sciendo

herba polonica
International journal edited by the Institute of Natural Fibres and Medicinal Plants
Vol. 66 No. 3 2020

Received: 2020-08-15
Accepted: 2020-09-10
Available online: 2020-09-30

DOI: 10.2478/hepo-2020-0011

EXPERIMENTAL PAPER

In vivo immuno - and angiomodulatory effects of *Aloe arborescens folii recentis extractum siccum* (AAES) in mice

ROBERT ZDANOWSKI[1], BARBARA J. BAŁAN[2], KARINA SCHÖNKNECHT[3], PIOTR SKOPIŃSKI[4], MARTA STELMASIAK[5], EWA SKOPIŃSKA-RÓŻEWSKA[6], SŁAWOMIR LEWICKI[5,6*]

[1]Laboratory of Molecular Oncology and Innovative Therapies
Military Institute of Medicine
Szaserów 128
04-141 Warszawa, Poland

[2]Department of Immunology, Biochemistry and Nutrition
Medical University of Warsaw
Oczki 3
02-007 Warszawa, Poland

[3]Department of Scientific Information
Phytopharm Klęka S.A.
Klęka 1
63-040 Nowe Miasto nad Wartą, Poland

[4]Department of Histology and Embryology
Medical University of Warsaw
Chałubińskiego 5
02-004 Warszawa, Poland

[5]Faculty of Medical and Health Sciences
Kazimierz Pułaski University of Technology and Humanities
Bolesława Chrobrego 27
26-600 Radom, Poland

[6]Department of Regenerative Medicine and Cell Biology
Military Institute of Hygiene and Epidemiology
Kozielska 4
01-163 Warszawa, Poland

Corresponding author: e-mail: lewickis@gmail.com

Summary

Introduction: AAES is a powdered form of Biostymina, herbal medicinal product of Phytopharm Klęka S.A., a water extract of *Aloe arborescens* Mill. leaves. *Aloe arborescens* Mill. (woody aloe, tree-like aloe) is known to have several traditional medicinal properties including anti-inflammatory, immunomodulatory, antiviral and antimicrobial activity.

Objective: The aim of this work was to study the *in vivo* effect of AAES on cellular (leukocyte-induced cutaneous angiogenesis, LIA test, and proliferative response to PHA) and humoral (anti-SRBC antibody response)immunity in mice.

Methods: Balb/c mice were fed AAES from 0.5 to 75 mg/kg body mass for seven days before grafting their splenocytes intradermally to F1 (Balb/cxC3H) recipients (LIA test). Neovascular reaction was evaluated 72 h later in dissection microscope. Spleen cell cultures were incubated with 0.5, 1 and 2 μg/ml of PHA. After 48 h of incubation, tritiated thymidine was added. After further 24 h, cells were harvested (Skatron) and incorporation of tritiated thymidine was measured using Beta-scintillation counter. Balb/c mice were fed for 7 days with AAES, then immunized intraperitoneally with 5% SRBC suspension and 7 days later the antibody response was measured with hemagglutination test.

Results: Neovascular reaction was significantly higher in groups grafted with splenocytes collected from all AAES fed donors than from the controls. The proliferation of splenocytes taken from mice fed AAES at doses ranging from 0.5 mg/kg to 7.5 mg/kg was stimulated in all cultures. Suppression of proliferation was observed in cell cultures derived from mice fed with higher doses of AAES. Stimulation of anti-SRBC antibody production was seen in mice fed both 2.5 and 7.5 mg/kg dose of AAES.

Conclusion: Powdered form of Biostymina (AAES) might be useful in the treatment of patients with ischaemia of tissues and organs (myocardial infarction, stroke, necrosis) and in deficiency in the production of immune cells and growth factors (infections, chronic wound healing, ulceration and bone fusion).

Key words*: **Aloe arborescens water extract, mice, splenocytes, angiogenesis (LIA test), cell proliferation (PHA), antibody production***

Słowa kluczowe: ***wyciąg wodny z Aloe arborescens, myszy, splenocyty, angiogeneza (test LIA), proliferacja komórek (PHA), produkcja przeciwciał***

INTRODUCTION

Aloe arborescens Miller (woody aloe, also known as tree-like aloe, Krantz aloe or Kidachi aloe), and *Aloe barbadensis* Miller (*Aloe vera*), both belonging to the *Aloe* genus, *Asphodelaceae* (*Liliaceae, Xanthorrhoeaceae*) family, are perennial plants native to Africa with traditionally grounded therapeutic properties, including facilitation of the wound healing process, anti-inflammatory, antiviral and antimicrobial activity. Nowadays, these properties have been confirmed in many studies conducted in experimental and clinical conditions, both *in vitro* and *in vivo* on various species of animals [1-9]. *A*. genus comprises about 400 species, native to Africa. *A. arborescens* is one of the main species of *A*. used in traditional medicine. *A. arborescens* is indigenous to southern Africa, the Arabian Peninsula, and many islands of the Western Indian Ocean. However, it actually grows in a variety of climates and its crops are widespread in many countries. It has the third largest distribution amongst the *Aloe* genus. Its natural habitat usually consists of mountainous areas but can vary and *A. arborescens* is one of only a few species of aloe that is found growing from sea level up to the tops of mountains.

