



Research Article

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***In vitro* anti-leishmanial activity of *Onosma stenosphon* extract against**

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ABSTRACT

The clinical appearances of human Leishmaniasis implicate a wide spectrum of simple cutaneous self-healing (Cutaneous Leishmaniasis) to a visceral (Visceral Leishmaniasis) disease where the parasites are outbreak into the reticuloendothelial system with a fatal outcome. It is wide range, worldwide, without drug, vaccine, septicide and has not sterile immunity and Efforts in this field has not been successful. Research on efficacy of medicinal plants, is including the research infrastructure. Herbaceous plants tomentose perennial local name Khouchoobe in Kerman and official Zangoolaei Looleh baric and scientific name *Onosma stenosphon* "Traditionally in the past as a poultice to treat skin, superficial cuts, wounds and acne has been used. It is disinfectant properties at the site of the wound has been proved. Purpose of this was in vitro antileishmanial activity of *Onosma stenosphon* extract against *Leishmania major*. Sufficient root of *Onosma stenosphon* were minced, sterilized and prepared as tropica with concentration of 0.8, 4 and 20 and 100 µg. *Leishmania major* [MRHO/IR/75/ER] Amastigote was isolated from mice spleens and then transformed to promastigotes in Novy-Nicolle-Mac Neal (NNN) medium, then RPMI₁₆₄₀ and supplemented with penicillin (100 U/ml), streptomycin (100µg/ml) and 20% heat-inactivated fetal calf serum (FCS) at 25°C. A fixed initial density of the parasites (both, Logarithmic and Stationary phases) was transferred to screw-capped vials containing 5 ml of RPMI₁₆₄₀ media to which different concentrations of 0.8, 4, 20 and 100 µg OSE were added and each concentration was done in triplicates. Each run also included control. The mortality of parasitoids was measured by the enzyme-linked assay (ELISA) methods. The percentage viability of OSE and stationary and logarithmic phases of *Leishmania major* [MRHO/IR/75/ER] showed that viability of parasites in PMs significantly decreased in NSE 0.8, 4, 20 and 100 µg treatment compare to control group ($P<0.05$). As shown, after 48hour, the percentage inhibition of OSE and stationary and logarithmic phases of *Leishmania major* [MRHO/IR/75/ER] that the percent inhibition is density in time ($P<0.05$). According to the results, OSE is effect in inhibiting and the duration of exposure and concentration correlates with inhibition.

Keywords: Antileishmanial, Cutaneous Leishmaniasis, *Onosma stinosiphon*, *Leishmania major*, In vitro.

INTRODUCTION

Since 1989(117 years ago) that the first explanation of the perfect clinical course of *Leishmania tropica* infection by Peter Borovsky [1] Leishmaniasis is wide range, worldwide, without drug, vaccine, and has not sterile immunity and efforts in this field have not been successful. It is a complicated disease induced by an obligate intracellular parasite from the genus *Leishmania*. Leishmaniasis is related to environmental changes such as urbanization, deforestation, building of dams and irrigation schemes. An estimated 1.3 million new cases and 20 000 to 30 000 deaths occur

annually. Cutaneous Leishmaniasis (CL) is the most common form of Leishmaniasis and causes ulcers on exposed parts of the body, leaving life-long scars and serious disability. About 95% of CL cases occur in the Americas, the Mediterranean basin, the Middle East and Central Asia. Over two-third of CL new cases occur in six countries: Afghanistan, Algeria, Brazil, Colombia, Iran and Syria. An estimated 0.7 million to 1.3 million new cases happen worldwide annually. Preventive measures are aimed at reducing contact with sand flies by using personal protective measures [2, 3, 4, 5 and 6]. According to the official reports of the Ministry of Health, the average incidence rate of CL is usually between 20 and 40 cases per 100 000 population. The endemic regions in the central and south-western parts of the country (including: Yazd, Semnan, Fars, Ilam, Khoozestan, and Isfahan), with an average incidence of more than 150/100 000 population, have the highest rates of CL. The number of reported CL cases increased from 13729 in 2002 to more than 24000 in 2006 and thereafter, and the disease prevalence is increasing and new foci of CL emerging in Iran (7 and 8).



