old before the experiment. Mice were kept in optimum condition with regard to temperature and humidity. They were randomly divided into five groups of four each. One group is considered as the control group and others were divided into four groups according to the extract dose they received.

Infecting animals with *Candida albicans*

Amounts of 10^6 - 10^7 of *Candida albicans* counted in RPMI medium, were injected into all mice peritoneum and after a week they were investigated.

Preparation of purified Aloe extract

Aloe vera gel obtained from fresh leaves was mixed with distilled and deionized water with ratio of 1:1 and then centrifuged at 14000 g for 20 min to remove insoluble particles. This soluble was passed through 0.2 μ m portions. This study procedure is similar to previous work that performed before.

Determining the extract dose

Normal saline was injected to the control group. Purified Aloe vera extract (100 mg/kg dose) in each injection was considered for the first group. The extract was diluted to 1/2, 1/5 and 1/10 ratio and doses of 50, 20, and 10 mg/kg were prepared for the second to fourth groups, respectively.

Extract administration

About 0.1 mL of normal saline was injected into each mice in the control group for 14 days. Then 0.1 mL of extract with 1/1, 1/2, 1/5 and 1/10 dilution of extract were injected into mice in the first to the fourth experimental groups. Table 1 presents the characteristics of mice groups.

Anesthesia and sampling

Twenty-four hours after the last injection, mice were anesthetized by cotton impregnated with ether. Then the tissues were sampled and kept in the falcon tube under sterile condition.

Macrophages separation

About 5 mL normal saline was injected into each of mice peritoneal cavity and then macrophages were col-

Table 1. Classification of groups received different doses of Aloe vera extract

Groups	Treated Dose (mg/kg)	Average Weight (g)
Control	0	25
1	100	25
2	50	25
3	20	25
4	10	25

lected under sterile condition. The tubes containing macrophages must be maintained at cold temperature to avoid macrophages sticking to tube wall.

Macrophages culture

The obtained suspension from mice peritoneal cavity was centrifuged at 1500 rpm at 8°C for 10 min and supernatant was discarded. For the first washing, 5 mL of cold normal saline was added to sediment macrophages then centrifuged again at 1500 rpm at 8°C for 10 min. Afterward, tube was washed by cold RPMI (Gibco company) and after discarding supernatant, 2 mL cold RPMI containing FBS10% was added to each of mice specific tube and shaken.

Finally, macrophages were counted by Neubauer lam and the obtained macrophage cells at 2 mL of culture medium was counted. Then, a volume of 2×10^5 macrophage cells were counted. In order to culture macrophage cells, we used sterile 96-well plate. The plates were placed in an incubator at 37°C with 5% CO₂. For lymphocytes separation, macrophage cells were sank into the wells bottom and after 1 to 2 hours, the lymphocytes remained stick to the wells. Next, the wells were washed by normal saline to separate lymphocytes from wells. After that, wells volume reached to 200 µL and LPS mitogen was added into three second wells until final concentration reach to 10 µg/mL. The plates were placed in an incubator at 37°C with 5% CO₂.

NO measurement

Sixteen hours after macrophage cell culture, 50 µL of supernatant were transferred into ELISA plates and culture plate was returned for MTT assay. To measure Nitric Oxide (NO) concentration in supernatant, 50 µL of 1% sulfonyl amide solution was added into 5% acid phosphoric and immediately 50 µL of 1% NEDA solution was added into 5% acid phosphoric. After 5 minutes, the optical absorption was measured by ELISA reader at 492 nm wavelength.

MTT assay

After incubating for 24 h, upper supernatant was collected to measure Tumor Necrosis Factor alpha (TNF-α) cytokine. Then, RPMI with 10% FBS in the same volume of supernatant was immediately added to wells and 20 µL of MTT solution were added into each well, then the plates were incubated for 4 h. Finally, 100 µL of isopropranolol were dispensed into each well and optical density was read by ELISA reader at 492 nm wavelength.

NBT measurement

Upper supernatant of the plates was collected and 50% RPMI with 10% FBS plus 50% sterile NBT solution were added into each well to measure NBT. After incubating for 1 h, upper supernatant was discarded and bottom cells were solved by pyridine and optical density was read at 493 nm wavelength.