A. vera is used mainly as a skin treatment, promoted as a moisturizer and anti-irritant, present in many hygiene and cosmetic products. *A. arborescens* is used for pharmaceutical purposes mainly. Oral ingestion of *A. vera* succus is potentially toxic (high amount of anthracene glycosides). Isolated from leaves the colorless substance (Aloe gel) containing polysaccharides is used for the treatment of thermal and radiation burns, inflammatory skin disorders, and for improve wound healing [4, 5, 9]. A randomized, comparative double-blinded 8-week study of patients with psoriasis revealed that the effect of *A. vera* cream was similar to that of a cream with a steroid [10].

Although less characterised than *A. vera, A. arborescens* is known to be richer in phytotherapeutic properties. In mice and rats, Beppu *et al.* observed antidiabetic effects of administration of *A. arborescens* components, connected with antioxidant activity which could protect islets of Langerhans, and with an inhibitory influence on glucose absorption in the jejunum [11]. In rats with alloxan-induced diabetes, Mogale *et al.* presented that *A. arborescens* aqueous gel extract influenced the activity of hepatic enzymes and blood concentration of triglycerides, glucose and insulin [12].

In our previous papers for the first time it was shown that *A. vera* and *A. arborescens* extracts suppressed tumour angiogenesis in mice experiments [13, 14, 32].

Lissoni *et al.* in patients with metastatic cancer suggest that *A. arborescens* may be successfully associated with chemotherapy to increase its efficacy in tumor regression rate and survival time [15].

Immunomodulatory properties of plants of *Aloe* genus (strongly expressed for *A. arborescens*) were discovered in 20th century [16-24]. In 1935 in Odessa, W. P. Filatov used tree-like aloe as an inhibitor of corneal transplants rejection [25]. According to Filatov's "tissue therapy", highly active immunological factors arise in the tissues of aloe as a result of biochemical reactions under the influence of cold and darkness. Particularly young tissues separated from the plant are capable to produce such "biogenic stimulants". When such tissues are incorporated into a foreign organism, they show strong therapeutic and stimulatory activity by strengthening the cellular mechanisms and physiological functions of its body. In the case of pathological changes, they increase the regeneration of organs and accelerate the effectiveness of disorders treatment. Since then, some experimental and clinical trials have been conducted in which the immunotherapeutic value of *A. arborescens* extracts, including Biostymina, has been presented. In the following years, numerous studies of immunotropic, anti-inflammatory, antiviral and antimicrobial activity of Biostymina and other *A. arborescens* extracts were carried out in animals and humans [1-4, 26-34]. As a result, it allows to conclude that the use of these drugs is safe in different groups of patients and also can be potentially used in patients who, due to use of cytostatic drugs, have some immunological impairment.

The change of chemical composition of *A. arborescens* by biostimulation (cold stress) was studied by Olennikov *et al* [35]. They observed that this procedure over 5-10 days affected the quantitative composition of many compounds. The content of phenolic compounds reached a maximum on the 5th day of the experiment, of organic acids on the 10th day, of carbohydrate components on 15th day. Cold stress longer than 5–10 days increased biostimulating activity of the raw material, evaluated using yeast test.

Using Filatov's experience, in 1949 professor Jan Muszyński developed a product containing biologically active ingredients obtained from the leaves of tree-like aloe, that was introduced as a medicinal product to treatment under the name Biostymina in 1956 in Poland. The raw material for the production of medicines Bioaron C® and Biostymina is obtained from Phytopharm Klęka S.A. own greenhouse cultivations. Greenhouse cultivation allows maintaining the genetic homogeneity of the raw material and prevents the creation of interspecific hybrids, which allows to maintain constant chemical composition of the obtained extracts [33].

Phytochemical studies on composition of *A. arborescens* leaves revealed the presence of a number of components, from which the highest activity is demonstrated by glycoproteins (lectins) and polysaccharides [33, 34]. They are the main biologically active substances of *A. arborescens*, responsible for the majority of its therapeutic and immunotropic effects. Water-soluble and alkaline-soluble polysaccharides from *A. arborescens* leaves enhanced lymphocyte transformation and phagocytosis [18]. However, many publications indicate that the chemical compounds present in the *A. arborescens* leaf act synergistically and that no single component of the extract is in itself responsible for the action of the plant raw material. In order to discover the activity and prove the effectiveness of preparations based on *A. arborescens* and its components, numerous biological, pharmacological and clinical studies have been carried out so far in many research centers around the world. All of them are valuable evidence that the use of *A. arborescens* extract in medicine as an immunomodulator is right. It should be emphasized, that opposite to typical anthraquinone aloe, such as *A. vera* and *A. ferox*, the therapeutic activity of *A. arborescens* products does not depend on the content of anthraquinones, because *A. arborescens* has low content of them [3].

The aim of our present study was to evaluate the *in vivo* effect of desiccated form of Biostymina, AAES, on the *ex-vivo* leukocyte-induced angiogenesis, lymphocyte proliferation and anti-SRBC humoral response in mice.

