

The In Vivo Antileishmanial Activity of Alcoholic Extract from *Onosma Stenosiphon* Root

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Received: September 12, 2014

Accepted: November 3, 2014

ABSTRACT

Onosma stenosphon is locally known as "kho Choubeh" in areas southern of Kerman province, Iran, which has been used by the people as anti-inflammation and antiseptic to treat skin burns and wound healing. Leishmaniasis is wide range, worldwide, without drug, vaccine, and has not sterile immunity. It has disinfectant properties at the site of the wound and its antimicrobial has been proved. The objective present laboratory study was the in vivo antileishmanial activity of alcoholic extract from *Onosma stenosphon* root. Sufficient of *Onosma stenosphon* root were minced and they were dissolved in Ethanol 80%. It prepared as topica with concentration of 40, 60 and 80%. in the laboratory, 40 BALB/c mice were infected with the parasite *Leishmania (L) major [MRHO/IR/75/ER]*, they were divided into four groups, control group and three groups that received 40, 60, and 80% concentrations of the extract *Onosma stenosphon* root. As well, the foot and the size of the lesion and weight measured every week until the death of the last mouse in the control group. The present study was carried out to investigate the in vivo Antileishmanial activity of alcoholic extract from *Onosma stenosphon* root. Average weight of mice receiving extracts each other and with an average weight of control mice showed no significant difference ($P > 0.05$). Average diameter ulcer mice receiving extracts each other and with an average diameter size of mice control mice showed significant difference ($P = 0.000$). Determine the average size of the spleen (mm) and compare survival time (days) in any study group which was not significant ($P > 0.05$). The results show that strain of leishmania major was sensitive to *Onosma stenosphon* root extract, topical use of *Onosma stenosphon* root extract in the treatment of cutaneous leishmaniasis wounds causes to be the growth slower diameter size but it Do not, None whole parasite removal from the body.

KEYWORDS: *Onosma stenosphon* root, Antileishmanial, in vivo, cutaneous Leishmaniasis.

Running Title: Antileishmanial activity of extract from *Onosma stenosphon* root

INTRODUCTION

Leishmaniasis is a complex of disease that induced by an obligate intracellular parasite from the genus *Leishmania*. It is one of the major infectious diseases affecting the continuing countries populations throughout the world and there are two million annual new reports in 88 countries. Cutaneous Leishmaniasis (CL) is the most common form of Leishmaniasis. It 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 occur worldwide annually. For more than 60 years, pentavalent antimonials (Sbv) were the major therapeutic agents for the treatment of the disease. However, in the early 1980s, ineffectiveness of these agents was reported, but unfortunately, there is still no development in the production of newer drug. Although studies regarding the production of an effective vaccine to control Leishmaniasis have been extensively conducted over the past decades, there is still no vaccine against any form of Leishmaniasis for general human use. Since vaccines against this parasite are not yet in sight, its control relies mostly on chemotherapy, so, there is an urgent need to develop new and better drugs to combat this infectious disease (1 and 2).

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Fig.1 *Onosma stenosphon* (Root)Fig. 2 *Onosma stenosphon* (Stems & leaves)

Onosma is a genus of flowering plants in the family Boraginaceae that found in the Asia, Mediterranean regions and Europe. Some *Onosma* species are used as herbs, folk medicines and dyes. Dried flowers of *Onosma stenosphon* are used in folk medicine to treat respiratory ailments. *Onosma stenosphon* (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 (3, 4, 5, 6 and 7). The present study was carried out to investigate the *in vivo* Antileishmanial activity of alcoholic extract from *Onosma stenosphon* root

MATERIALS and METHODS

The design of the present probe was experimental (laboratory-trial); therefore, Iranian. Endemic species including *Leishmania (L) major* [MRHO/IR/75/ER] was proliferated, and maintained in the standard culture.

Preparation of plant extract

Onosma stenosphon root (OSR) of Iranian origin was prepared from areas of the southern in Kerman province, and then it was authenticated by the herbal medicine research center in the School of Pharmacy, Shahid Sadoughi University of Medical Sciences. The roots after three times grind, 200 cc ethanol per 100 grams of powder OSR, was added and the mixture is poured into the flask on a magnetic stirring (Stirrer) containing magnets placed 72 hours were dried. Then the liquid in the water bath at 45 ° C for 48 h and extracts were prepared. The extracts were filtered and the solvents were evaporated in vacuum with a rotatory evaporator that yielded a blackish-brown concentrates and kept at 4°C prior to use. The extractive values (%w/w) of the ethanol dry extracts were 4.3 and 7.5%, respectively. The extract was concentrated under reduced pressure of 22 to 26 mmHg at 45°C to yielded 40, 60 and 80% of OSR extract in ointment base (Emami, Activor, USA) and the residue obtained was stored at 4°C (Sudeendrabhat *et al.*, 2007).

