The antileishmanial activity of *Aloe vera* leaf exudates: *in vitro* and *in vivo*

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Background: According to the drug resistance and side effects of the standard treatments for leishmaniasis, achieving effective treatment with less side effects and more benefits is of paramount importance. The present study aimed to evaluate the effect of *Aloe vera* leaf exudate on *Leishmania major* under *in vitro* and *in vivo* models, in inbred BALB/c mice.

Methods: Different concentrations of both *Aloe vera* leaf exudates (AVL) and the standard drug meglumine antimoniate (Glucantime®;Sanofi-Aventis, France) were prepared (9.375l, 18.75, 37.5, 75, 150, and 300 µg/ml) for *in vitro* model and then were applied to the fixed number of promastigotes. The promastigotes were counted after 24, 48, and 72 h. The viability of promastigotes was tested by MTT. A total of 20 mice with cutaneous leishmaniasis were divided into four groups for *in vivo* model, 1: positive group (treatment with Glucantime®), 2: negative group (without treatment), and 3 and 4: experimental groups (treatment with AVL1% and AVL4%, respectively). The size of the ulcers were recorded at the beginning of the experiment on a weekly basis for four weeks.

Results: The results of *in vitro* model indicated that both AVL and Glucantime® reduced the number of promastigotes such that there was the lowest number of parasites in the concentration 300 µg/ml of AVL and Glucantime®; however, the difference between them was not statistically significant. *In vivo* model demonstrated that AVL4% and Glucantime® decreased significantly the size of ulcers more than negative (P=0.000) and AVL1% groups (P=0.000 and P=0.004, respectively).

Conclusions: There was no significant difference between AVL4% and Glucantime® (P=0.634). Therefore, AVL could control the *Leishmania major*.

Keywords: *Aloe vera* leaf exudate; *in vitro*; *in vivo*; *Leishmania major*

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INTRODUCTION

Leishmania species as flagella and protozoan parasite are seen in two forms during their life cycle: one named promastigote within the phlebotomus vector digestive tract and the other named amastigote within the host mammalian macrophages [1]. This protozoan causes a wide spectrum of disease, including cutaneous, mucocutaneous, and visceral leishmaniasis [1-3].

Patients with leishmaniasis should be followed because sometimes, treatment does not provide satisfactory results, especially in immunodeficient cases. Similar to other opportunistic parasites such as *Toxoplasma gondii* [4], reactivation of disease and producing atypical clinical manifestation may occur and leads to its spread to other organs easily [4]. Leishmaniasis is a health problem in the Middle East, Central Asia, Africa, and many tropical and subtropical regions of the world [5]. According to the reports of the World Health Organization (WHO), it is among the six most important tropical diseases [6].

Regarding the adverse side effects, resistance to commonly used drugs against leishmaniasis, toxicity, and cost, achieving natural bioactive compounds with less toxicity and high efficacy has recently received much attention [1, 5, 7-11] and led to more interest in medicinal plants. According to the report of WHO, about 80% of the world's population uses traditional medicine [5]. In this regard, the results of some studies have suggested using medicinal herbs in the management of some skin diseases [12]. As cutaneous leishmaniasis is endemic in some parts of Iran [13], the management and treatment of this disease can be effectively done using herbal drugs. Some studies have successfully reported the discovery of products from plants and used them against *Leishmania* [14], but some did not show the therapeutic effect on cutaneous leishmaniasis. For example, Ahmadi [3] extracted the hydro-alcoholic of *S. Rosmarinus* with a concentration below 15% and showed that using a higher concentration of *S. Rosmarinus* or combining it with nanoparticles would lead to ideal results. Aloe vera (AV) is among the herbal disinfectants with the great history of therapeutic properties. AV has diverse biological activities, including purgative, antimicrobial and antifungal, immunostimulatory, anti-inflammatory, anti-tumor, anti-diabetic activities, and wound healing [13,15]. Moreover, some studies have reported AV as a potent anti- leishmaniasis agent in different aspects [13,15]. AV has a direct parasiticidal effect on all forms of *Leishmania*, enhances activated macrophages, and leads to increasing $TNF_{-\alpha}$, which is the main mediator of acute inflammation. Hence, it could have positive effects on antigen processing and cause resistance against infection [15,16]. Moreover, some studies have demonstrated that AV had no deleterious effects to the host cell [5,13] while its extract with acetone has an antimicrobial activity [17].

