Oral administration of *Aloe vera* gel, anti-microbial and anti-inflammatory herbal remedy, stimulates cell-mediated immunity and antibody production in a mouse model

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**Abstract**

**Introduction:** *Aloe vera* (L.) Burm. f. (*Aloe barbadensis* Mill) Liliaceae, succulent plant native to northern Africa, is presently cultivated in many regions of the world. Traditionally, its inner part of parenchyma, which contains aloe gel, was used for the treatment of minor wounds, inflammatory skin disorders, thermal and radiation burns and to alleviate chronic osteoarthritis pain. It also possesses some antimicrobial activity. Now, aloe gel is also increasingly consumed as a dietary supplement. Some data suggest its immunomodulatory properties.

The aim of the study was to evaluate the influence of orally administered aloe gel on some parameters of cellular and humoral immunity viz. mitogen-induced proliferation of splenic lymphocytes and their chemokinetic activity, and anti-sheep red blood cells (SRBC) antibody production in Balb/c mice.

**Results:** Daily treatment of mice for 14 and 21 days with 50 µl or 150 µl of aloe gel dose resulted in enhanced chemokinetic activity and stronger response of their splenic lymphocytes to mitogen PHA and enhancement of anti-SRBC antibody production.

**Key words:** aloe gel, antibody production, chemokinesis, mice, proliferation, splenocytes.

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**Introduction**

Immunomodulation is an important factor for overcoming various acute and chronic infections, especially in the present times when various bacterial species have increasingly become resistant to antibiotics. Taking this into consideration, it has been proved that herbs showing both immunostimulatory and antimicrobial properties might provide useful alternative infections treatment. *Aloe vera* (*Aloe barbadensis*, Liliaceae) is a perennial succulent plant in tropical climates. Aloe gel, the colorless substance obtained from the parenchymatous cells in the fresh leaves of *Aloe vera*, contains polysaccharides (pectins, hemicelluloses, glucomannan, acemannan, and other mannose derivatives) and it should not be confused with the laxative drug “Aloë” (bitter yellow exudate containing anthracene glycosides, product of specialized resin canal cells in the thick leaf epidermis). These two products, despite the fact that they share certain components, have distinctly different properties. Traditionally, aloe gel was mainly used for treatment of inflammatory skin disorders, thermal and radiation burns, arthritis and for wound healing [1].

Recently, it has been reported that *Aloe vera* possess, both *in vitro* and *in vivo*, antimicrobial properties. Its *in vitro* inhibitory activity on some clinically isolated carcinogenic and periodontopathic bacteria was described [2]. In other study, the bacteriostatic effect on *Listeria monocytogenes*, a bacteria responsible for foodborne diseases was observed [3]. Aloe gel and its extracts also exert antimicrobial activity against multidrug-resistant bacteria (MDR) from clinical isolates [4, 5]. In the study of Kwon *et al.* [6], the antimicrobial activity of *Aloe vera* peel extract in distilled water against *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus spp.*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Vibrio spp.*
was ascertained. The number of bacterial colonies was significantly reduced and the observed effect was especially strong against E. coli and Vibrio spp. (p < 0.01). Authors observed also in vivo antimicrobial activity of this extract against Salmonella typhimurium in mice. Gupta et al. [7] reported growth inhibition activity of aloe gel against MDR Mycobacterium tuberculosis isolates. On the other hand, it was observed [8] that Aloe vera juice incorporated into the growth media of Lactobacilli at a concentration of 5% was effective in promoting the growth of L. acidophilus, L. plantarum and L. casei. Whereas, concentration higher than 25% showed an inhibitory effect. Authors concluded that aloe gel in a lower concentration might be used in combination with probiotic Lactobacillus strains as a therapy for gastrointestinal disorders. Banu et al. observed aloe gel-mediated suppression of growth of MDR bacteria in human infected leg ulcers in 28 of 30 patients, by day 11, after daily topical treatment. Cultures of the control group, treated with topical antibiotics, did not show any decrease in the bacterial growth by day 11 [9].

Aloe gel possesses anti-inflammatory properties. It was demonstrated in vitro that aloe gel suppressed bacterial-induced pro-inflammatory [tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β)] cytokines production in human immune cells [10]. In vivo, a protective effect of Aloe vera on polymicrobial sepsis in mice was reported [11]. In this study, administration of Aloe vera reversed the lethality and ameliorated the multiple organ dysfunction syndrome both by enhancing bacterial clearance and by attenuation of pro-inflammatory cytokines production. In vitro, an anti-inflammatory effect of Aloe vera was connected with down-regulation of MMP-9 activity [12].