Parasite culture

Leishmania (L) major strain (MRHO/IR/75/ER) was maintained in BALB/c mice. Amastigotes were isolated from mice spleens, and then transformed to promastigotes in Novy-Nicolle-Mac Neal (NNN) medium supplemented with penicillin (100 U/ml), streptomycin (100µg/ml) and 20% heat-inactivated fetal calf serum (FCS) at 25°C. Subsequently, the third passage from NNN medium was progressively adapted to RPMI 1640 media (GIBCO) supplemented with antibiotics, glutamine and FCS (complete medium).

Experimental infection and monitoring

A total of 40 BALB/c mice (8 weeks old) were randomly and equally divided into 3 cases, receiving the ointment containing 40%, 60% and 80% of OSR extract and 1 control group (receiving the ointment base) groups. Mice were subcutaneously inoculated with 0.1 ml =10⁶ infective promastigotes at the dorsal base of the tail. As soon as the symptom appeared, lesion development was monitored three times a day by a direct-reading vernier caliper gauge. The largest diameter of the lesions was calculated as the ulcer size. Animals were daily weighed and then the wounds were covered by the ointment. This monitoring was continued until the death of last mice in the control group. Finally, the spleen size of all animals was measured as an index for the severity of disease progress.

Statistics

Data are expressed as Mean ± SEM of lesion's diameter and animal's weight, and were statistically analyses by multivariate test for analyzing the effect of time within-subjects and multiple comparisons test for comparing the variables between groups. p < 0.05 was considered as the significant level.

RESULTS

Fifteen weeks of weight and wound monitoring showed a significant time dependent weight loss and ulcer development. Average weight of mice, receiving 40% OSR extract was the same as those receiving 60% and 80% of OSR extract and control mice ($p > 0.05$). Also, there was no significant (figure.1). In other words there was not a significant difference between the average weight of mice receiving 40%, 60% and 80% of OSR extract as compared with the average weight of control group ($p > 0.05$). Average diameter of ulcer mice receiving 40% of OSR extract with a mean diameter of ulcer mice receiving extracts 60% and 80% of OSR extracts and the control group mice showed a significant difference ($p < 0.05$). Average diameter ulcer mice receiving 60% OSR extract with a mean diameter of ulcer mice receiving extracts 40% and 80% of OSR extracts and the control group mice showed a significant difference ($p < 0.05$). Average diameter ulcer mice receiving 80% of OSR extract with a mean diameter of ulcer mice receiving extracts 40% and 60% of OSR extracts and the control group mice showed a significant difference ($p < 0.05$). Average diameter wound control mice with a mean diameter of ulcer mice receiving extracts 40, 60 and 80% of OSR extracts showed significant difference ($p < 0.05$). At the end of experiments, average diameter of ulcer in mice receiving 40% OSR extract showed a significant difference as compared to those receiving 60% and 80% of OSR extract ($p > 0.05$). Ulcer's diameter in animals receiving 80% of OSR extract was significantly less than those receiving 60% of OSR extract ($p > 0.05$). Ulcer's diameter in all test groups was significantly less than the control group ($p > 0.05$, which received 40%, 60% and 80% of OSR extracts). Spleen size in test groups was smaller than the control group. It showed that the more the of the

Extract was higher, the more the spleen size was smaller. Our findings also revealed that there is a negative relation between the OSR extract percentage and the spleen size. Wounds in mice receiving different concentrations of OSR extracts also showed less secondary infection, necrosis, stiffness, redness, swelling and inflammation as compared with the control group (Figures 1 to 6).