Fig.1 *Onosma stenosiphon* (Stems, Leaves and flowers), Kerman, Iran

Onosma is a genus of flowering plants in the family Boraginaceae that initiate in the Asia, Mediterranean regions and Europe. Some *Onosma* species are preloved as herbs, dyes and folk medicines (fig.1). Dried flowers of *Onosma stenosiphon* (OS) are used in folk medicine to treat respiratory ailments. OS is locally known as "kho Chobeh" in areas of Kerman province, Iran, which has been used by the rural people as anti-inflammation and antiseptic to treat skin burns and wound healing. It is used for the treatment of wounds and burns in rural areas in Turkey and shows high antioxidant and antimicrobial activities (9, 10, 11 and 12). The present study was carried out to in vitro antileishmanial activity of *Onosma stenosiphon* extract against *Leishmania major*.

EXPERIMENTAL SECTION

Preparation of OSE

OS prepared from Iranian origin was purchased from an herbal shop in Yazd, Iran and was authenticated by the herbal medicine research center in the School of Pharmacy, Shahid Sadoughi University of Medical Sciences. The seeds were dried and crushed into coarse powder. Five hundred grams of the powder were extracted with ethanol (95% v/v). The extracts were filtered and the solvents were evaporated in vacuum with a rotator evaporator that yielded a blackish-brown and kept at 4°C prior to use. The extractive values (%w/w) of the ethanol dry extracts were 4.3 and 7.5%. The extract was concentrated under reduced pressure of 22 to 26 mmHg at 45°C to yielded 0.8, 4, 20 and 100 µg of OSE obtained was stored at 4°C [13].

Source of parasites

Leishmania (L) *major* strain [MRHO/IR/75/ER] promastigotes were obtained from the medical Parasitology department/school of medicine/Shahid Sadoughi University of medical sciences. *Leishmania major* strain (MRHO/IR/75/ER) was maintained in BALB/c mice. Amastigote were isolated from mice spleens, and then transformed to promastigotes in Novy-Nicoll-Mac Neal (NNN). The Third passage promastigotes from NNN medium were progressively adapted to RPMI 1640 media (gibco) with antibiotics, glutamine and FCS supplemented with penicillin (100 U/ml), streptomycin (100 µg /ml) and 20% heat-inactivated fetal calf serum (FCS) at 25°C [14].

The cell proliferation ELISA, Nrd (chemiluminescent) method

The cell proliferation of enzyme-linked immunosorbent assay (ELISA), Nrd (Chemiluminescent) was performed as described by Roche Diagnostics GmbH Roche Applied Science 68298 Mannheim Germany (Version march 2005, Cat. No. 11 669 915 001) the cell proliferation ELISA, Nrd (chemiluminescent) method is a Quantitative determination of DNA synthesis in cell cultures is now a routine procedure in many laboratories. Protocols are available for various applications, especially in cell culture systems. The effects of growth factors, inhibition of cell division by exogenous factors, stimulation/inhibition by cytokines, development of serum-free media, influence of hormones and receptor activity on proliferation, and selection advantages for aneuploid cells in vitro are just a few examples where the determination of DNA synthesis provides important information. That in brief is:

- A fixed initial density of the parasites was transferred to screw-capped vials containing 5 ml of liquid medium to which different concentrations of 2.5, 5, 10 and 20 μg of asafetida were added. Each concentration was done and each run included control
- It was stimulated with acetone in the period
- Dioxy bromouridin was added and it was incubated at 37°C for 8 hours
- Supernatant was removed. Fixator was added to the permeable membrane
- Anti-oxibromouridin conjugated with POD was Added and incubated for 3 hours
- Chromogen was added and incubated. And finally, it was terminated and read at 450 nm.