MATERIAL AND METHODS

Plant material

Aqueous extract from fresh leaves of *A. arborescens* (Biostymina, Phytopharm Klęka S.A.) in desiccated form (AAES)was prepared from the leaves of 2.5-3-year-old plants, cultivated, collected and identified in greenhouse and laboratories of Phytopharm Klęka S.A. Specification of quality requirements for *A. arborescens* leaves is kept in the Quality Assurance Department of Phytopharm Klęka S.A. After collection, the leaves were stored for several days in cooling conditions. The technological process is confidential. Generally, you can designate production stages such as: crumbling, extraction, maceration, filtration, densification, pasteurization. For experiments, extract was dissolved in distilled water. According to the manufacturer's specifications, Biostymina was free of pathogenic bacteria; aloenin A (marker of activity) was present at minimum levels of 2 mg%, aloin and aloe-emodin (anthra-compounds) were present at maximum levels of 1.32 mg% and 1.10 mg%, respectively [36].

AAES was administered to the 5 experimental groups of 6 Balb/c mice each, for 7 days, orally, by Eppendorff pipette, in 0.5, 2.5, 7.5, 25 and 75 mg/kg body mass, adjusted to 100 μl with water. Mice from the control group received 100 μl of water.

Animals, feeding, and housing

The study was performed on 108 inbred female Balb/c mice (20–22 g of body mass, 7–8 week-old) and on 36 (Balb/c x C3H) F1 hybrids of both sexes (5–6-week-old) delivered from National Institute of Hygiene and from Oncology Center, Warsaw, Poland. During experiments, animals were handled according to the Polish ethical regulations concerning the wellness of laboratory animals (Polish National Institute of Health and NIH standards). All experiments were accepted and conducted according to ethical guidance of the Local Ethical Committee (42/N). Mice were housed up to 6 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.

Preparation of splenocyte suspension was performed according to previously described in [20, 37, 38] with some minor modifications. Briefly, mice were fed with AAES and controls were retro-orbitally bled (3.6% chloral hydrate, Sigma) and euthanized (Morbital-pentobarbital 400 mg/kg, BIOWET, Puławy, Poland). Spleens were aseptically dissected and spleen cells suspension prepared, as described previously [20, 37]. Spleen leukocytes were isolated from aseptically dissected organ (stainless sieve and cotton gauze, Histopaque 1077) rinsed twice and resuspended in Parker culture medium (TC 199, BIOMED, Lublin, Poland) for LIA test, or in RPMI 1640 (BIOMED, Lublin, Poland) for cell culture experiments.

Leukocyte-induced angiogenesis (LIA)

Cell suspensions were pooled within a group and 0.05 ml samples, containing $5x10^5$ spleen cells, each grafted intradermally into narcotized (chloral hydrate) F1 recipients, cells from each pool into 6 recipient mice (3–4 injections per mouse).

After 3 days, graft recipients were sacrificed (Morbital) and on the inner skin surface newly-formed blood vessels were counted in dissection microscope [38].

Cell culture study

Mitogen-induced (PHA) splenocytes proliferation assay was performed as previously described [20]. Briefly, spleen cell cultures (in 12-16 repetitions) with PHA(0, 0.5, 1 and 2 μg/ml) were incubated at 37°C for 48 h, then (3HTdR, 0.2 mCi/ml, specific act. 2 Ci/mM) was added, 24 h later cells were harvested (Skatron) and incorporation of tritiated thymidine measured using Beta-scintillation counter (Rack Beta 1218, LKB Wallac). The arithmetic mean of quadruplicate count was calculated and expressed as counts per minute (CPM).

Study of antibody production (hemagglutination test) was performed as previously described [20]. Briefly, Balb/c mice fed for 7 days with AAES, then immunized with 5% SRBC, bled 7 days later. The hemagglutination titers of sera were established and reciprocal log titers of antibodies were calculated. Indices of stimulation (IS) were calculated dividing log titers of experimental sera by mean log titer of corresponding control group.

Statistical analysis

Bartlett's test for equal variances, one- way ANOVA and Tukey Multiple Comparison Post Test, two-way ANOVA and Bonferoni post-test (GraphPadPrism 5.01software, San Diego, CA). Differences were considered statistically significant at $p<0.05$.

RESULTS

In the present paper we show for the first time the *in vivo* modulatory effect of desiccated form of Biostymina, AAES, on the *ex-vivo* leukocyte-induced angiogenesis (LIA), lymphocyte proliferation and antibody production in mice.

LIA test was performed according to Sidky and Auerbach with some modifications [38]. In this test grafted Balb/c cells recognize foreign C3H histocompatibility antigens and produce many immunological mediators including angiomodulatory factors (immunological angiogenesis). The number of newly-formed blood vessels is the measure of donor cells ability to release pro-angiogenic mediators in this model of local graft-versus-host reaction.