In the present study, the anti-leishmanial activity of AV is evaluated by *in vitro* and *in vivo* models.

MATERIALS AND METHODS

Preparation of plant

AV was prepared and identified by using a standard key. Then, it was transmitted to the pharmacology laboratory inside the Department of Pharmacology, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences. The leaves of AV, which contain alkaloids, triterpenes, cyanidins, proanthocyanidins, tannins, and saponins [8], were crushed in an electric grinder and the resultant slurry was used as the aqueous extract from this plants. Then, it was changed in the form of lyophilized (Aloe vera leaf exudates= AVL), stored at 4°C until further use. It was dissolved in Dimethyl sulfoxide (DMSO) at the time of using, and AVL IC_{50} (inhibitory concentration) was computed for the promastigote (i.e., 117µg/ml).

For the in vivo study, the ointment was synthesized in two percentages by a mixture of AVL and vaselin-eucerin: Ointment 1%, AVL (1gr) + vaselin- eucerin (99gr) and ointment 4%, AVL (4gr) +vaselin- eucerin (96gr). For in vitro study, different concentrations were prepared (9.375µg/ml, 18.75µg/ml, 37.5µg/ml, 75µg/ml, 150µg/ml, and 300µg/ml).

Parasites

Leishmania major strain (MRHO/IR/75/ER) promastigotes were purchased from the Department of Parasitology, Faculty of hygiene, Tehran University of Medical Sciences, Tehran, Iran. It was cultivated in Novy-MacNeal- Nicolle (NNN) medium and then in Roswell Park Memorial Institute medium ($RPMI_{1640}$) supplemental with 10% fetal bovine serum (FBS) and 2Mm L-glutamine. Finally, the stationary phase of parasites was obtained.

Animals

Twenty female BALB/C mice (3- 4 weeks old)

were purchased from pasture institute, Tehran, Iran. The animals were maintained under standard condition (temperature 22 ± 20°C, lights on at 6:0-18:00 h) with free access to food and water. The experiments started after 1 week of acclimatization period in the Faculty of Veterinary, Urmia University, Urmia, Iran.

In vitro model

The effect of AVL on the standard number of parasites was evaluated. First, AVL in different concentrations was added to the culture contained promastigotes (n= $2\times10^{6)}$ and then incubated at 22°C. The number of promastigotes was counted 24, 48, and 72 h after adding AVL. Besides, the viability of alive promastigotes was assayed by MTT test.

In vivo model

Mice were inoculated subcutaneously in a shaved and disinfected area at the base of the tail with approximately 2×10^6 stationary phase of *Leishmania major* (MRHO/IR/75/ER) promastigotes. Then, they were randomly divided into 4 groups (n=5 for each group): Group 1: treated with vaseline-eucerin (negative control group); Group 2: treated with meglumine antimoniate (Glucantime®; Sanofi-Aventis, France) as a standard drug (positive control group); Group3: treated with AVL1% ointment (experimental group); and Group 4: treated with AVL4% ointment (experimental group).

After observing the ulcers in the local inoculation, the treatment started on a daily basis for 4 weeks. Two diameters – small (d) and big (D) –of the ulcers were metered by the caliper at the beginning and weekly for four weeks. Then, the size of them was calculated by using the following formula: D+d/2 (mm)

Statistical methods

All measurements were expressed as the mean (Mean ± SEM). A two-way repeated measures ANOVA model was used for statistical analyses via SPSS v. 22 (SPSS Inc, Chicago, Illinois, USA).

Ethical considerations

The entire procedures were carried out in accordance with the Ministry of Health and Education of Iran Animal Care Guidelines. The study procedures were approved by the Ethics Committee, AJA University of Medical Sciences, Tehran, Iran.