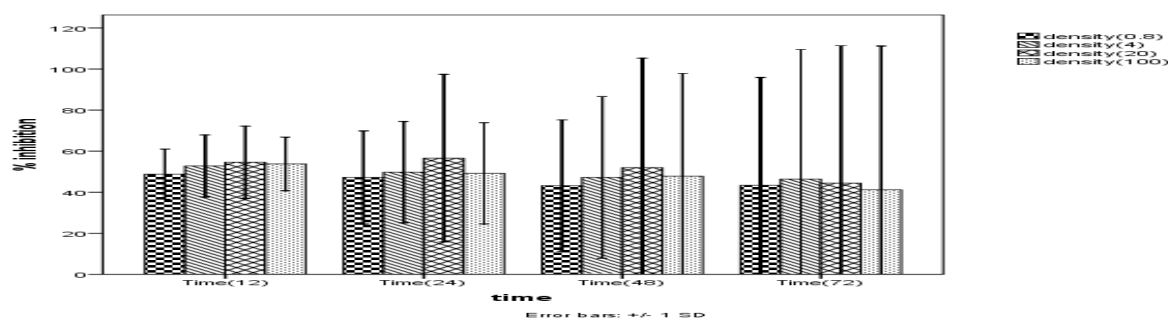
Statistical analysis

The results were expressed as mean \pm SEM. Comparisons among the experimental groups were done by one-way ANOVA test using graph pad prism5 software program. The upper level of significance was chosen as $P < 0.05$.

RESULTS

The percentage inhibition of OSE and stationeries and logarithmic PMs of *Leishmania major* is presented in figure 2. Our results showed that the %inhibition is time dependent in time (hour). After 72 h, the %inhibition was upper 90% in all doses in stationary and logarithmic phase. The result of ELISA measurement is showed in figure 3. As shown in these figures, after 48h, viability of parasites in PMs significantly decreased in OSE 5, 10T 20 and 100 $\mu\text{g/kg}$ treatment compare to control groups.

Figure 2: % inhibition OSE against Logarithmic Phase *Leishmania major* in concentrations of 0.8, 4, 20 and 100 μg . Results are compared to the control on 6-72 hours



The percentage inhibition of OSE and stationeries and logarithmic PMs of *Leishmania major* is presented in figure 1. Our results showed that the %inhibition is time dependent in time (hour). After 72 h, the %inhibition was upper 90% in all doses in stationary and logarithmic phase. The result of ELISA measurement is showed in figure 5. As shown in these figures, after 48h, viability of parasites in PMs significantly decreased in OSE 5, 10 and 20 and 100 $\mu\text{g/kg}$ and counted after 48 hours.

Figure 3: % inhibition OSE against stationary Phase *Leishmania* major in Concentrations of 0.8, 4, 20 and 100 μ g. Results compared to the control on 6-72 hours

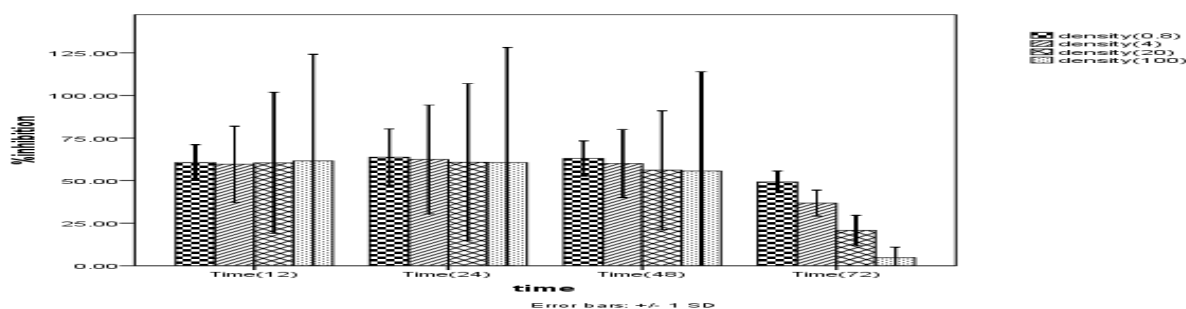


Figure 4: Effect of NSE on viability *Leishmania* major in Logarithmic of Phase. Each bar represents means \pm SD. promastigotes were cultivated in the presence of different concentrations of the 0.8, 4, 20 and 100 μ g and counted after 48 hours