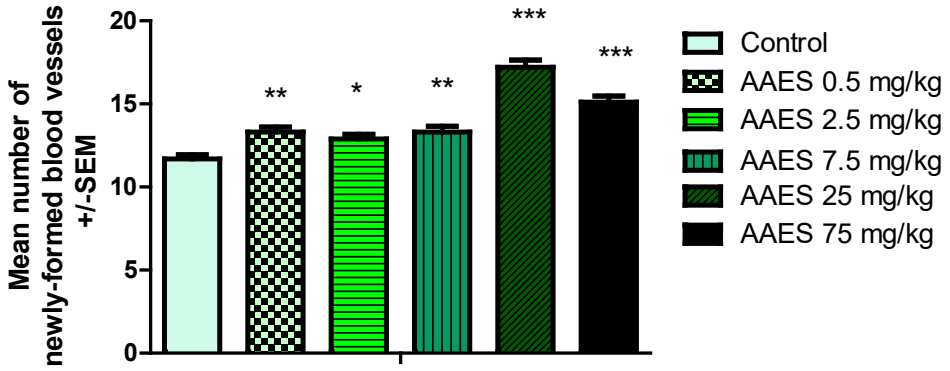
The results obtained for the mice grafted with spleen cells derived from AAES – fed donors are presented in the figure 1 as a mean number of newly-formed blood vessels ± standard error of the mean (SEM). In this experimental design, stimulatory effect was present in all groups of mice injected intradermally with splenocytes deriving from remedy- fed donors, as compare to the recipients of cells from distilled water-fed mice. The highest stimulatory effect was observed in the group grafted with cells obtained from Balb/c mice fed 25 mg/kg of body mass daily.

The proliferation of splenocytes taken from mice fed for 7 days AAES at doses ranging from 0.5 mg/kg to 7.5 mg/kg was stimulated in all cultures. Suppression was observed in cell cultures derived from mice fed with higher doses of AAES (fig. 2).

AAES stimulated the production of anti-SRBC antibodies in groups of mice fed 2.5 and 7.5 mg/kg body mass (fig. 3). Higher doses were not tested.

DISCUSSION

The angiogenesis is a physiological process in which endothelial cells over-proliferate and form new blood

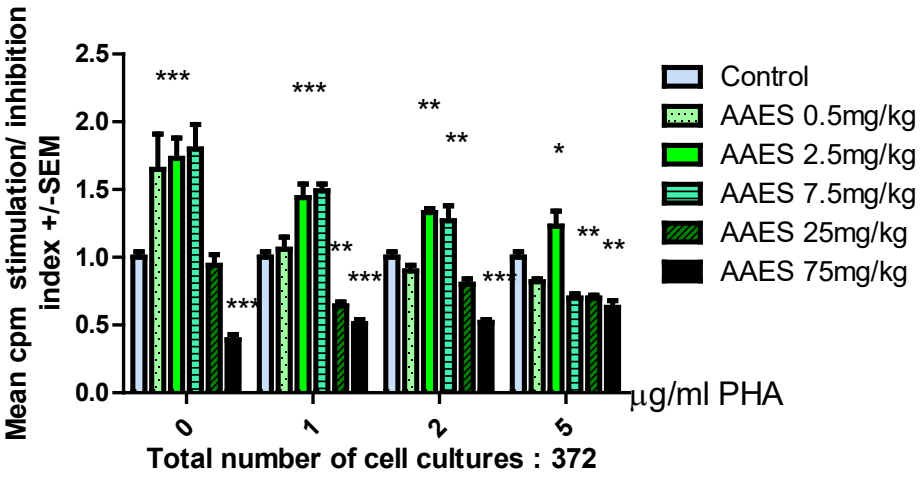


Total number of LIA tests: 136

One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	31.92
R squared	0.5511

Figure 1

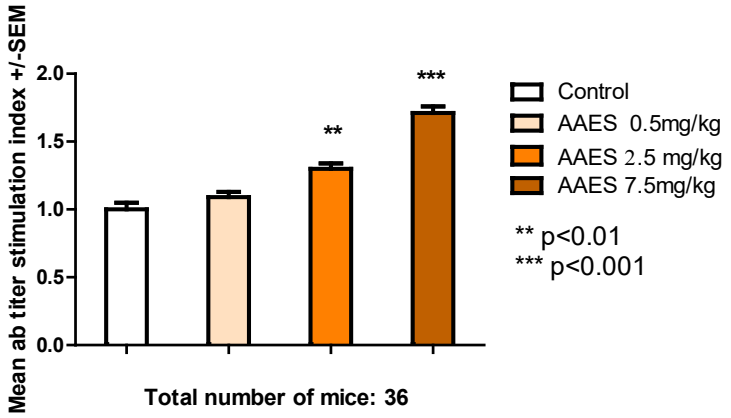
The effect of feeding Balb/c mice with AAES for 7 days on the ability of their spleen cells to induce local GVH reaction in the skin of (Balb/c x C3H)F1 hybrids (leukocyte-induced angiogenesis, LIA test). Data are presented as a mean number of newly-formed blood vessels. *$p<0.05$; **$p<0.01$; ***$p<0.001$



Two-way ANOVA		
Source of Variation	% of total variation	P value
Interaction	10.53	< 0.0001
Dose of drug	31.95	< 0.0001
PHA concentration	7.06	< 0.0001
Source of Variation	P value summary	Significant?
Interaction	***	Yes
Dose of drug	***	Yes
PHA concentration	***	Yes

