Feeding mice with Aloe vera gel diminishes L-1 sarcoma-induced early neovascular response and tumor growth

JANUSZ KOCIK¹, BARBARA JOANNA BALAN², ROBERT ZDANOWSKI¹, LESZEK JUNG³, EWA SKOPIŃSKA-RÓŻEWSKA¹,¹, PIOTR SKOPIŃSKI⁵

¹Military Institute of Hygiene and Epidemiology, Warsaw, Poland
²Department of Immunology, Biochemistry and Nutrition, Warsaw Medical University, Warsaw, Poland
³CKR Rehabilitation Center, Konstancin-Jeziorna, Poland
⁴Pathology Department, Center for Biostructure Research, Warsaw Medical University, Warsaw, Poland
⁵Department of Histology and Embryology, Center for Biostructure Research, Warsaw Medical University, Warsaw, Poland

Abstract
Aloe vera (Aloe arborescens, aloe barbadensis) is a medicinal plant belonging to the Liliaceae family. Aloe vera gel prepared from the inner part of Aloe leaves is increasingly consumed as a beverage dietary supplement. Some data suggest its tumor growth modulatory properties. The aim of the present study was to evaluate in Balb/c mice the in vivo influence of orally administered Aloe vera drinking gel on the syngeneic L-1 sarcoma tumor growth and its vascularization: early cutaneous neovascular response, tumor-induced angiogenesis (TIA test read after 3 days), and tumor hemoglobin content measured 14 days after L-1 sarcoma cell grafting.

Feeding mice for 3 days after tumor cell grafting with 150 µl daily dose of Aloe vera gel significantly diminished the number of newly-formed blood vessels in comparison to the controls. The difference between the groups of control and Aloe-fed mice (150 µl daily dose for 14 days) with respect to the 14 days’ tumor volume was on the border of statistical significance. No difference was observed in tumor hemoglobin content.

Key words: Aloe vera gel, mice, tumor growth, angiogenesis.

Introduction
Aloe vera gel is a colorless substance obtained from the parenchymatous cells in fresh leaves of Aloe vera (L) Burm. f. (Aloe barbadensis Mill) Liliaceae. Native to North Africa, Aloe has been introduced and is being cultivated in the warmer areas of the world. Aloe vera gel, rich in polysaccharides (pectins, hemicelluloses, glucomannan, acemannan, and other mannose derivatives) should not be confused with the drug “Aloe” – dried juice of Aloe vera leaves, bitter yellow exudate containing anthracone glycosides, mainly of the aloe-emodin anthrone 10-C-glucoside type [1, 2].

Aloe vera gel is commonly consumed as a beverage, dietary supplement. It is a traditional herbal remedy without unwanted side-effects. Consumed as a beverage it was not toxic in vivo for mice [3]. On the contrary, aloe latex and its hydroxyanthrone derivatives (aloin, aloe-emodin etc.) have strong laxative properties and their longer use requires medical supervision [2].

Traditionally, Aloe gel was widely used for the treatment of minor wounds, inflammatory skin disorders, and thermal and radiation burns. In vitro, Aloe gel suppressed bacteria-induced pro-inflammatory [tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β)] cytokines and matrix metalloproteinase 9 (MMP-9) production in human mononuclear leukocytes [4, 5].

In vivo, polysaccharides derived from Aloe vera gel, injected into mice, potently stimulated migration of macrophages to the peritoneal cavity [6].

In human, oral Aloe vera gel was used by patients with inflammatory bowel disease [7], osteoarthritis, and other inflammatory conditions. Oral and topical administration of Aloe vera gel diminished inflammation and eased joint immobility and pain [8-11].

In ophthalmology, Aloe vera extracts may be used in eye drops to treat inflammations and other cornea ailments [12].

Besides its anti-inflammatory activity, Aloe vera gel has antimicrobial properties and in vivo exerts a protective
Feeding mice with Aloe vera gel diminishes L-1 sarcoma-induced early neovascular response and tumor growth

effect on polymicrobial sepsis in mice [13-17]. Anthraquinones, compounds present in the outer part of Aloe leaves and in their succus or extract, have been shown to have direct anti-cancer activity in different kinds of human cancer cell lines [18]. Moreover, aloe-emodin, a hydroxyanthraquinone from Aloe vera, can act as an anti-angiogenic agent [19].