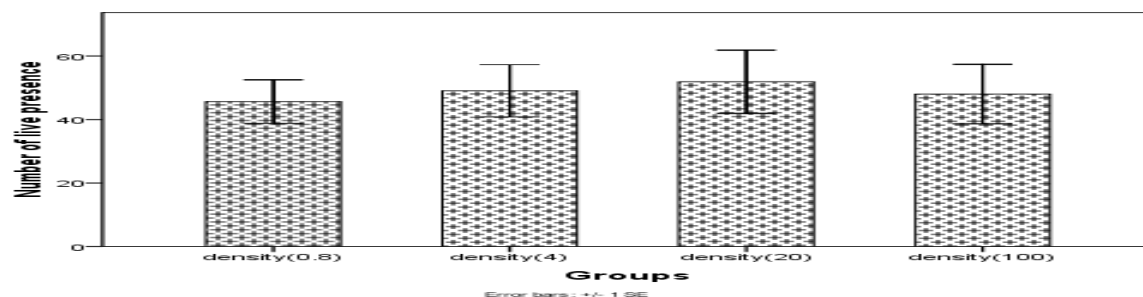
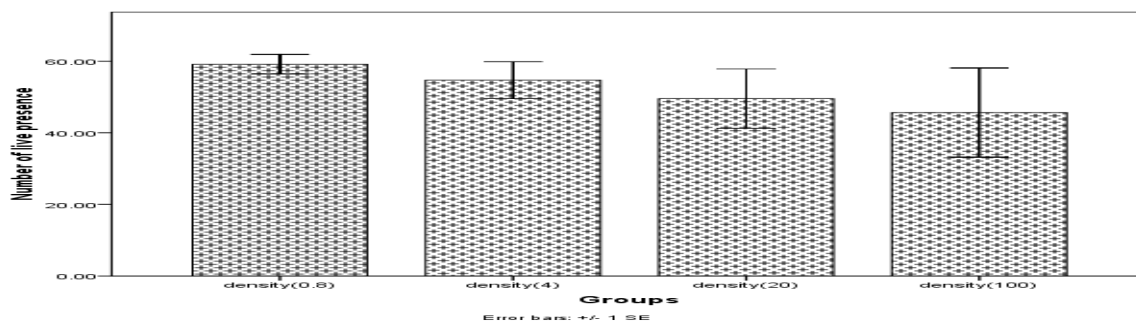


Figure 5: Effect of OSE on viability *Leishmania* major in Stationary of phase. Each bar represents means \pm SD. promastigotes were cultivated in the presence of different concentrations of the 0.8, 4, 20 and 100 μ g and counted after 48 hours



DISCUSSION

The main drugs used to treat leishmaniasis are antimony-containing compounds and include; antimonite (Glucantime) and Sodium stibogluconate (pentostam). Despite the recent developments, the effective therapy for cutaneous leishmaniasis has been yet based on long parenteral courses of these drugs for six decades, even though these are fairly costly, toxic and inconvenient to use, Along with inadequate knowledge on their pharmacokinetics or mechanism of action [15]. The time appertain effect of these plant products may be due to the uptake of the active moiety which progressively increases the amount of active component in PMs with increase in exposure period or it may be possible that the active component (s) could change into more toxic forms in the PMs[16]. Our result indicated that OSE increase mortality of *Leishmania* major and this effect was dependent to time. The time dependent effect of these plant products may be due to the uptake of the active moiety, which progressively increases the amount of active component in PMs with increase in exposure period or the possibility that the active component (s) could change into more toxic forms in the PMs, by the action of different enzymes. Cytotoxic, antibacterial, antimicrobial, antioxidant, antiparasitic activities of PMs was researched in several different studies. Tosun et al, 2008, demonstrated that some of the *Onosma* species displayed remarkable anti-inflammatory, anti-

oxidants and antinociceptive activities [17]. Zarghami et al, 2012, showed that the use of OSR can be a confirmation for using this plant in traditional medicine, as antiseptic and antioxidant effect [18]. Carlos Di Giorgio et al, 2008 their studies have been conducted on the effect of anti-Leishmania *Onosma* spp; Lebanese plants were investigated for their in vitro immunomodulatory and antileishmanial activities as compared to their toxicity against human cells [19]. B Ahmad et al, 2009, showed that *Onosma* has potent antileishmanial and moderate antifungal and antibacterial activities that strongly encourage the activity guided isolation of biologically active compounds [20].

CONCLUSION

According to the results, OSE is effect in inhibiting and the duration of exposure and concentration correlates with inhibition. Using different concentrations of OSE was due to the fact that OSE was each that compared with control group showed a significant difference and that implied OSE was effectively shown against the parasite. However, necessary to elucidate the mechanism of action

In parasite body

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