Figure 2

The effect of feeding Balb/c mice with AAES for 7 days on the response of their spleen cells to PHA in in vitro cell culture (incorporation of 3 H thymidine). Data are expressed as the mean cpm stimulation/inhibition indices, calculated in relation to the parallel control groups. *p<0.05; **p<0.01; ***p<0.001 (Bonferroni post-test, comparison to control group)



One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
F	27.39
R squared	0.7197

Figure 3

The effect of feeding Balb/c mice with AAES for 7 days on the serum level of anti-SRBC antibodies evaluated 7 days after i. p. immunization. Data are expressed as the mean antibody titer stimulation indices, calculated in relation to the parallel control groups

vessels. There is a major group of compounds and more complicated systems which may initiate and prolong formation of new vessels in organism. One of them is the immune system where stimulation of immune-competent cells causes the production of cytokines, chemokines or growth factors which accelerate the angiogenesis. The immunostimulatory activity of Biostymina as well as other extracts from *A. arborescens* has been confirmed by many authors, however, their relationships with angiogenesis are still poor-known. It is known that Biostymina affects both innate and adaptive components of immune system. The first tests assessing rabbits cellular and humoral immune response after Biostymina administration (innate immunity) were performed by Kucharska in 1980 [28]. The studies have shown that administration *i. m.* of liquid extract from fresh leaves of tree-like aloe caused initially (for 3 doses) increases the granulocytes phagocytic capacity in animals. Also Kołacz *et al.* [30] showed an increased activity of neutrophils after the administration of this drug. Similar trend was showed for components of adaptive immunity. Białas-Chromiec *et al.* confirmed that Biostymina in fluid form increased the migration ability of mouse splenocytes and production of anti-SRBC antibodies [32]. It also affected the number and activity of B lymphocytes [28]. Biostymina research conducted by Furowicz *et al.* [29] on calves suffering from bronchopneumonia, subjected to liquid extract from fresh leaves of tree-like aloe, Biostymina, revealed that supplementation was effective in the treatment and prevention of bronchopneumonia and showed greater resistance to infection of lower respiratory tract with the virus, as well as increased the weight gain.

This study presents for the first time the *in vivo* stimulatory effect of *A. arborescens* leaves desiccated extract (Biostymina) on the leukocyte-induced cutaneous angiogenesis in mice. We observed significant stimulatory effect in all groups of mice injected intradermally with splenocytes deriving from remedy-fed donors, as compared to the recipients of cells from distilled water-fed mice. The highest stimulatory effect was observed in the group grafted with cells obtained from Balb/c mice fed AAES 25 mg /kg of body mass daily. These results are in agreement with our previous studies [32] showing stimulated angiogenic activity of human leukocytes isolated from the blood of healthy people and patients with oral infections after the treatment with Biostymina fluid. On the other hand, our other studies revealed different inhibitory effect of *A. vera* and *A. arborescens* fluid extracts on angiogenesis induced in mice skin after grafting of animal or human tumour cells [13, 14, 32]. Moreover, *A. vera* gel administered to mice of the parental strain for a longer time (14-21 days) caused the development in their spleens of a population of cells actively inhibiting the LIA reaction [38].

It corresponds with finding of Kucharska that administration *i. m.* of liquid extract from fresh leaves of tree-like aloe caused initially (for 3 doses) to animals increases granulocytes phagocytic capacity and then, further administration reduced it. This confirms the regularity of most immunomodulators, because repeated administration for a long time is pointless and may lead to a reduction in the effectiveness of the cellular immune system. Findings of study performed in rats by Halder *et al.* suggest that high doses of *A. vera* can modulate immune response by augmenting humoral immunity and decrease the cell-mediated immunity [39]. In present study we also observed lower proliferative activity (both spontaneous and PHA-induced) in cell cultures obtained from animals fed high doses of AAES, than from the control mice spleens.

In the rat model of wound healing, Atiba *et al.* showed that *A. vera* oral administration accelerates acute radiation-delayed wound healing by stimulating the transforming growth factor-ß and fibroblast growth factor (bFGF) production [40]. It corresponds with our previous papers reporting the role of various cytokines and growth factors produced by immunologically competent cells, in cutaneous angiogenesis (LIA test). The most important role in this model of immunological angiogenesis play bFGF, IL-18 and vascular endothelial growth factor (VEGF) [41-43].

Recently, partly indirect (through VEGF induction) role of interleukin 1 cytokine family in angiogenesis was described by Fahey and Doyle [44].

Wahedi *et al.* studied the effect of aloesin from *A. vera* on skin wound healing in mice. Aloesin is a phenolic component of the outer pulp region below the leaf epidermis. Its belongs to phytochemical group known as chromones, and is known as commonly found aloe leaf constituent. Aloesin positively regulated the release of cytokines and growth factors (IL-1β, IL-6, TGF-β1 and TNFα) from macrophages (RAW264.7) and enhanced angiogenesis in endothelial cells. Authors conclude, that aloesin ameliorates each phase of the wound healing process, including inflammation, proliferation and remodeling [45, 46].

