

Evaluation of immunological activities of an organic freeze dried inner leaf Aloe vera L. powder

Vaclav Vetvicka

University of Louisville, Department of Pathology, Louisville, KY, USA

Abstract: Aloe vera L. (hereinafter referred to as “Aloe” or “Aloe vera”) has been used for millennia for medicinal purposes both topically and internally. Previous publications have shown a great deal of inconsistency of quality and therefore biological activity from commercially available Aloe vera products. The purpose of this study was to determine the immunomodulatory activity of an organic freeze dried inner leaf Aloe vera powder, which claims to have the highest immunomodulatory Acemannan (Beta 1,4 Acetylated Polymannan) fraction of the commercially available aloes. To accomplish this we chose well established biomarkers and a suitable model to measure phagocytosis, IL-2 production, antibody production, superoxide and nitrite production. The tests chosen were a combination of both in vivo and in vitro models using live mice as well as mouse and human cells. The results clearly demonstrated that BiAloe[®] is a biologically active material in both cellular and humoral immunity.

Background

The healing properties of Aloe vera have transcended the ages since the time of the Egyptians, earning it the name “Plant of Immortality”.¹ Aristotle convinced Alexander the Great to overtake the Isle of Socotra for their Aloe supply.² Ancient Chinese doctors called Aloe the “harmonic remedy” due to its therapeutic properties.³ Current research has largely upheld the therapeutic claims made in earlier papers concerning how the Aloe gel helps in the treatment of inflammation. A common theme running through much recent research is the immunomodulatory properties of the gel polysaccharides due to the acetylated mannans found in Aloe vera.⁴ There are also cautionary investigations warning of possible allergic effects on some patients presumably due to differences in what part of the plant is being used and the processing techniques employed in production. In industrial practice various methods of processing the Aloe vera inner leaf gel result in inconsistency in quality and efficacy.⁵

Introduction

Recently, there has been an increasing interest in using complementary and alternative medicine. The most studied biological response modifiers are natural immunomodulatory polysaccharides. Polysaccharides expressed in Aloe vera are one of those immunomodulatory molecules. They are mostly monoacetyl mannose polymers with β -(1,4)-D-linkage.⁶ This main fraction of the Aloe Polysaccharides is often referred to as Acemannan.

Aloe vera polysaccharides are known to have significant immunoregulatory and immunostimulatory activities.⁷ The main effects include stimulation of phagocytosis, oxidative effects, and stimulation of humoral immunity.^{8,9,10}

In addition, Aloe vera increases collagen content and degree of collagen cross-linkage within the wound, therefore supporting faster wound healing.¹¹ Furthermore, solid data on rats, mice and dogs showed no significant signs of intoxication or death even after massive doses.¹²

Despite clear beneficial effects of Aloe vera polysaccharides, some problems remain. There are virtually hundreds of different preparations on the market, vastly differing in types and quality of isolation, purity, and subsequently, their biological activities. Based on severely limited published comparison of individual samples, we decided to evaluate a commercial sample of an organic freeze dried inner leaf Aloe vera powder with the trade name of BiAloe[®] using the same experimental design as used in a previous study which was a comparative evaluation of another natural immunomodulator β -glucan.¹³

Material and Methods

Aloe vera powder:

BiAloe[®] an organic Aloe vera inner leaf freeze dried powder with minimum Acemannan content of 15%, (Lorand Laboratories LLC, Houston, TX).

Animals:

Female, 8 week old BALB/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All animal work was done according to the University of Louisville IACUC protocol. Animals were sacrificed by CO₂ asphyxiation.

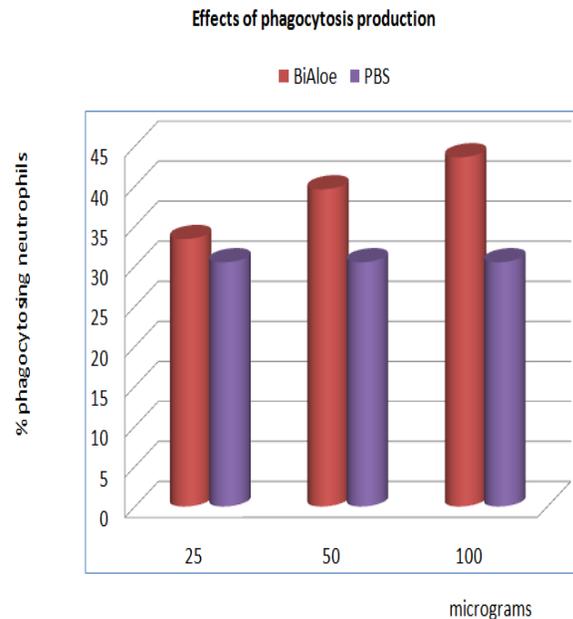
Cell lines:

Human neutrophil cell line HL-60 was obtained from the ATCC (Manassas, VA). The cell lines were maintained in RPMI 1640 (Sigma Chemical Co., St. Louis, MO) medium containing HEPES (Sigma) buffer supplemented with 10% heat-inactivated FCS (Hyclone Lab., Logan, UT), 100U/ml penicillin (Sigma) and 100 μ g/ml streptomycin (Sigma), in plastic disposable tissue culture flasks at 37° C in a 5% CO₂/95% air incubator.

