

Full Length Research Paper

The effect of *Satureja khuzestanica* essential oil on the lesions induced by *Leishmania major* in BALB/c mice

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Accepted 11 May, 2011

Cutaneous leishmaniasis is the most common form of leishmaniasis that is still treated with expensive drugs, which present side effects. Conventional healers usually are cheap and sometimes more effective than chemical drugs. *Satureja khuzestanica* is an endemic plant in the west of Lorestan Province, Iran. This plant has therapeutic value. This study was carried out for the first time to assess the *in vivo* efficacy of *S. khuzestanica* in animal model of leishmaniasis. Mice were inoculated with *Leishmania major* promastigotes and then when a lesion developed, the mice were divided randomly into treatment and control groups. The mice were treated with different concentrations of *S. khuzestanica* essential oil (SKEO). The results showed that the lesions' size in SKEO treated groups was restrained but not significantly different from the control group which might be due to the low sample size or concentration of SKEO. The mortality rate in treated groups was clearly less than the control. The results indicated that SKEO has an effect on preventing death in infected mice. Also the survival rate in the treated groups compared with the control groups was more. According to the results, it seems that not only SKEO is antifungal, antiviral, antibacterial but also antiparasitical.

Key words: *Satureja khuzestanica*, *Leishmania major*, cutaneous leishmaniasis, mice.

INTRODUCTION

Protozoa of *Leishmania* spp. are obligate intracellular parasites which survive and multiply in the phagolysosome compartment of macrophages and are transmitted by the bite of female sand flies. Depending upon the *Leishmania* causative agent and host genetic background, clinical manifestation of leishmaniasis ranges from a self-healing cutaneous lesion to a fatal visceral form of disease (Desjeux, 2004).

Leishmaniasis affects more than 12 million people in 88 countries (WHO, 2009). Cutaneous leishmaniasis (CL) with 1 to 1.5 million annual cases is the most common form of leishmaniasis (Desjeux, 2004). There is no vaccine available against any form of leishmaniasis and chemotherapy is the only available treatment (Noazin et al., 2009; Croft et al., 2006). The standard treatment for CL is antimonate which is toxic, required long term injections with low efficacy and moreover resistant is emerging which accompanies with side effects and is not always effective (Hadighi et al., 2007; Croft et al., 2006).

Leishmaniasis is endemic in many parts of Iran and CL caused by *Leishmania major* or *Leishmania tropica* is

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reported from at least 17 provinces of the country (Zoonoses Control Office, CDC, 2010). Search for new safe and inexpensive drugs to treat and control CL especially in anthroponotic CL is needed. Conventional healers usually are cheap and sometimes more effective than chemical drugs (Sandhu and Heinrich, 2005). The genus *Satureja* belongs to the family Lamiaceae, subfamily Nepetoideae and tribe Mentheae. One of the diagnostic characteristic of the subfamily Nepetoideae is that its representatives contain more than 0.5% essential oil (EL-Gazzar and Watson, 1970). It has been reported that there are marked differences between and within the subspecies of *Satureja* essential oil composition (Slavkovska et al., 2001).

In the recent years, component of the essential oils of *Satureja khuzestanica* have been determined (Farsam et al., 2004; Sefidkon and Ahmadi, 2000). *S. khuzestanica* is an endemic plant that is widely distributed in the west of Lorestan Province, Iran. This plant has therapeutic value, such as analgesic and antiseptic (Vosovgh-Ghanbari et al., 2010), antibacterial (Sahin et al., 2003; Azaz et al., 2002), antiviral (Abad et al., 1999), antifungal (Boyras and Ozcan, 2005; Zarrin et al., 2010; Sadeghi-Nejad et al., 2010).

The acute toxicity test (LD₅₀) determined that SKEO is not lethal up to 2 g/kg intra-peritoneally injection in rats and is not toxic (Abdollahi et al., 2003). Further evidences about usefulness of *Satureja* species have been reviewed (Momtaz and Abdollahi, 2008; 2010). This study was carried out to assess the *in vivo* efficacy of *S. khuzestanica* essential oil (SKEO) in murine model of leishmaniasis.