CONCLUSIONS

Our paper shows that powdered form of Biostymina (AAES) *in vivo* stimulated angiogenic and proliferative potential of splenic leukocytes and enhanced antibody production in mice. These facts may be important for the treatment of patients with ischaemia of tissues and organs (myocardial infarction, stroke, necrosis) as well as in other pathological conditions correlated with impairment od humoral and cellular immune response in which a deficiency in the production of immune cells and growth factors (infections, chronic wound healing, ulceration and bone fusion) is noted.

Conflicts of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. Mrs. K. Schönknecht is a researcher in Phytopharm Klęka S.A., cooperating in planning the study and discussing the results.

REFERENCES

1. Glatthaar-Saalmüller B, Fal AM, Schönknecht K, Conrad F, Sievers H, Saalmüller A. Antiviral activity of an aqueous extract derived from *Aloe arborescens* Mill. against a broad panel of viruses causing infections of the upper respiratory tract. Phytomedicine. 2015; 15;22(10):911-20. doi: http://dx.doi.org/10.1016/j.phymed.2015.06.006

2. Schönknecht K, Conrad F, Sievers H, Jambor J, Fal AM. Anti-viral activity of Biostymina (*Aloe arborescens* folii recentis extractum fluidum) against viruses causing upper respiratory tract infections tested *in vitro*. Postępy Fitoterapii 2014; 3:127-135.

3. Singab, ANB, El-Hefnawy HM, Esmat A, Gad HA, Nazeam JA. A Systemic review on *Aloe arborescens* pharmacological profile: biological activities and pilot clinical trials. Phytother Res 2015; 29(12):1858-1867. doi: http://dx.doi.org/10.1002/ptr.5483

4. Moriyama M, Moriyama H, Uda J, Kubo H, Nakajima Y, Goto A, et al. Beneficial effects of the genus aloe on wound healing, cell proliferation, and differentiation of epidermal keratinocytes. PLoS One 2016; 13;11(10):e0164799. doi: http://dx.doi.org/10.1371/journal.pone.0164799

5. Teplicki E, Ma Q, Castillo DE, Zarei M, Hustad AP, Chen J, Li J. The effects of *Aloe vera* on wound healing in cell proliferation, migration, and viability. Wounds 2018; 30(9):263-268.

6. Zhong J, Huang Y, Ding W, Wu X, Wan J, Luo H. Chemical constituents of *Aloe barbadensis* Miller and their inhibitory effects on phosphodiesterase-4D. Fitoterapia 2013; 91:159-165. doi: http://dx.doi.org/10.1016/j.fitote.2013.08.027

7. Ranjbar R, Arjomandzadegan M, Hosseiny H. Evaluation of antioxidant activity and growth control properties of nonoscale structure produced from *Aloe vera* var. littoralis extract on clinical isolates of *Salmonella*. Sci Pharm 2017; 31;85(3):28. doi: http://dx.doi.org/10.3390/scipharm85030028

8. Gawon-Gzella A, Michalak A, Kędzia A. Antimicrobial activity of Bioaron C. Acta Pol Pharm 2014; 71(5):795-802.

9. Di Luccia B, Manzo N, Vivo M, Galano E, Amoresano A, Crescenzi E, et al. A biochemical and cellular approach to explore the antiproliferative and prodifferentiative activity of *Aloe arborescens* leaf extract. Phytother Res 2013; 27(12):1819-1828. doi: http://dx.doi.org/10.1002/ptr.4939.

10. Choonhakarn C, Busaracome P, Sripanidkulchai B, Sarakarn P. A prospective, randomized clinical trial comparing topical *Aloe vera* with 0.1% triamcinolone acetonide in mild to moderate plaque psoriasis. J Eur Acad Dermatol Venereol 2010; 24(2):168-172. doi: http://dx.doi.org/10.1111/j.1468-3083.2009.03377.x

11. Beppu H, Shimpo K, Chihara T, Kaneko T, Tamai I, Yamaji S, et al. Antidiabetic effects of dietary administration of *Aloe arborescens* Miller components on multiple low-dose streptozotocin-induced diabetes in mice: investigation on hypoglycemic action and systemic absorption dynamics of aloe components. J Ethnopharmacol 2006; 20;103(3):468-477. doi: http://dx.doi.org/10.1016/j.jep.2005.10.034

12. Mogale MA, Lebelo SL, Shai LJ, Eloff JN. *Aloe arborescens* aqueous gel extract alters the activities of key hepatic enzymes and blood concentration

of triglycerides, glucose and insulin in alloxan-induced diabetic rats. Afric J Biotechnol 2011; 10(20):4242-4248.

13. Skopiński P, Zdanowski R, Bałan BJ, Siwicki AK, Kocik J, Lewicki S, et al. *Aloe arborescens* and American cranberry (*Vaccinium macrocarpon*) extracts inhibit tumor-induced cutaneous angiogenesis in mice. Cent Eur J Immunol 2013; 38(4):480-485. doi: http://dx.doi.org/10.5114/ceji.2013.39765

14. Kocik J, Bałan BJ, Zdanowski R, Jung L, Skopińska-Różewska E, Skopiński P. Feeding mice with *Aloe vera* gel diminishes L-1 sarcoma-induced early neovascular response and tumor growth. Cent Eur J Immunol 2014; 39(1):14-18. doi: http://dx.doi.org/10.5114/ceji.2014.42116

15. Lissoni P, Rovelli F, Brivio F, Zago R, Colciago M, Messina G, et al. A randomized study of chemotherapy versus biochemotherapy with chemotherapy plus *Aloe arborescens* in patients with metastatic cancer. In Vivo 2009; 23(1):171-175.