Some data suggest that the inner part of Aloe vera leaves, Aloe vera gel and their polysaccharide components also have tumor growth modulatory properties, probably connected with their immunomodulatory activity [20, 21].

In our previous paper we reported the inhibitory effect of Aloe barbadensis fresh leaves aqueous extract (herbal drug Biostymina) on tumor-induced cutaneous angiogenesis in mice [22].

The aim of the present study was to evaluate in Balb/c mice the in vivo influence of commercial Aloe vera gel product (Aloe vera drinking gel) on the syngeneic L-1 sarcoma tumor growth and its vascularization: a) early cutaneous neovascular response, tumor-induced angiogenesis (TIA test), and b) tumor hemoglobin content measured 14 days after L-1 sarcoma cell grafting.

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 59 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 law on the protection of animals and NIH (National Institutes of Health) standards. All experiments were accepted by the local Ethical 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.

Experiments were performed in anesthesia: ketamine 100 mg/kg (prep. Ketamina 10%, BIOWET, Pulawy, Poland); xylazine 10 mg/kg (prep. Sedazin, BIOWET, Pulawy, Poland); 3.6% chloral hydrate 0.1 ml per 10 g of body mass (Sigma Aldrich, USA); Morbital (BIOWET Pulawy, Poland).

Evaluation of sarcoma L-1 growth and angiogenic activity was performed as previously described [23, 24]. L-1 sarcoma cells were delivered from Warsaw Oncology Center collection, passaged twice in vivo and grafted subcutaneously (for evaluation of tumor growth and its hemoglobin (Hb) content) or intradermally (for evaluation of their angiogenic activity) to syngeneic Balb/c mice.

Preparation of tumor cells after in vivo passage. Briefly, sarcoma L-1 cells from in vitro stock were grafted (10⁶/0.1 ml) subcutaneously into the subscapular region of Balb/c mice. After 14 days the tumors were excised, cut to smaller pieces, rubbed through the sieve and suspended in 5 ml of phosphate buffered saline (PBS). The suspension was left for 10 min at room temperature. After sedimentation, the supernatant was collected and centrifuged for 10 min at 1500 rpm. Obtained sarcoma cells were washed once with PBS for 10 min, then centrifuged at 1500 rpm, and resuspended in Parker medium in concentration of 4 × 10⁹/ml or 10⁷/ml.

Cutaneous angiogenesis assay (tumor-induced angiogenesis, TIA test)

Multiple 0.05 ml samples of 200 thousand sarcoma cells were injected intradermally into partly shaved, narcotized Balb/c mice (at least 2-4 mice per group). In order to facilitate the localization of cell injection sites, the suspension was colored with 0.1% trypan blue. Mice obtained Aloe vera gel (150 µl for one mouse daily) in drinking water for 3 days. After 72 hours mice were sacrificed with a lethal dose of Morbital. All newly formed blood vessels were identified and counted in the dissection microscope, on the inner skin surface, at magnification of 6×, in 1/3 of the central area of the microscopic field. Identification was based on the fact that new blood vessels are thin, directed to the point of cell injection, with ramifications, and some of them are tortuous (Fig. 1). Test calculations were performed by two independent observers and the results were averaged.

Subcutaneous tumor growth assay

0.1 ml samples of 1 million sarcoma cells were grafted subcutaneously into the sub-scapular region of Balb/c mice. On the day of cell grafting and on the following 13 days mice obtained 150 µl of Aloe vera gel in drinking water, or water as a control. After 14 days mice were sac-
Hb concentration in tumors was done using the method described [25]. Briefly, tumors were homogenized in PBS using an ultrasonic sonificator (Virsonic, USA), then centrifuged for 20 min at 4000 × g. 20 µl of the supernatant was added to 5 ml of Drabkin reagent. The absorbance was read in a spectrophotometric reader Elx800 (Biotek Instruments, USA) at 570 nm. The reader for the Hb measurement was calibrated with hemoglobin standard solutions (Sigma). The results were shown as µg Hb in 1 mg of tumor mass.