METHODS

Preparation of essential oil

The aerial parts of the plant were collected pending the flowering stage from Khorramabad, Iran. The collected aerial parts were air-dried and hydrodistilled using a Clevenger type apparatus for 5 h, giving yellow oil in 0.9% yield. The oil was dried over anhydrous sodium sulfate and stored at 4°C until used. The density of the essence was 0.943 g/ml (Basiri et al., 2007).

Analysis of chemical composition of essential oil

Isolation and measurement of sample were done by GC/MS SHIMADZU 17 A apparatus by exposure of SHIMADZU mass spectrometry model QP5050A and isolation of composition was done in Fused Silica type DBX-5 95% polydimethylsiloxane with length of 30 mm and internal dimensions of 25% mm and film thickness of 25% micrometer. Column temperature increased from 5 to 20°C with speed of 5°C per minute then the temperature increased to 280°C and kept at 280°C for 2 min. Both the injection site and detector (transfer line) temperature was set to 260°C. Helium gas with speed of 0.9 ml/minute with 999 / 99% purity were used as carrier gas. Spectrometer conditions were exactly in accordance with gas chromatography, just ionization energy of 70 electron volts was used. Also for identification of spectrum with the retention indices, the injection of normal hydrocarbons (C8-C20)

under the conditions of sample injection was used.

Animals

BALB/c (Male, 6 to 8 weeks old) mice were purchased from Pasteur Institute, Tehran, Iran and maintained at the Center for Research and Training in Skin Disease and Leprosy, TUMS, Tehran, Iran. Mice were inoculated subcutaneously with 2×10^6 *L. major* promastigotes (MHROM/IR/75/ER) at the ramp of the tails. When the lesions developed at the site of *Leishmania* inoculation, the mice were randomly divided into different groups (7 mice per group):

- Group 1: Non-treated (control 1).
- Group 2: Treated with paraffin (control 2).
- Group 3: Treated with SKEO 0.01%
- Group 4: Treated with SKEO 0.001%
- Group 5: Treated with SKEO 0.0001%

The treatment was applied to the lesions on the daily bases for 7 weeks. Paraffin and SKEO were applied only to the lesions. The diameter of lesions was measured using vernier caliper before treatment and weekly thereafter.

Statistical analysis

Statistical significance between groups was analyzed using One-way Anova. Comparing different times regardless of the experimental groups were analyzed by repeated measures test. To analyze survival rate, the Log Rank test was used. For binary comparison of the experimental groups of treatment effect on mortality ratio, Chi-square test was used. The difference was considered to be statistically significant when the p-value obtained was less than 0.05.

RESULTS

The mean sizes of the lesions are shown in Table 1. Trend graphs of change in lesion diameter are shown in Figure 1. Since the average diameter of lesions in different groups at the beginning of the study was not identical, using Anova Model, the effects of this confounder factor was adjusted. Based on Repeated Measures Test, there are significant differences between the mean lesion diameters at different times ($p = 0.021$), mainly this change is observed as linear.

Despite the increasing trend in groups, Repeated Measures Test results showed that no interaction exists between treatment group and time ($p = 0.715$). It means that observed line trend in the different groups, are not significantly different. Based on One-Way ANOVA results between the size of the lesions in different experimental groups, there is no significant difference ($p = 0.741$). Using Chi-Square test for binary comparison of the experimental groups of treatment effect on mortality ratio, there is a significant difference in the group treated with SKEO 0.001% and control 1 group. So that the mortality in the group treated with SKEO 0.001% was significantly lower ($p = 0.05$). Also in comparison of the group treated with SKEO 0.01% and control 1 group, the significant

Table 1. Effect of SKEO on the size of lesions (mm) in Balb/c mice infected by *L. major*.