16. Strickland FM, Pelley RP, Kripke ML. Prevention of ultraviolet radiation-induced suppression of contact and delayed hypersensitivity by *Aloe barbadensis* gel extract. J Invest Dermatol 1994; 102(2):197-204. doi: http://dx.doi.org/10.1111/1523-1747.ep12371762

17. Stuart RW, Lefkowitz DL, Lincoln JA, Howard K, Gelderman MP, Lefkowitz SS. Upregulation of phagocytosis and candidicidal activity of macrophages exposed to the immunostimulant acemannan. Int J Immunopharmacol 1997; 19(2):75-82. doi: http://dx.doi.org/10.1016/s0192-0561(97)00010-6

18. Nazeam JA, Gad HA, Esmat A, El-Hefnawy HM, Singab AB. *Aloe arborescens* polysaccharides: *in vitro* immunomodulation and potential cytotoxic activity. J Med Food 2017; 20(5):491-501. doi: http://dx.doi.org/10.1089/jmf.2016.0148

19. Byeon SW, Pelley RP, Ullrich SE, Waller TA, Bucana CD, Strickland FM. *Aloe barbadensis* extracts reduce the production of interleukin-10 after exposure to ultraviolet radiation. J Invest Dermatol 1998; 110(5):811-817. doi: http://dx.doi.org/10.1046/j.1523-1747.1998.00181.x

20. Bałan BJ, Niemcewicz M, Kocik J, Jung L, Skopińska-Różewska E, Skopiński P. Oral administration of *Aloe vera* gel, anti-microbial and anti-inflammatory herbal remedy, stimulates cell-mediated immunity and antibody production in a mouse model. Cent Eur J Immunol 2014; 39(2):125-130. doi: http://dx.doi.org/10.5114/ceji.2014.43711

21. Hormozi M, Assaei R, Boroujeni MB. The effect of *Aloe vera* on the expression of wound healing factors (TGFβ1 and bFGF) in mouse embryonic fibroblast cell: *in vitro* study. Biomed Pharmacother 2017; 88:610-616. doi: http://dx.doi.org/10.1016/j.biopha.2017.01.095

22. Prakoso YA, Kurniasih. The effects of *Aloe vera* cream on the expression of CD4+ and CD8+ lymphocytes in skin wound healing. J Trop Med 2018; 6218303. doi: http://dx.doi.org/10.1155/2018/6218303.

23. Womble D, Helderman JH. Enhancement of allo-responsiveness of human lymphocytes by acemannan (Carrisyn). Int J Immunopharmacol 1988; 10(8):967-974.

24. Bastian P, Fal AM, Jambor J, Michalak A, Noster B, Sievers H, et al. Candelabra aloe (*Aloe arborescens*) in the therapy and prophylaxis of upper respiratory tract infections: traditional use and recent research results. Wien Med Wochenschr 2013; 163(3-4):73-79. doi: http://dx.doi.org/10.1007/s10354-012-0171-3

25. Degtiarenko TV, Ivanova AS, Skvortsov VIu, Masternak TV, Larin AS. Pervichnyĭ skrining immunofarmakologicheskoĭ aktivnosti preparatov tkanevoĭ terapii po V.P. Filatovu [Primary screening of the immunopharmacologic activity of Filatov tissue therapy preparations]. Oftalmol Zh 1989; 1:34-39 [in Russian].

26. Nersesian ON, Bogatyreva EV. Vliianie khimioterapii v komplekse s tkanevymi preparatami na nespetsificheskiĭ immunitet u boľnykh tuberkulezom legkikh [Effect of chemotherapy combined with the use of tissue preparations on nonspecific immunity in patients with pulmonary tuberculosis]. Probl Tuberk 1990; 1:28-31 [in Russian].

27. Fal AM, Schönknecht K, Jambor J. Immunomodulacyjna rola Biostyminy® i Bioaronu® C w profilaktyce i leczeniu infekcji górnych dróg

oddechowych [Immunomodulatory role of Biostymina® and Bioaron® C in the prevention and treatment of upper respiratory tract infections]. Wiad Lek 2016; 69(1 Pt 2):77-84 [in Polish].

28. Kucharska E. Wpływ biostyminy na niektóre procesy immunologiczne [Effect of biostymine on various immunological processes]. Ann Acad Med Stetin 1980; 26:369-386 [in Polish].

29. Furowicz AJ, Czernomysy-Furowicz D, Lewandowska S. Wykorzystanie biostyminy w terapii i profilaktyce bronchopneumonii cieląt. Przegląd Hodowlany 1989; 9:10-15 [in Polish].

30. Kołacz R, Rodak E, Świtała M, Gajewczyk P. Herbs as agents affecting the immunological status and growth of piglets weaned with body weight deficiency. J Animal Feed Sci 1997; 6:269-279.