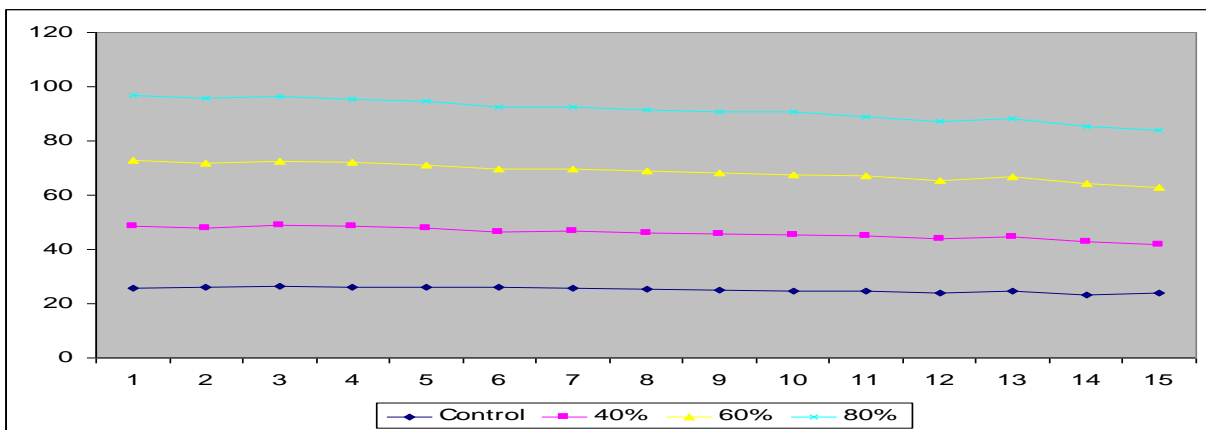


Fig.1 Effect of OSR extract on average weight of BALB/c mice received 40, 60, 80% of and the control groups.

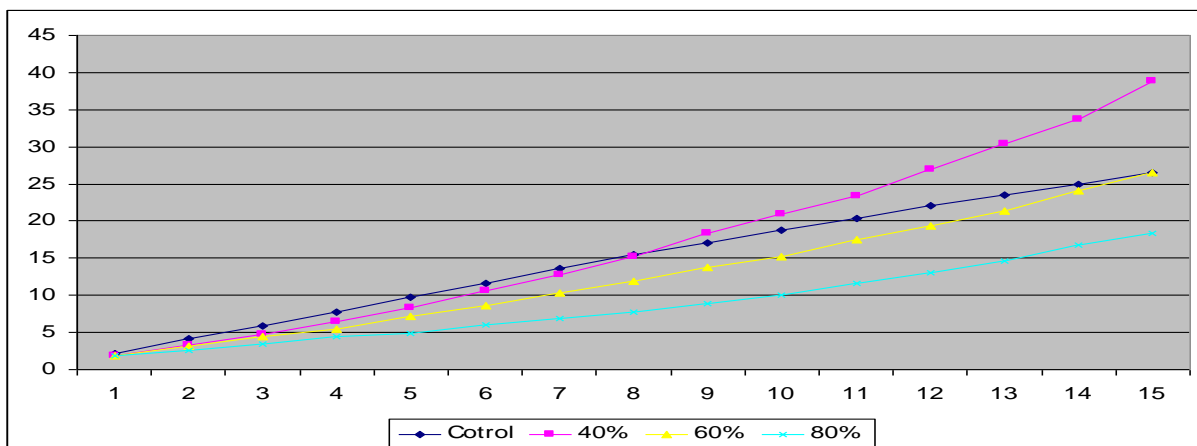


Fig. 2- Effect of OSR extract on average diameter of ulcer BALB/c mice received 40, 60, 80% of and the control groups.

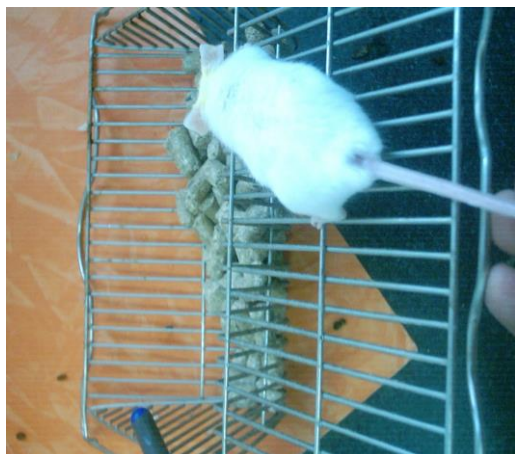


Fig. 3- Control group.