**Statistical analysis**

Statistical evaluation of the results was performed by unpaired t test (GraphPadPrism).

**Results**

The results of the TIA test were evaluated by unpaired t test. Two-tailed P value was lower than 0.0001 (\(t = 4.467; \text{df} = 70\)). Hence, the mean number of newly formed blood vessels in the experimental, Aloe-fed group of mice was highly significantly lower than in the corresponding controls (Fig. 2).

The difference between the groups of control and Aloe-fed mice (150 µl daily dose for 14 days) with respect to the 14 days’ tumor volume was on the border of statistical significance (Fig. 3). No difference was observed in hemoglobin content between control and experimental tumors (21.3 ±3.1 vs. 24.1 ±3.6 µg/mg, respectively).

**Discussion**

It was shown by other authors that some *Aloe vera* active components slow down the experimental tumor growth. Three anthraquinones (aloesin, aloe-emodin and barbaloin) extracted from *Aloe vera* leaves may exert their chemo-preventive effect through modulating antioxidant and detoxification enzyme activity levels [18]. Aloe-emodin induces cell death through S-phase arrest and apoptosis in the dose- and time-dependent manner [26]. Other researchers describe the anti-tumour effect of specific derivatives of the *Aloe vera* plant. Di(2-ethylhexyl)phthalate isolated from *Aloe vera* Linne may have anti-leukemic and anti-mutagenic properties [27].

The anti-tumor effect was also documented for the *Aloe vera* leaf pulp extract and the main lectin (Aloctin I) present in it, in the Ehrlich ascites tumor model [28]. Acemannan, the compound of the extract from the parenchyma of *Aloe vera/aloe barbadensis*, stimulates the synthesis of monokines and recruitment of immune cells and, by this mechanism, necrosis and regression of murine sarcoma [29]. The results of these studies suggest that this effect could be due to its immunomodulatory activity. Acemannan has been approved by the FDA-US as a potent immunomodulating and anti-viral agent. It was approved as an aid in the treatment of canine and feline fibrosarcoma [30].

The anti-tumor effect was also documented for the *Aloe vera* leaf pulp extract and the main lectin (Aloctin I) present in it, in the Ehrlich ascites tumor model [28]. Acemannan, the compound of the extract from the parenchyma of *Aloe vera/aloe barbadensis*, stimulates the synthesis of monokines and recruitment of immune cells and, by this mechanism, necrosis and regression of murine sarcoma [29]. The results of these studies suggest that this effect could be due to its immunomodulatory activity. Acemannan has been approved by the FDA-US as a potent immunomodulating and anti-viral agent. It was approved as an aid in the treatment of canine and feline fibrosarcoma [30].

However, the critical condition for the tumor to effectively metastasize is formation of the new vessels prompted by a group of cancer cells derived from the primary, transported by the blood circulation and grafted in "per-
missible” tissue environment. This “permissiveness” is conditioned by the agents released by the tumor cells that drive recipient tissue to facilitate new vessel growth in it. We have been able to show that Aloe vera drinking gel slows down an early phase of new vessel formation and their in-growth in hosting tissue. This may also explain Aloe vera gel anti-tumor activity. However, Aloe vera drinking gel has not caused the necrotical effect on the tumor volume and it has not influenced the vascularity of the mature tumor (as indicated by the lack of differences in hemoglobin content of tumors between groups). It may suggest that its effect is exerted only on the newly forming vessels during micrometastasis implantation.