Groups	Before treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Mean	p-value
	(Mean± S.D)									
SKEO 0.0001%	11.77 ± 12.59	16.91 ± 17.36	19.29 ± 19.67	21.57 ±20.33	21.22±20.41	23.28±20.44	26.02±21.04	18.85±5.38	20.59±16.25	0.74
SKEO 0.001%	8.97±4.64	11.36±6.58	12.70 ±6.46	15.13 ±7.98	16.61±1093	15.04±5.77	15.51±4.90	17.37±4.58	13.99±5.65	
SKEO 0.01%	12.33 ±6.39	13.60±6.54	14.50 ±5.45	14.56 ±3.36	15.57±3.56	15.74±4.63	18.26±5.91	19.85±6.91	15.42±4.58	
Control 1	13.61 ±8.50	26.03±22.72	36.26± 26.40	35.24 ±31.58	36.60± 32.02	42.62± 35.14	18.85± 17.32	25.95± 27.08	33.27±21.04	
Control 2	14.81 ±6.30	17.86±8.19	21.83± 12.90	22.19 ±12.10	23.60± 12.02	27.54± 16.34	34.05± 24.81	28.13± 28.73	23.41±12.92	
Total	12.30 ±7.92	17.15± 14.04	20.91±17.46	20.90 ±16.98	21.92± 17.33	23.80± 19.49	22.54± 16.24	21.05±13.80	21.34±14.57	

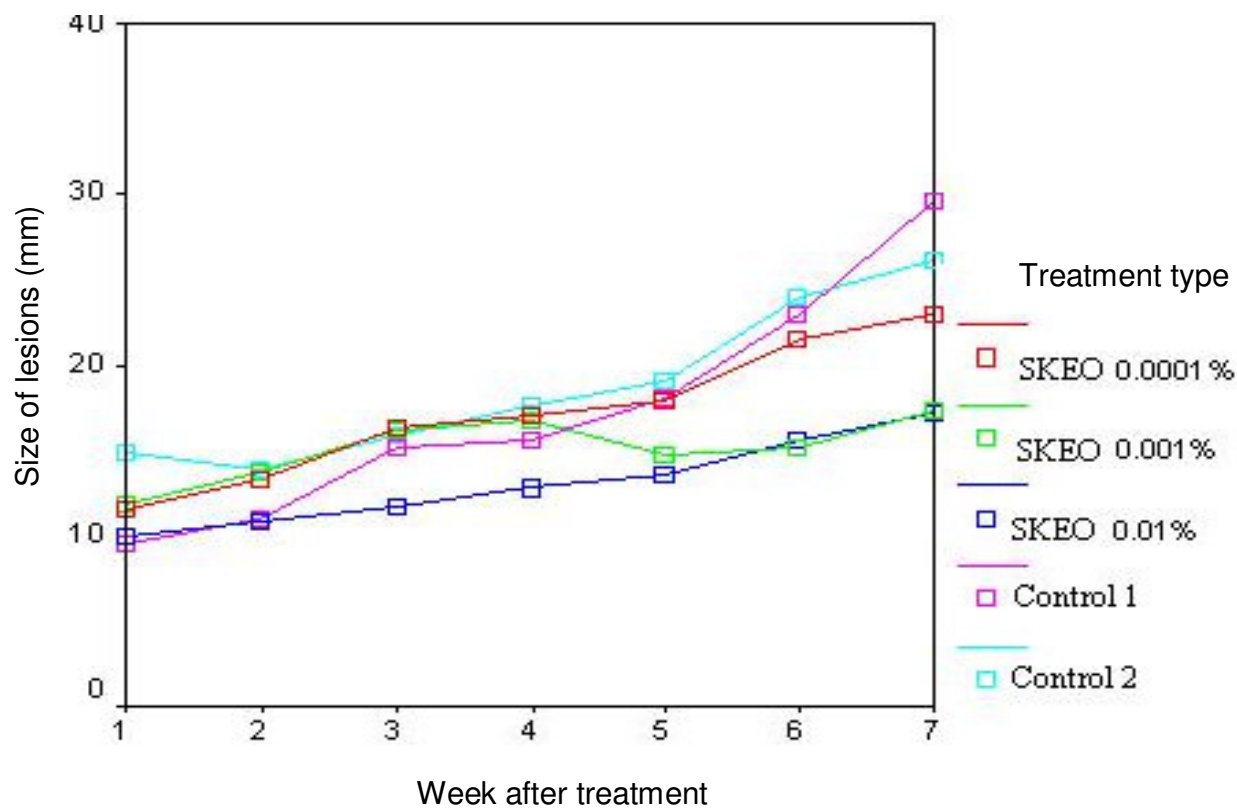


Figure 1. Effect of treatment with SKEO on size of lesion during seven weeks after the beginning of treatment.