RESULTS

Antipromastigote effect

The mean numbers of promastigotes after 24, 48, and 72 h exposure to different concentrations of AVL, Glucantime® (positive control), and negative control are shown in Figures 1, 2, and 3 and Table 1. The effectiveness of the 300 µg/

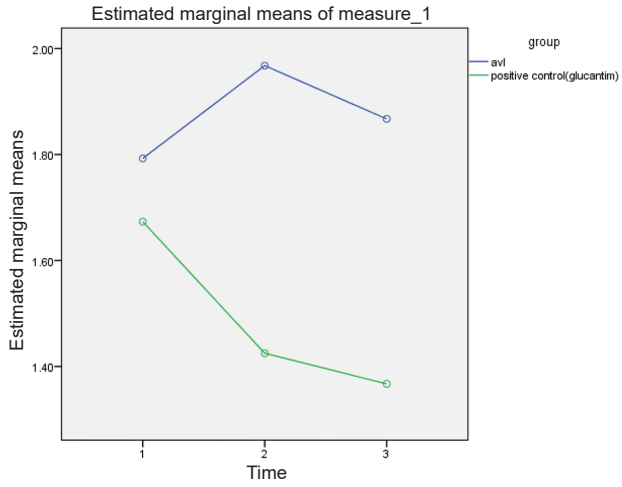

Figure 1. The mean number of promastigote after 24, 48 and 72 hours confronted with AVL and standard drug (Glucantime®)

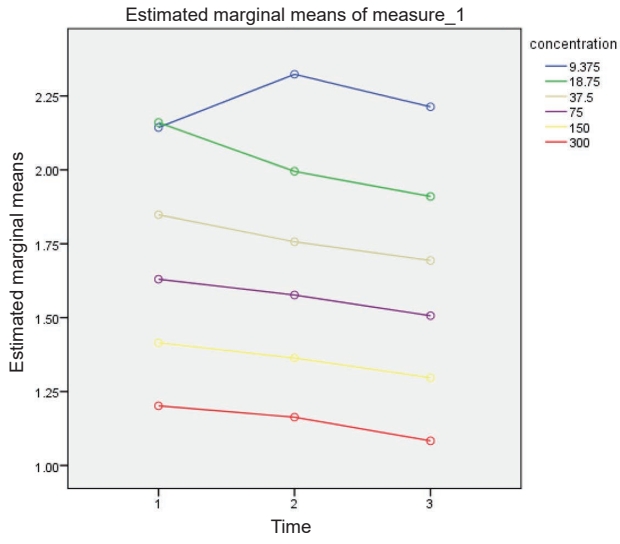

Figure 2. The mean numbers of promastigote after confronted with different concentration of AVL

Table 1. The mean number of promastigotes after 24, 48, and 72 hours of exposure to different concentrations of AVL extract and Glucantime® (numbers of initiation: 2×10^6)

Concentration (µg/ml)	Drug	The number at different times (hour)		
		24	48	72
9.375	Aloe vera	2.68±0.80	2.55±0.16	2.51±0.10
	Glucantime®	1.96±0.10	1.87±0.03	1.77±0.06
18	Aloe vera	2.32±0.09	2.22±0.07	2.16±0.55
	Glucantime®	1.67±0.10	1.60±0.08	2.16±0.05
37.5	Aloe vera	2.02±0.09	1.92±0.08	1.85±0.68
	Glucantime®	1.49±0.92	1.46±0.06	1.85±0.60
75	Aloe vera	1.83±0.18	1.76±0.09	1.63±0.32
	Glucantime®	1.32±0.02	1.26±0.06	1.63±0.02
150	Aloe vera	1.55±0.09	1.46±0.05	1.41±0.45
	Glucantime®	1.17±0.35	1.13±0.04	1.41±0.07
300	Aloe vera	1.39±0.08	1.29±0.04	1.20±0.11
	Glucantime®	0.93±0.07	0.87±0.05	1.20±0.07

ml concentration of AVL and Glucantime® after 72 h were 1.2 and 0.82, respectively (Figure 1). Inhibitory concentration (IC_{50}) was computed for the promastigotes, which was determined to be 117 µg/ml. Using the three-way repeated measures ANOVA model (with one within-subject factor and two between-subject factors), the effect of time, group, interaction with the group time and interaction with time in concentration were found to be statistically significant (Figures 1, 2, and 3).