The key symptom of rheumatoid arthritis is painful inflammation of the joints. There have been some scientific studies about Aloe vera and its use for easing arthritis pain. Oral Aloe vera could be used in the treatment of chronic non-cancer pain, particularly that caused by osteoarthritis [13]. Aloe vera administration topically also inhibits inflammation [14]. The gel of the Aloe vera plant can be also applied directly on the swollen and painful joints. The gel will provide relief of joint immobility and pain, due to its anti-inflammatory properties. Recently, it has been demonstrated that aloe gel acts as an effective gel base to prepare nimesulide emulgel with a significant anti-inflammatory effect for topical delivery in rheumatoid arthritis and other inflammatory conditions [15].

In ophthalmology, Aloe vera extracts may be used in eye drops to treat inflammations and other cornea ailments [16]. Data about the effect of aloe gel and its compounds on immunity are scanty, and mostly connected with its stimulatory effect on macrophages activity [17-19]. Primary screening of the immunotropic activity of aloe preparations elaborated by V.P. Filatov (tissue therapy) was done by Degtiarenko et al. [20]. They found that the studied bioregulators possess immunomodulating action on primary humoral immune response and do not increase a delayed type hypersensitivity reaction. Nersessian and Bogatyrieva showed, in 143 new cases of pulmonary tuberculosis that a combination of chemotherapy and aloe preparations had an immunomodulating effect [21].

The aim of the present study was to evaluate the in vivo influence of orally administered Aloe vera drinking gel on some parameters of cellular and humoral immunity: mitogen-induced proliferation and chemokinetic activity of splenic lymphocytes, and antibody production in Balb/c mice.

Material and methods

Drug

Tru-Alo 99% Aloe vera Drinking Gel (Aloe barbadensis Miller folium succus), Aloin content < 40 ppm; produced by HI TECH ALOE VERA PTY LTD, Bundaberg, Australia.

Animals

The study was performed on 91 female inbred Balb/c mice 6-8 weeks old, weighing about 20 g, delivered from the Polish Academy of Sciences breeding colony. For all performed experiments animals were handled according to the Polish regulations concerning the wellness of laboratory animals (Polish National Institute of Health) standards. All experiments were accepted and conducted according to ethical guidance of the Local Bioethical Committee. Mice were housed 4-5 per cage and maintained under conventional conditions (room temperature 22.5-23.0°C, relative humidity 50-70%, 12 h day/night cycle) with free access to standard rodent diet and water.

Cell culture experiments

Balb/c mice were fed for 14 or 21 days with aloe gel (50 μl or 150 μl daily dose) in drinking water, or water (controls), then bled in anaesthesia (ketamine 100 mg/kg-Ketamina 10%, Biowet Pulawy, Poland and xylazine 10 mg/kg, Sedazin, Biowet, Pulawy, Poland) and sacrificed by cervical dislocation. Aloe gel doses corresponded to 25 ml or 75 ml given to a person weighing 70 kg (applying the counter 7 for differences between mouse and human in relation of the surface to body mass). Splenocytes were isolated from their spleens under the sterile conditions by straining through stainless sieve and cotton gauze and centrifugation on Histopaque 1077 (Sigma-Aldrich, USA) for 8 min at 400 g in order to remove erythrocytes. Isolated splenocytes were resuspended in Parker culture medium (TC 199, BIOMED, Lublin, Poland).

Mitogen-induced (PHA) splenocytes proliferation assay was performed as previously described with some modifications [22, 23]. Briefly, before establishing the cultures, splenocytes from 2-3 Balb/c mice were pooled. Spleen cell cultures (in multiple repetitions) were incu-
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Spleen cells chemokinesis (spontaneous migration) assay

Chemokinesis assay was performed in vitro according to the Sandberg method [24] in own modification [25]. Briefly, splenocytes were resuspended in Parker culture medium with 5% inactivated FCS, at the final concentration of 30 × 10^6 cells/ml. Afterwards, siliconized capillary tubes were filled with cell suspension, sealed with plasticine, centrifuged (5 min, 450 g) and fixed on the glass plates. Cell levels were marked. After 24 h incubation (37°C, 5% CO₂ humidified atmosphere) the distances of migration were measured in millimeters (mm) at a magnification of 6.5 × and presented as migration units (1 MU = 0.18 mm).

Study of antibody production was performed as previously described with some modification [26]. Briefly, Balb/c mice were fed for 14 days with aloe gel 50 µl or 150 µl daily dose in drinking water, or water (controls), then immunized with 10% SRBC (0.2 ml intraperitoneally) and 7 days later bled in anaesthesia (ketamine 100 mg/kg-Ketamina 10%, Biowet, Pulawy, Poland and xylazine 10 mg/kg – Sedazin, Biowet, Pulawy, Poland) from retro-orbital plexus.

The antibody level was evaluated with hemagglutination assay in inactivated (56°C, 30 min) sera. A number of serum dilutions was performed (from 1 : 1 to 1 : 1024), applying PBS or 0.1 M of 2-mercaptoethanol in PBS. After 60 min incubation at room temperature, 0.5% SRBC were added and the mixtures were incubated for another 60 min at room temperature, then centrifuged (10 min, 150 g) and shaken. The hemagglutination titer was evaluated in a light microscope – as the last dilution, in which at least 3 cell conglomerates were present in at least 3 consecutive fields at objective magnification 20×.