Few cytotoxic and targeted drugs have been proven effective in adjuvant systemic therapies after most of the tumor was removed by surgery or radiotherapy. The presence of micrometastases at the time of primary therapy is emphasized as the cause of failure of loco regional therapies. The effective chemoprevention should be directed at the micrometastasis priming mechanism, that is among most important angiogenesis. Aloe vera drinking gel, having a low profile of side effects, may be a good candidate for supplemental therapy. However, Aloe vera is also known for its beneficial wound healing impact that might be partly attributed to its compound, β-sitosterol, pro-angiogenic properties. It was shown that in the presence of heparin, beta-sitosterol stimulated neovascularization in the mouse Matrigen plug assay, and the motility of human umbilical vein endothelial cells in an in vitro wound migration assay [31]. Therefore, further detailed studies on the specific compounds’ contribution of the antiangiogenic effect and its mechanism are warranted.

Lissoni et al. performed a randomized study of chemotherapy versus chemotherapy plus Aloe arborescens, in 240 patients with lung, colorectal, gastric and pancreatic metastatic cancers [32]. Aloe arborescens was given orally at a dose of 10 ml thrice daily of a mixture consisting of 300 g of Aloe fresh leaves in 500 g of honey plus 40 ml of 40% alcohol, every day without interruption, either during or after chemotherapy, until the progression of disease, starting 6 days prior to the onset of chemotherapy. The results of this study suggest that Aloe may be successfully associated with chemotherapy to increase the tumor regression rate and survival time.

Authors declare no conflict of interest.

References
1. WHO Monographs on Selected Medicinal Plants, 1999, vol. 1: Aloe vera gel.
2. WHO Monographs on Selected Medicinal Plants, 1999, vol. 1: Aloe.
3. Seghai I, Winters WD, Scott M, Kousoulas K (2013): An in vitro and in vivo toxicologic evaluation of a stabilized Aloe vera gel supplement drink in mice. Food Chem Toxicol 55: 363-370.
4. Habeeb F, Stables G, Bradbury F, et al. (2007): The inner gel component of Aloe vera suppresses bacterial-induced pro-inflammatory cytokines from human immune cells. Methods 42: 388-393.
5. Damodharan V, Ramamurthy D, Sivallingam J, et al. (2012): In vitro anti-inflammatory activity of Aloe vera by down-regulation of MMP-9 in peripheral blood mononuclear cells. J Ethnopharmacol 141: 542-546.
6. Liu C, Leung MY, Koon JC, et al. (2006): Macrophage activation by polysaccharide biological response modifier isolated from Aloe vera L. var. chinesis (Haw) Berg. Int Immunopharmacol 6: 1634-1641.
7. Langmead L, Makins RJ, Rampton DS (2004): Anti-inflammatory effects of Aloe vera gel in human colorectal mucosa in vitro. Aliment Pharmacol Ther 19: 521-527.
8. Alvarez-Hernandez E, Cesar Casasola-Vargas J, Lino-Perez L, et al. (2006): Complementary and alternative medicine in patients attending a rheumatology department for the first time. Analysis of 800 patients. Reumatol Clin 2: 183-189.
9. Cowan D (2010): Oral Aloe vera as a treatment for osteoarthritis: a summary. Br J Community Nurs 15: 280-282.
10. Davis RH, Rosenthal KY, Cesario RL, Rouw GA (1989): Processed Aloe vera administered topically inhibits inflammation. JAPMA 79: 395-397.
11. Vandana K, Prasanna Raju Y, Sundaresan C, et al. (2013): In vitro assessment and pharmacodynamics of Nimesulide Incorporated Aloe vera Transmuelgel. CurrDrug Discov Technol (Epub ahead of print).
12. Wozniak A, Puduch R (2012): Aloe vera extract activity on human cornal cells. Pharm Biol 50: 147-154.
13. Gupta R, Thakur B, Singh P, et al. (2010): Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant Mycobacterium tuberculosis isolates. Indian J Med Res 131: 809-813.
14. Shilpakala SR, Pruthiba J, Malathi R (2009): Susceptibilities of Escherichia coli and Staphylococcus aureus to Aloe barbadensis. Eur Rev Med Pharmacol Sci 13: 461-464.
15. Fani M, Kohanteb J (2012): Inhibitory activity of Aloe vera gel on some clinically isolated cariogenic and periodontopathic bacteria, J Oral Sci 54: 15-21.
16. Banu A, Sathyaranyarana B, Chattanavar G (2012): Efficacy of fresh Aloe vera gel against multi-drug resistant bacteria in infected leg ulcers. Australas Med J 5: 305-309.
17. Yun N, Lee CH, Lee SM (2009): Protective effect of Aloe vera on polymicrobial sepsis in mice. Food Chem Toxicol 47: 1341-1348.
18. El Shemy HA, Aboul-Soud MA, Nassr Allah AA, et al. (2010): Antitumor properties and modulation of antioxidant enzymes activity by Aloe vera leaf active principles isolated via supercritical carbon dioxide extraction. Curr Med Chem 17: 129-138.
19. Lin SZ, Wei WT, Chen H, et al. (2012): Antitumor activity of emodin against pancreatic cancer depends on its dual role: promotion of apoptosis and suppression of angiogenesis. PLoS One 7: e42146. doi: 10.1371/journal.pone.0042146. Epub 2012 Aug 2.
20. Im SA, Oh ST, Song S, et al. (2005): Identification of optimal molecular size of modified Aloe polysaccharides with maximum immunomodulatory activity. Int Immunopharmacol 5: 271-279.
21. Talmadge J, Chavez J, Jacobs L, et al. (2004): Fractionation of *Aloe vera* L. inner gel, purification and molecular profiling of activity. Int Immunopharmacol 4: 1757-1773.