MMT test

The mean ± SD viability of parasites in 300 µg/ml concentration after 72 h for AVL and Glucantime® was 15 and 8, respectively. Descriptive statistics are shown in Table 2. According to the factorial design and by using two-way ANOVA analyzing method, the effects of group, concentration, and interaction of the group concentration were significant (Figure 4).

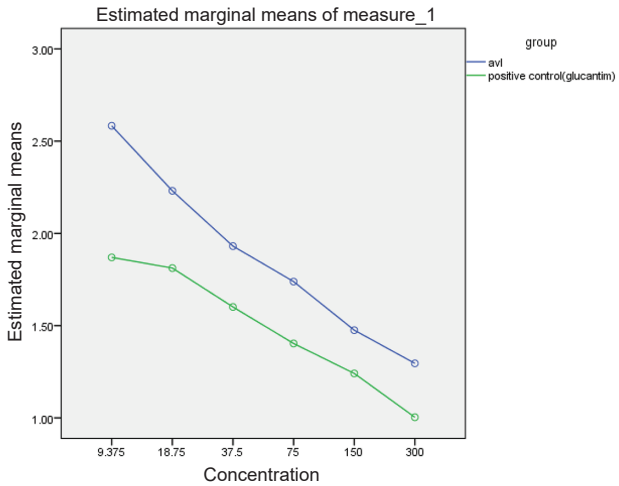

Figure 3. The mean numbers of promastigote after 24, 48 and 72 hours confronted with different concentration of drugs (numbers of initiation: 2×10^6)

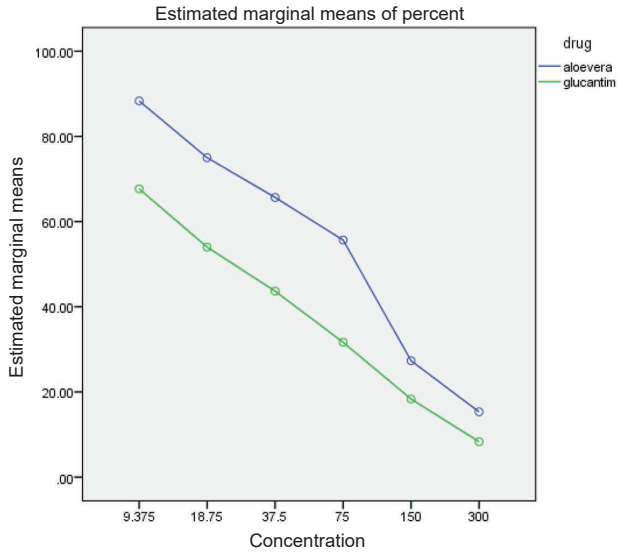

Figure 4. The mean viable of promastigote after 72 hours confronted with different concentration of drugs (AVL, Glucantime®)

Table 2. The mean viable promastigotes after 72 hours of exposure to different concentrations (µg/ml) of products

Drug	Concentration					
	9.375	18.75	37.5	75	150	300
AVL	88±4.5	75±2.2	66±2.5	56±4	27±2.6	15±2.5
Positive Control	68±8.2	54±2.7	44±4.2	32±4	18±4.2	8±2.1

Table 3. The mean size of ulcers (mm) at different times and concentrations of drugs

Drug	Time (week)				
	0	1	2	3	4
AVL1%	6.38±0.26	6.34±0.36	6.2±0.38	5.74±0.42	5.32±0.24
AVL4%	10±0.75	8.8±0.49	8.12±0.5	7.22±0.79	6.08±0.83
Positive Control	8.78±0.49	7.44±0.38	6.84±0.18	5.88±0.60	4.26±0.39
Negative Control	6.6±0.45	6.76±0.53	6.78±0.51	6.84±0.35	6.86±0.27

In vivo model

The mean size of ulcers in different groups at different times is presented in Table 3. According to the two-way RM-ANOVA model and Tukey test (F= 188.65; P= 0.000), the time has a healing effect on the ulcers and demonstrates a time-dependent reduction in the size of ulcers in experimental and positive groups. In this regard, the results of treating the ulcers with AVL4% (experimental group) and Glucantime® (positive control) were similar and there was no significant difference between these two groups (P= 0.634) however, they were more effective than AVL1% (P= 0.000 and P= 0.000, respectively) and negative control groups (P= 0.000 and P= 0.000, respectively) (Figures 5 and 6). The AVL1% significantly decreased the size of ulcers in comparison to the negative control group (P= 0.011) (Table 3).