Cytokine measurement

Cytokine measurement was performed by ELISA assay based on manufacturers' instruction. At first, the supernatant was collected from infected mice with *Candida albicans* which were under Aloe vera treatment. To measure TNF-α (Biosource company), first the antibody with certain concentration was coated on the bottom of 96-wells plate and kept them in the refrigerator for 18 h. After that, blocking level was performed by BSA solution. Next, the fluid inside the wells was moved out then the sample and standard were dispensed into each determined well. The plates were incubated. After washing three times, the second antibody was added and the plates were incubated at room temperature. After washing, enzyme, substrate, and stopping solution were added into wells consecutively at regular intervals. Optical density was read by ELISA reader (ICNflow Company). Interlukin-12 (IL-12) cytokine level was measured by ELISA as in TNF-α cytokine measurement.

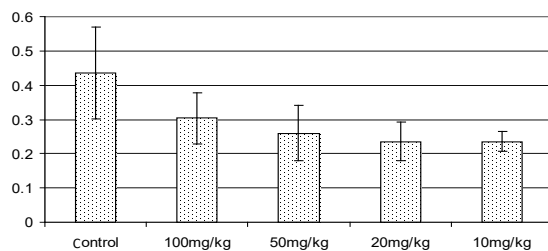
Results

Macrophage vital activity

Different doses of Aloe vera extract significantly decreased macrophage vital activity without mitogen compared to the control group in model mice infected with *Candida albicans* ($P < 0.025$). However, there was significant increase in macrophage vital activity in doses of 20, 50 and 100 mg/kg in the presence of mitogen ($P < 0.034$) (Figure 1). Also, the effect of Aloe vera extract on macrophage vital activity in mice infected with *Candida albicans* demonstrated a significant increase in doses of 20, 50 and 100 mg/kg in comparison to the control group ($P < 0.041$) (Figure 2).

NO production

Results of Aloe vera extract effect on NO production without mitogen showed significant decrease in NO production at treated doses of 10, 20 and 50 mg/kg of Aloe vera as compared to the control group ($P < 0.001$) (Figure 3). Also, there was significant decrease in NO produc-



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Figure 1. Results of MTT assay without mitogen stimulation. The results of macrophage vital activity demonstrated significant decrease in all treated doses of Aloe vera as compared with the control group.

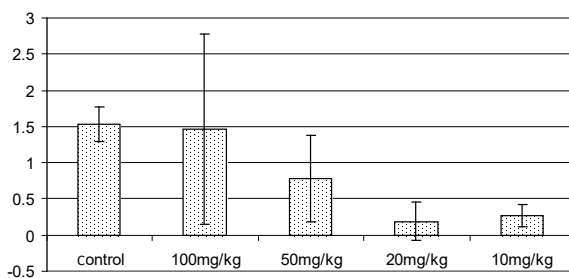
tion at all doses as compared to control group with mitogen stimulation ($P < 0.0001$) (Figure 4).

Cytokine measurement

Results of TNF- α assay with mitogen showed a significant increase in TNF- α in 20 mg/kg treated dose of Aloe vera as compared to the control group ($P < 0.013$) (Figure 5). Also, results of IL-12 with mitogen indicate significant increase in IL-12 at 10 mg/kg dose as compared to the control group ($P < 0.001$). But in treated dose of 100 mg/kg of Aloe vera, significant decrease in IL-12 was observed compared to the control group ($P < 0.01$). Moreover, results of IL-12 without mitogen showed significant decrease in groups which were received Aloe vera extract in doses of 50 or 100 mg/kg ($P < 0.001$).

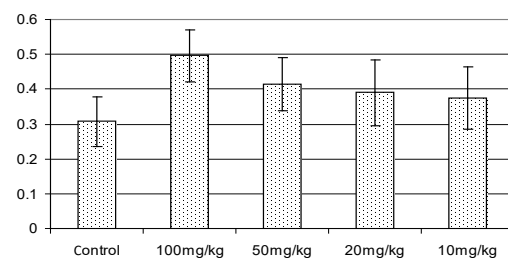
Discussion

Candida albicans is one of the most frequent pathogens among *Candida* spp, causing severe candidiasis in susceptible patients, like those who undergone transplant, have malignancy, or hospitalized in ICU units. There are several approaches to treat or prevent *Candida albicans*. Recent efforts have focused on immunotherapy in *Can-*



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Figure 3. Results of NO production. NO production assay without mitogen stimulation showed significant decrease in treated doses of Aloe vera of 10, 20 and 50 mg/kg compared to the control group without mitogen.