22. Skopinski P, Zdanowski R, Balan BJ, et al. (2013): *Aloe arborescens* and American cranberry (*Vaccinium macrocarpon*) extracts inhibit tumor-induced cutaneous angiogenesis in mice. Centr Eur J Immunol 38: 480-485.

23. Skopińska-Różewska E, Skurzak H, Wasiuṭyński A, et al. (2007): Sarcoma L-1 in mice as a model for the study of experimental angiogenesis. Centr Eur J Immunol 32: 77-83.

24. Skopińska-Różewska E, Malinowski M, Wasiuṭyński A, et al. (2008): The influence of *Rhodiola quadri̇rida* 50% hydro-alcoholic extract and salidroside on tumor induced angiogenesis in mice. Pol J Vet Sci 11: 97-104.

25. Rogala E, Sommer E, Radomska-Leśniewska D, et al. (2004): Immunomodulatory effects of *Panax ginseng* preparations on the mouse. Herba Polonica 50: 40-46.

26. Chiu TH, Lai WW, Hsia TC, et al. (2009): Aloe-emodin induces cell death through S-phase arrest and caspase – dependent pathways in human tongue squamous cancer SCC-4 cells. Anticancer Res 29: 4503-4511.

27. Lee KH, Kim JH, Lim DS, Kim CH (2000): Anti-leukaemic and anti-mutagenic effects of di(2-ethylhexyl)phthalate isolated from *Aloe vera* L. J Pharm Pharmacol 52: 593-598.

28. Akev N, Turkay G, Can A, et al. (2007): Tumor preventive effect of *Aloe vera* leaf pulp lectin (Aloctin I) on Ehrlich ascites tumours in mice. Phytother Res 21: 1070-1075.

29. Peng SY, Norman J, Curtin G, et al. (1991): Decreased mortality of Norman murine sarcoma in mice treated with the immunomodulator, Acemannan. Mol Biother 3: 79-87.

30. King GK, Yates KM, Greenlee PG, et al. (1995): The effect of Acemannan immunostimulant in combination with surgery and radiation therapy on spontaneous canine and feline fibrosarcomas. J Am Anim Hosp Assoc 31: 439-447.

31. Moon EJ, Lee YM, Lee OH, et al. (1999): A novel angiogenic factor derived from *Aloe vera* gel: beta-sitosterol, a plant sterol. Angiogenesis 3: 117-123.

32. Lissoni P, Rovelli F, Brivio F, et al. (2009): A randomized study of chemotherapy versus biochemotherapy with chemotherapy plus *Aloe arborescens* in patients with metastatic cancer. In Vivo 23: 171-175.