Figure 5. The size of ulcer before treatment by AVL4%

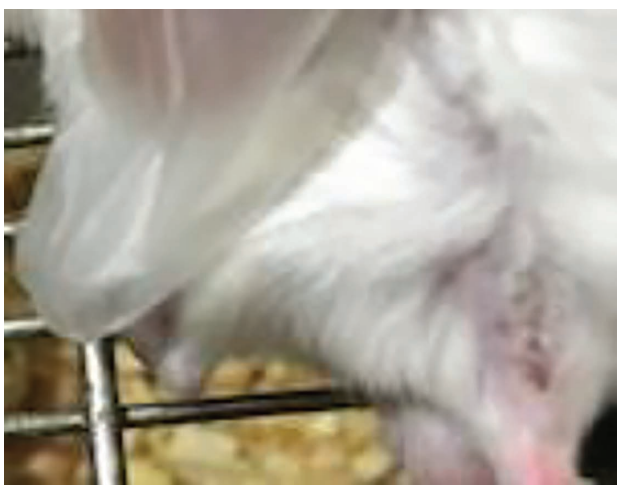

Figure 6. The size of ulcer after treatment by AVL4%

DISCUSSION

There is some difficulty in the treatment of leishmaniasis, for instance, drug resistance, and side effects of standard drugs. In this study, it was tried to assess the effect of Aloe Vera as a herbal drug against the *Leishmania major*. The results showed that both AVL and Glucantime® reduced the number of promastigotes and concentration 300µg/ml of AVL and Glucantime® had the lowest number of parasites, and there were no significant differences between them.

Treatment of promastigotes with AVL demonstrated a dose- and time-dependent inhibition of parasite growth, such that decreasing the number of promastigotes increases the concentration of AVL and time of exposure to AVL; this result also was confirmed by some other studies [13, 15,18]. AVL has a direct leishmanicidal effect. In this regard, previous studies reported that AVL induced programmed cell death via apoptosis. It increases the cytochrome C in the cytosol, followed by disruption of the outer mitochondrial membrane; however, it did not generate reactive oxygen. It could induce caspase-independent cell death in *Leishmania donovani* promastigote [15]. Meanwhile, it bulges the nuclei of DNA after 72 h and led to increasing the fragmentation of nuclear DNA [5,15,17]; these findings are in line with those of the present study.

In vivo model demonstrated that AVL4% and Glucantime® decreased the size of ulcers more than negative and AVL1% significantly. There were no significant differences between AVL4% and Glucantime® (p= 0.634).

AVL4% as a herbal drug and Glucantime® as a standard treatment had similar results. This data is

confirmed by some studies [13,15] that reported AVL could heal the ulcers of the skin and repair some damage in skin. In a study, Dalimi et al. used the inner gel of plant and showed that the emodin compounds of it can effectively reduce the size of ulcers in cutaneous leishmaniasis; however, in the present study, we used the leaf of the plant instead of its gel. Moreover, there were some differences in study design such as the lack of control in the study by Dalimi et al [13].

In addition to the direct parasiticidal effect of AVL on Leishmania, it had immunomodulatory activity and contained two low and high molecular weight components. Low molecular weight component has anti-inflammatory and immunosuppressive properties, but a high molecular weight component has immunostimulating properties, increases activated macrophages, and enhances the reactive oxygen species (ROS) and reactive nitrogen species (RNS) production in macrophages, which lead to increasing the secretion of IL12, IL1, IL6, and $TNF_{-\alpha}$ [15,16,19,20]. This result is in line with the results of the *in vivo* model in the present study.

The results of the present study showed the potential of AVL to control the *Leishmania major in vitro* and *in vivo* models. Moreover, in the treatment of challenging cases of cutaneous leishmaniasis, the use of AVL will be suggested as a complementary treatment.

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Conflict of Interest: None declared.

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