31. Picchietti S, Bernini C, Belardinelli MC, Ovidi E, Taddei AR, Guerra L, et al. Immune modulatory effects of *Aloe arborescens* extract on the piscine SAF-1 cell lone. Fish Shellfish Immunol 2013; 34(5):1335-44. doi: http://dx.doi.org/10.1016/j.fsi.2013.02.019

32. Białas-Chromiec B, Skopińska-Różewska E, Strzelecka H, Filewska M, Radomska D, Mierzwińska-Nastalska E, et al. The immunomodulatory effect of Biostimine-water soluble extract of the leaves of triennial plants *Aloe arborescens* Mill. Onkologia Polska 2000; 3(2):85-89.

33. Anuszewska E, Drozd J, Drozd E, Gruber B. Immunomodulatory activity of two polysaccharide fractions isolated from Biostymina (*Aloe arborescens* folii recentis extractum fluidum). Postępy Fitoterapii 2015; 16(2):83-88.

34. Koike T, Beppu H, Kuzuya H, Maruta K, Shimpo K, Suzuki M, Titani K, Fujita K. A 35 kDa mannose-binding lectin with hemagglutinating and mitogenic activities from "Kidachi Aloe" (*Aloe arborescens* Miller var. natalensis Berger). J Biochem 1995; 118(6):1205-1210. doi: http://dx.doi.org/10.1093/oxfordjournals.jbchem.a125008

35. Olennikov DN, Ibragimov TA, Chelombit VA, Nazarova AV, Rokhin AV, Zilfikarov IN. Chemical composition of *Aloe arborescens* and its change by biostimulation. Chem Natur Comp 2009; 45(4):478-482.

36. Kodym A. Biologically active substances of the leaves of the tree aloe (*Aloe arborescens* Mill.) grown in greenhouses of Herbapol Klęka. Farmacja Polska 1998; 54(19):887-892.

37. Lewicki S, Stankiewicz W, Skopińska-Różewska E, Wilczak J, Leśniak M, Suska M, et al. Spleen content of selected polyphenols, splenocytes morphology and function in mice fed *Rhodiola kirilowii* extracts during pregnancy and lactation. Pol J Vet Sci 2015; 18(4):847-855. doi: http://dx.doi.org/10.1515/pjvs-2015-0110

38. Skopiński P, Lewicki S, Bałan BJ, Kocik J, Zdanowski R, Skopińska- Różewska E, et al. *In vivo* inhibitory effect of *Aloe vera* gel on the ability of mouse parental splenic lymphocytes to induce cutaneous angiogenesis in recipient F1 mice. Pol J Vet Sci 2014; 17:131-136. doi: http://dx.doi.org/10.2478/pjvs-2014-0017

39. Halder S, Mehta AK, Mediratta PK. Augmented humoral immune response and decreased cell-mediated immunity by *Aloe vera* in rats. Inflammopharmacology 2012; 20(6):343-346. doi: http://dx.doi.org/10.1007/s10787-012-0134-8

40. Atiba A, Nishimura M, Kakinuma S, Hiraoka T, Goryo M, Shimada Y, et al. *Aloe vera* oral administration accelerates acute radiation-delayed wound healing by stimulating transforming growth factor B and fibroblast growth factor production. Am J Surg 2011; 201(6):809-818. doi: http://dx.doi.org/10.1016/j.amjsurg2010.2010.06.017

41. Skopiński P, Skopińska-Różewska E, Sommer E, Siwicki AK, Wasiutyński A. The effect of some diet-derived angiogenesis inhibitors and sulindac sulfone on the ability of VEGF, bFGF and IL-18 to induce cutaneous neovascular response in mice. Pol J Environ Studies 2005; 14 (Suppl. II):325-329.

42. Skopiński P, Szaflik J, Partyka I, Chorostowska-Wynimko J, Duda-Król B, Lipińska A, et al. Serum *in vivo* angiogenic activity and some proangiogenic cytokine levels in diabetes mellitus type 2 patients with or without background retinopathy. Centr Eur J Immunol 2007; 32(2):48-52.

43. Skopińska-Różewska E, Chorostowska-Wynimko J, Skopiński P, Sommer E, Bałan BJ, Bany J. Important role of bFGF in the angiogenic activity of human serum evaluated by the mouse cutaneous test. Centr Eur J Immunol 2007; 32(2):45-47.

44. Fahey E, Doyle SL. IL-1 family cytokine regulation of vascular permeability and angiogenesis. Front Immunol 2019; 10:1426. doi: http://dx.doi.org/10.3389/fimmu.2019.01426

45. Wahedi HM, Jeong M, Chae JK, Do SG, Yoon H, Kim SY. Aloesin from *Aloe vera* accelerates skin wound healing by modulating MAPK/Rho and Smad signaling pathways *in vitro* and *in vivo*. Phytomedicine 2017; 28:19-26. doi: http://dx.doi.org/10.1016/j.phymed.2017.02.005

46. Salehi B, Albayrak S, Antolak H, Kręgiel D, Pawlikowska E, Sharifi-Rad M et al. *Aloe* genus plants: from farm to food applications and phytopharmaco-therapy. Int J Mol Sci 2018; 19(9):2843. doi: http://dx.doi.org/10.3390/ijms19092843