Statistical analysis

Statistical evaluation of the results was performed by two-way ANOVA and Bonferroni Multiple Comparison PostTest (GraphPadPrism).

Results

The results of splenocytes proliferation in cell cultures established with mitogen PHA are presented in Fig. 1. Cells collected from aloe-fed animals responded more vigorously to mitogen than cells isolated from the spleens of control mice. Feeding mice with a lower dose of aloe gel resulted in better splenocytes response to a lower dose of PHA than feeding with a higher one. Response to a higher dose of PHA was the same in both aloe-fed groups.

The results of experiments performed for evaluation of the influence of in vivo aloe gel administration to mice on the in vitro spontaneous migration of their splenocytes in tissue culture, are presented in Fig. 2. In this type of experiment, a stimulatory effect was observed only in mice fed with a higher (150 µl) daily dose of aloe gel, disregarding whether they belong to the group fed for 14 or 21 days.

![Fig. 1. The effect of aloe gel mice feeding for 21 days in 50 µl or 150 µl daily dose on the proliferation of their splenocytes in in vitro culture with mitogen PHA](image1)

![Fig. 2. The effect of aloe gel mice feeding for 14 or 21 days on the ex vivo chemokinetic (spontaneous migratory) activity of their splenic cells in 24 h tissue culture](image2)
Feeding mice with *Aloe vera* gel for 14 days enhanced 2-mercaptoethanol + resistant, anti-SRBC 7 S (IgG) antibody production, disregarding the aloe daily dose (Fig. 3).

### Discussion

The aloe leaf can be processed into two types of juices for commercial use: inner gel juice, produced from gelatinous parenchyma of the leaf, and decolorized whole leaf juice, produced by treatment of ground leaf with activated charcoal, to remove aloe latex. Toxicological studies of aloe gel and its main polysaccharide (acemannan) did not reveal adverse effects associated with a high intake of these products at acute or subchronic periods [27-29].

In the present paper we have found for the first time that aloe gel feeding of experimental mice increased their splenic cells motility and response to mitogen PHA, and anti-SRBC antibody production, disregarding the aloe daily dose (Fig. 3).

Recently, Halder *et al.* has reported, in rats fed with the aqueous extract of *Aloe vera*, augmented anti-SRBC antibody production and decreased delayed-type hypersensitivity to keyhole limpet haemocyanin [32]. Enhancement of anti-SRBC antibody production and health improvement were also observed in chickens suffering from coccidiosis, administered with the *Aloe vera* extract [33]. Similarly, the protective effect of *Aloe vera*-based diet in *Eimeria maxima*-infected broiler chickens was observed [34].

*In vitro*, Topman *et al.* did not observe the effect of *Aloe vera* on other cell (fibroblast) motility [35], in vivo, in agreement with our results obtained ex vivo with splenic lymphocytes, but in a different experimental model, Liu *et al.* reported stimulation of macrophage migration by polysaccharides derived from aloe gel [18]. In humans, the *in vitro* and *in vivo* impact of *Aloe vera* juice plus vitamin C on natural killer (NK) cells activity, was assessed in 27 healthy volunteers. Cytotoxicity of NK cells was significantly increased compared to the pre-supplementation values [36].

*Aloe vera* is a rich source of polysaccharides, of which mannose-6-phosphate and acemannan are major constituents responsible for its immunomodulatory activity and other, which e.g. enhance the antitumor activity of chemotherapy drugs [37-39]. It was reported that acemannan, a major carbohydrate fraction of aloe gel, promoted differentiation of immature dendritic cells generated from mouse bone marrow, what might partly explain its immunostimulatory activity [40].

Oligosaccharides prepared from purified aloe polysaccharide prevented suppression of DTH responses *in vivo* and reduced the amount of IL-10 observed in ultraviolet irradiated murine epidermis [41]. High-mannose biological response modifiers, purified from *Aloe vera*/*barbadensis* leaf parenchyma gel, expressed adjuvant immune activity [42], enhancing anti-*coxackievirus* antibody titers in mice [43], improving both the quality of life and the survival rate of *oncornavirus*-infected cats [44] and, in a murine model, stimulating peritoneal macrophages and splenic T and B cells to secrete TNF-α, IL-1β, INF-γ, IL-2 and IL-6 [45]. *Im et al.* have found that polysaccharides between 400 and 5KDa of molecular size exhibited the most potent macrophage-activating activity *in vitro* and most potent antitumor activity *in vivo* [46].

### Conclusions

These data demonstrate that aloe gel administered orally to mice behaves as a stimulator of cellular and humoral immunity, increasing their splenic cells motility and response to mitogen PHA, and anti-SRBC antibody production.

The authors declare no conflict of interest.

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