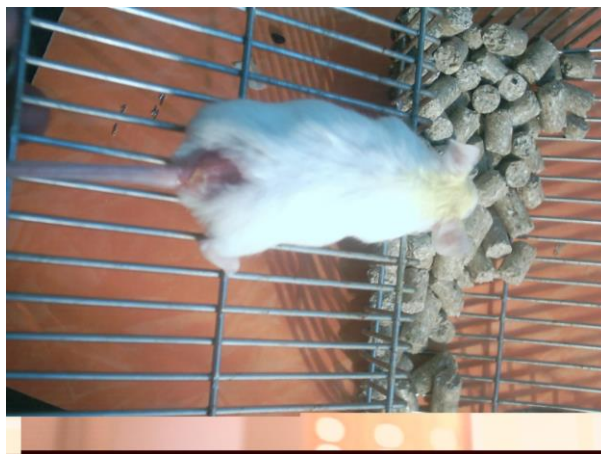


Fig. 4- Received group 80% of OSR extract

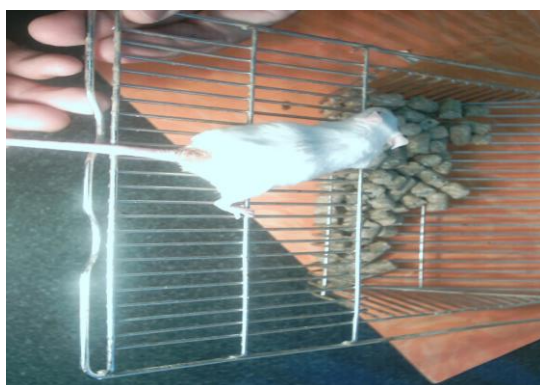


Fig. 5- Received group 60% of OSR extract.



Fig. 6- Received group 40% of OSR extract.

DISCUSSION

Antimony-containing compounds that are the main drugs used to treat leishmaniasis include: Melamine 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. Different concentrations (40%, 60% and 80%) of OSR extracts did not show any significant change in weight loss in BALB/c this result indicates that the OSR extracts could not prevent the spread of parasite inside the animal's gut and failed, this means that Skin parasites spread throughout the body and their proliferation decreases were dying host. But on the other, different concentrations levels were inhibiting the increase in lesion diameter this means that damage to macrophages, inflammation control, and it is preventing the spread of cutaneous leishmaniasis. *Onosma stenosisiphon* has been used as herbal medicine for healing skin cuts and rashes. However, burn healing effect of this herb has not been yet reported in pharmaceutical texts. The aim of this study was to find out the effect of herb ointment on burn healing the superficial wounds post type II burns.¹⁶ some of the experimental data demonstrated that some of the *Onosma* species displayed remarkable anti-inflammatory, anti-oxidants and antinociceptive activities¹⁷. The use of OSR can be a confirmation for using this plant in traditional medicine, as antiseptic and antioxidant effect¹⁸. Several 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. *Onosma* spp antileishmanial activities on the intracellular Amastigote form of the parasite it was shown¹⁹. Different parts of 65 plant species, in addition *Onosma* spp from the Greek island of Crete have been extracted and the 249 extracts obtained have been investigated for in-vitro antiprotozoal activity, none of the extracts was cytotoxic.²⁰ *Onosma griffithii* was screened for possible pharmacological activities. *Onosma* has potent antileishmanial and moderate antifungal and antibacterial activities that strongly encourage the activity guided isolation of biologically active compounds²¹. In many studies is referred to affecting of anti-inflammatory, anti-microbial, anti-parasitic, anti fungal, antibiotics and toxicity of OSR.

CONCLUSION

Using different concentrations of OSR, in BALB/c mice infected with cutaneous leishmaniasis, was not due to the stoppage or slow changes of their weight loss, but was due to the fact that OSR was less in wound diameter. Separately, each concentration that was used with control group showed a significant difference. Even among groups, concentrations

Used were also significantly different, which implied that OSR was effectively shown against the parasite in macrophages. At the result of intense parasite proliferation, when the immune cells are attracted to the place of conflict, it is possible to prevent the development of inflammation, swelling and ulcer.

ACKNOWLEDGEMENT

The authors would like to thank the authorities of Infectious and Tropical Diseases Research Center, Yazd Shahid Sadoughi University of Medical Sciences, for financial support. This article is based on a Project submitted (No. 975) in The School of medicine, Yazd Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Conflict of Interest

The authors report no conflicts of interest.

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