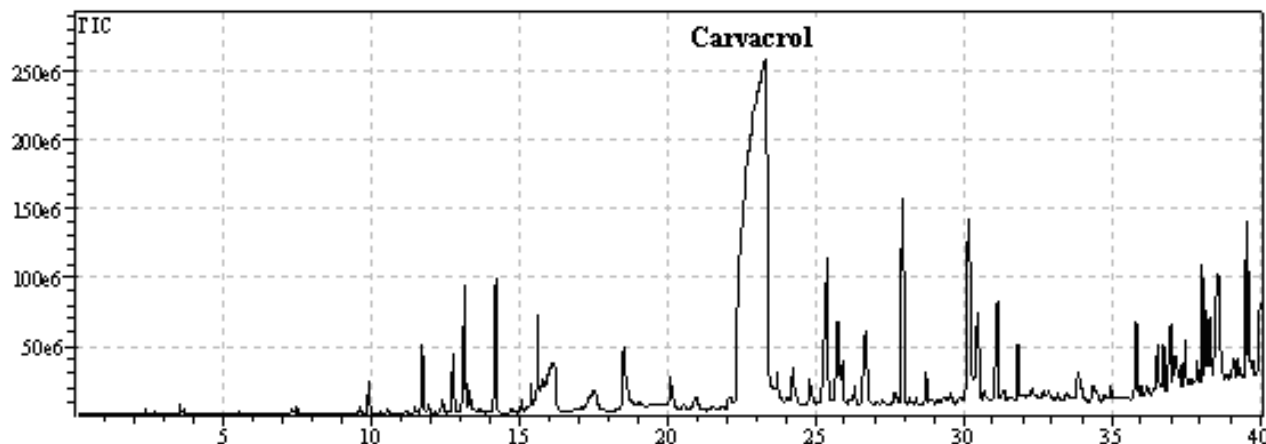


Figure 2. Chromatogram of cultured SKEO compounds by using GC method.

difference was seen so that the mortality in the group treated with SKEO 0.01% was clearly less ($p = 0.09$). Of course this 5% significance level is not significantly different.

In general comparison of survival curve in the study groups using the Log Rank Test at 0.05 significance level, experimental groups in terms of survival are not significantly different ($p = 0.07$), however, according to the p -value close to 0.05 these differences can be fairly be considered significant. The binary comparison of the experimental groups using Log Rank Test, significant difference between the group treated with SKEO 0.001% and control 1 group was seen ($p = 0.02$). Also between the group treated with SKEO 0.01% and control 1 group, significant difference was observed ($p = 0.04$). Chromatogram of cultured SKEO compounds by using GC method in Figure 2 has been shown. The highest peak was related to major compound that was carvacrol (56.33%) and other peaks were shown other compounds. Chemical compounds identified cultured SKEO has been shown in Table 2.

DISCUSSION

Cutaneous leishmaniasis is the most common form of leishmaniasis that are still treated with expensive drugs, which present side effects. There is an urgent need to search new compounds against this disease. *S. khuzestanica* is an endemic plant, which is widely distributed in the west of Lorestan Province, Iran. This plant showed therapeutic values for several diseases (Vosovgh- Ghanbari et al, 2010; Sahin et al., 2003; Azaz et al, 2002; Abad et al., 1999; Boyraz and Ozcan, 2005; Zarrin et al., 2010; Sadeghi- Nejad et al., 2010).

The genus *Satureja* belongs to the subfamily Nepetoideae and one of the diagnostic characteristics of this subfamily is that its representatives contain more

than 0.5% of essential oil (EL-Gazzar and Watson, 1970). Traditional uses of this plant suggest that it favors non toxicity to humans (Abdollahi et al., 2003). *Satureja* species have a variety of biologically active elements such as essential oil, triterpenes, rosmarmic acid and flavonoids (Skocibusic and Bazic, 2004). Carvacrol concentration of active substance in SKEO; the essence used in the study was 56.33%. Results from analysis of data showed that the size of lesions has increased in all groups ($p = 0.021$). However, it was observed that the size of lesions in the groups treated with SKEO was restrained but statistically not different ($p = 0.741$) which might be due to small sample size or concentration of the SKEO.