Phagocytosis:

Peripheral blood cells were incubated *in vitro* with 0.05 ml of 2-hydroxyethyl methacrylate particles (HEMA; 5×10^8 /ml, Cesio, Czech Republic). These particles were prepared according to Molday¹⁴ by gamma irradiation-activated copolymerization of a nitrogen-bubbled aqueous solution of a mixture consisting of 2-hydroxyethyl methacrylate, methacrylic acid, and methylene-bis-acrylamide (Sigma). The test tubes were incubated at 37° C for 60 min., with intermittent shaking. Smears were stained with Wright stain (Sigma). The cells with three or more HEMA particles were considered positive.

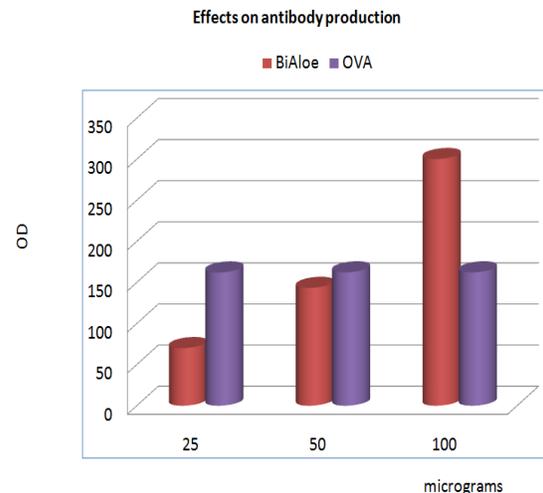
All experiments were performed in triplicate. At least 200 cells in 60 high power fields were examined in each experiment.¹⁵ Samples were diluted in PBS (Sigma) and were administered an intraperitoneal injection at three different concentrations – 25, 50, and 100 micrograms/mouse.



Antibody formation:

Formation of antibodies was evaluated using ovalbumin as an antigen. Mice were injected twice (two weeks apart) with 100 μ g of albumin and the serum was collected 7 days after last injection. Experimental groups were getting daily intraperitoneal (ip.) injections of tested sample.

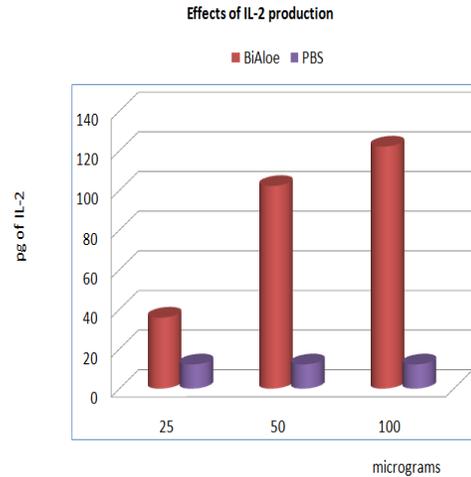
Level of specific antibodies against ovalbumin was detected by ELISA. As positive control, combination of ovalbumin and Freund's adjuvant was used. Samples were diluted in PBS (Sigma) and were administered an intraperitoneal injection at three different concentrations – 25, 50, and 100 micrograms/mouse.



IL-2 production:

Purified spleen cells (2×10^6 /ml in RPMI 1640 medium (Sigma) with 5% FCS, Hyclone Laboratories, Logan), from mice injected tested samples were added into wells of a 24-well tissue culture plate. After addition of 1 μ g of Concanavalin A (Sigma), cells were incubated for 48 hrs in a humidified incubator Hereaus (37° C, 5% CO_2).

At the endpoint of incubation, supernatants were collected, filtered through 0.45 μ m filters and tested for the presence of IL-2 using a Quantikine mouse IL-2 kit (R&D Systems, Minneapolis, MN).