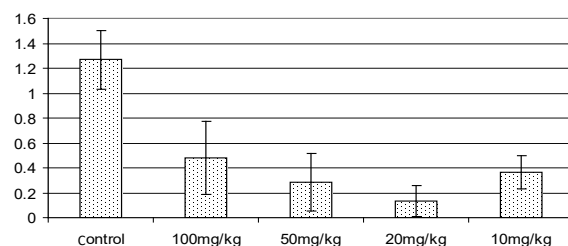
IMMUNOREGULATION

Figure 2. Results of MTT assay with mitogen stimulation. The results of macrophage vital activity showed significant increase in treated doses of Aloe vera of 20, 50 and 100 mg/kg.

didia albicans infection [12]. An increase in macrophage cell viability against *Candida albicans* has been reported after Aloe vera consumption [4, 13, 14]. Furthermore, TNF- α can remove fungal infections and IL-12 secreted by macrophages could help secrete IFN- γ as cellular immunity against fungal infections [6-8].

In the present study, we tried to determine the effect of Aloe vera herbal extract on innate immunity. In this regard, TNF- α and IL-12 cytokine secretion were measured as index of innate immunity and inflammatory responses. Results demonstrated that macrophage vital activity elevated after stimulation with mitogen. But, NO production decreased significantly with or without mitogen. In this regard, Karace et al. reported that carbohydrate derived from Aloe vera could induce NO production from macrophage [15]. In addition, effect of isolated fractions on TNF- α and IL-12 secretion by macrophages against *Candida albicans* was evaluated.

The results showed that TNF- α and IL-12 cytokine increased after stimulating by mitogen in certain doses. The optimum effect of Aloe vera extract on TNF- α secretion by macrophage were obtained at 20 mg/kg dose. Also, the optimum effect in IL-12 secretion was 10 mg/kg with mitogen stimulating but in doses of 50 and 100 mg/kg were the optimum points to produce IL-12 cytokine from macrophage.



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Figure 4. Results of NO production. NO production assay with mitogen stimulation showed significant decrease in all treated doses of Aloe vera compared to the control group.

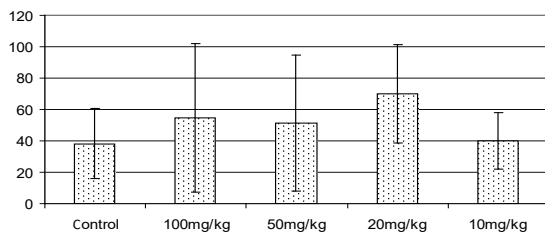


Figure 5. Results of TNF- α assay
The results showed significant increase in 20 mg/kg treated dose of Aloe vera compared to the control group with mitogen.

Mawarti et al. reported that injection of Aloe vera extract could suppress TNF- α secretion in tuberculosis infection [16]. In another study, Habeeb et al. reported that Aloe vera can suppress TNF- α production and modulate inflammatory responses in bacterial infection [17]. Similarly, our results revealed that Aloe vera could promote innate immune responses through increased production of IL-12 and TNF- α cytokines. Macrophage activity was potentiated by these cytokines and could eliminate fungal infections such as *Candida albicans* [18, 19].

Actually, the increased production of TNF- α cytokines is associated with better performance of innate immunity, especially macrophage cells. In addition, IL-12 is able to activate antimicrobial peptides such as cathelicidins, promote autophagy, and inhibit intracellular infectious disease [19, 20]. IL-12 cytokine also leads to differentiate TH1 cells [21]. Taken together, Aloe vera extract can stimulate innate and adaptive immunity to respond the infection with more efficiency. Our findings revealed that Aloe vera has significant effect in eliminating fungal infections via elevated secretion of key cytokines such as TNF- α and IL-12 in vivo study model. Further studies are necessary to evaluate which Aloe vera ingredient(s) has the ability to stimulate immune responses against fungal infectious diseases.

Ethical Considerations

Compliance with ethical guidelines

The study was approved by the ethical committee of Research Ethics Board of Shahed University; with the code of IR.SHAHED.REC.1397.024.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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