On the other hand, the binary comparison of the experimental groups about the effectiveness of treatment effect on mortality ratio, significant difference ($p = 0.05$) between groups treated with SKEO 0.001% and control 1 was seen, so that mortality with treated group rather than control 1 group was significantly lower. Also significant difference between groups with treated SKEO 0.01% and control 1 with significant level of 1% was seen, so the mortality in treated groups clearly was less than control 1 ($p = 0.09$). The results indicated that SKEO effect on preventing death of mice infected with *L. major*, as it was observed that concentrations of 0.001 and 0.01% were effective, it might be concluded that with increasing essence concentration, the anti-Leishmania effect might be increased. Different in survival rate between the study groups on the base of Log Rank Test results with regard to close $p = 0.07$ in to significant level of 0.05 considered to be significant.

It means that the survival rate in the SKEO treated groups compared with the control groups was more ($p = 0.07$). In comparison of binary experimental groups with significant level of 0.05 between control 1 and treated with SKEO 0.001% groups ($p = 0.02$) and in the group treated with SKEO 0.01% and control 1 group ($p = 0.04$),

Table 2. Chemical compounds identified cultured SKEO.

No	Compound name	Ret. time	Similarity	KI _{STD}	KI _{CAL}	Area (%)
1	α-Pinene	9.93	98	945	946	0.36
2	Myrcene	11.70	98	991	1002	0.92
3	α-Terpinene	12.76	95	1017	1034	0.75
4	para, Cymene	13.15	93	1091	1046	2.44
5	γ-terpinene	14.20	94	1060	1078	2.06
6	Linalool	15.64	97	1097	1122	1.86
7	Hotrienol	15.80	91	-	1127	0.72
8	Decanal	17.51	91	1202	1181	1.44
9	4-Terpineol	18.54	96	1177	1214	1.93
10	Rosifoliol	20.11	80	-	1265	0.88
11	α-Humulene	20.98	91	1455	1294	0.47
12	Carvacrol	23.33	85	1299	1376	56.33
13	6-Methyl γ- inone	24.26	81	1482	1408	1.01
14	endo-Isofenchol	24.81	68	-	1429	0.70
15	Tetradecane	25.40	92	1400	1451	4.94
16	Tridecanal	26.69	96	1510	1498	2.03
17	β-Bisabolene	28.00	94	1506	1549	3.87
18	α-Bisabolene	28.76	90	1507	1579	0.57
19	Hexadecane. 5-butyl	30.23	91	-	1639	5.42
20	2-Pentadecanone	30.50	93	1711	1650	2.29
21	Tetradecanal	31.13	96	1619	1676	2.18
22	2-Heptadecanone	33.88	87	-	1794	1.01
23	1-H,inden, 2-butyl-4-hexyloctahydro	34.40	74	-	1818	0.48
24	γ- Dodecalactone	36.75	88	-	1940	0.83
25	Methyl hexadecanoate	36.97	74	1922	1954	1.61
26	γ-Stearolactone	38.32	88	-	-	0.98
27	Alkaninn	39.14	61	-	-	0.49
28	γ-Palmitolactone	39.63	92	-	-	1.43
						100

significant difference was seen. The results indicated that SKEO has some effects on survival as in the groups treated with SKEO; survival rate is significantly higher than the control groups. Since this plant is native to Iran and traditionally used in the area with no toxicity or side effect report (Abdollahi et al., 2003) an effective formulation developed could be used in human with no limitation. According to the results, it seems that not only SKEO is antifungal, antiviral, antibacterial but also antiparasitical. Since the most concentrated essence was more significantly effective, it seems that in order to achieve more effective results a higher concentrations of the essence must be used.

ACKNOWLEDGEMENTS

This study was financially supported by Deputy for research and technology affairs of Lorestan University of Medical Sciences. The authors of this article appreciate vice-chancellor for research and health and Razi Herbal

Medicines Research Center, of Lorestan University of Medical Sciences, and also appreciate our co-workers in Center for Research and Training in Skin Disease and Leprosy, Tehran University of Medical Sciences, for their sincere cooperation. Also we thank all of those people who helped us in this research.

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