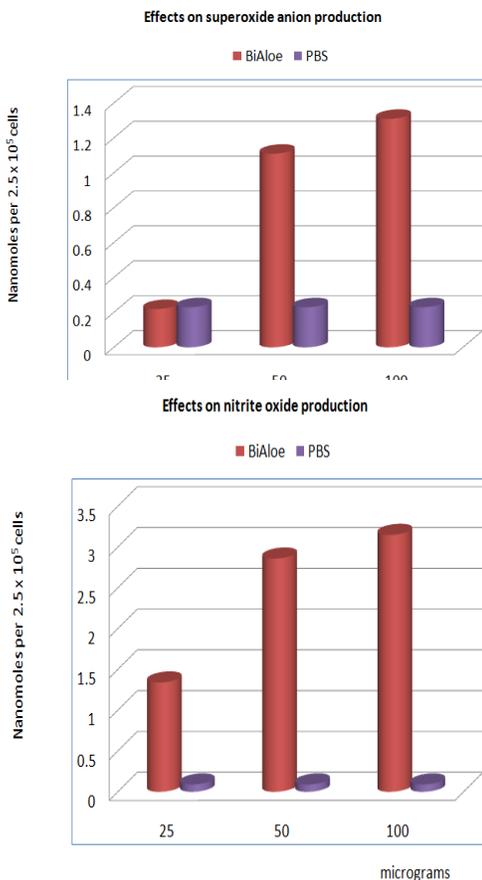


Superoxide and nitrite production:

Cells were incubated in a final volume of 200 μ l of medium containing 0.1 % gelatin and 100 μ M cytochrome C (Sigma, St. Louis, MO, USA). Mice were challenged with individual samples 24 hrs earlier (at concentrations mentioned earlier). Cell lines were incubated with 1 μ g/ml of BiAloe (at concentrations mentioned earlier) for 24 hrs. For the superoxide production, the reaction was initialized by addition of 5 ng/ml PMA (Sigma). After gentle mixing, the absorbance was measured 30 minutes after incubation at 37° C using multiwell spectrophotometer at 550 nm.

Results are expressed as nanomoles of cytochrome C reduced/ 2.5×10^5 cells/30 minutes, after subtraction of the SOD and spontaneous release controls.¹⁶

For a nitrite (NO_2^-) formation we used a technique described by Green and Nacy¹⁷ with LPS (Sigma) as the triggering agent.



Results

First, we evaluated the effects on phagocytosis by peripheral blood neutrophils. We used a well-established technique of synthetic polymer HEMA particles, as their slight negative charge eliminates false positivity. In these tests, the tested sample showed clear dose-dependency. Doses from 50 to 100 μg showed significant effects.

Next, we studied the effects on antibody formation using ovalbumin as an antigen. Lowest two doses produced almost no effects, but the highest dose showed significant stimulation of antibody production (compared to antigen only).

Next experiments focused on secretion of IL-2 by spleen cells. The production of IL-2 was measured 48 hrs after in vitro incubation of splenocytes from BiAloe[®]-stimulated mice. Since the IL-2 production of non-stimulated spleen cells is very low (app. 20 pg), the effects were significant.

Superoxide anion formation was tested on mouse neutrophils and on neutrophil cell line HL-60. In both cases, the BiAloe[®] showed strong activation of superoxide anion formation.

In case of nitrite oxide formation, the situation was identical. We showed strong activation, which was clearly dose-dependent.

Discussion

Despite the extensive amount of scientific reports about Aloe vera products and their biological activities, it is imperative to know the biological effects of every sample prepared for a human market. This extract is known to modulate cellular immunity; therefore we started with evaluation of phagocytosis. We used HEMA particles with no nonspecific adherence on cell surface, which guarantees that only phagocytosing cells will engulf these particles.

Ligand-induced interactions occurring during phagocytosis result in a substantial outburst of metabolic activity often known as an oxidative burst. During these stages, the phagocytosis cells exhibit large increase in oxygen consumption and production of active oxygen species. As numerous immunomodulators are known to stimulate an oxidative burst according to Nerren¹⁸, we decided to evaluate this sample on stimulation of superoxide anion and nitrite oxide. Clearly, BiAloe[®] was highly active with a strong dose dependency.

In addition to the direct effects on various cells involved in immune reactions, Aloe vera extracts are also known for indirect effects causing potentiation of synthesis and subsequent release of cytokines. We focused on the stimulation of IL-2 production by spleen cells in vitro and found that the tested material

significantly stimulated production of IL-2. In the last part of our study we focused on potential effects on antibody response. Using a model of ovalbumin immunization, we showed that BiAloe[®] significantly increase the specific antibody response.

Data presented in this study clearly demonstrated that BiAloe[®] is a biologically active material, particularly in doses of 50 and 100 µg. This material affects both branches of the immune reaction, i.e. the cellular and humoral immunity. Further experiments demonstrating the effects of BiAloe[®] on wound healing, NK cells and additional cytokine synthesis are currently being prepared.

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