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ORIGINAL ARTICLE

Effect of Boswellia thurifera oleo gum resin on the viability of *Leishmania (L) major* promastigotes an *in vitro* study

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ABSTRACT

Cutaneous Leishmaniasis (CL) is an endemic disease in developing countries. Glocantime has been recommended for CLtreatment by W.H.O, there are some restrictions in this case including high expense, frequent injections need, side effects, and incomplete efficacy. Considering different methods which have been used for disease treatment thus far; in present study, the Boswellia thurifera oleo gum (BTG) effect on rural strain CL' Viability of old world in vitro is under investigation. Iranian endemic species including Leishmania (L) major [MRHO/IR/75/ER] was appropriately provided. The results of different concentrations of BTG on stationary phase PMs of Leishmania (L) major strain [MRHO/IR/75/ER]. In geometrically increasing concentrations, dose dependently inhibited the growth of Leishmania (L) major strain [MRHO/IR/75/ER] PMs. The results show strain of Leishmania (L) major strain [MRHO/IR/75/ER] was sensitive to BTG, increasing concentrations; dose dependently inhibited the growth of parasite. BTG concentrations 0.1, 0.5, 1, 5 and 10 mg/ml was added to cultured parasite and counted PMs with the Cell proliferation ELISA, Nrdu (Chemiluminescent) method. There was statistically significant difference between BTG and MA groups and Control (P=0.000). BTG may make it possible to use them in the treatment of Cutaneous Leishmaniasis as a complementary or alternative therapy; however, further studies are necessary and should be evaluated in cell culture and in vivo conditions to confirm it.

Keywords: *Boswellia thurifera, Leishmania (L) major, Promastigotes, in vitro.*

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INTRODUCTION

Boswellia thurifera gum (BTG) is edible and is used in traditional medicines in Asia and Africa for digestion and healthy skin. For internal consumption, it is recommended that f BTG be translucent, with no black or brown impurities. It is often light yellow with a (very) slight greenish tint. It is often chewed like gum, but it is stickier. In Ayurvedic medicine BTG, commonly referred to in India as "*dhoop,*" has been used for hundreds of years for treating arthritis, healing wounds, strengthening the female hormone system and purifying the air. The use of f BTG in Ayurveda is called "*dhoopan*". In Somali, Ethiopian, Arabian, and Indian cultures, it is suggested that burning BTG daily in the house brings good health[1-3]. There is a growing use of complementary and alternative medicines in different countries, especially

developing countries. *Boswellia* has been reported traditionally to have beneficial effects like antitumor, analgesia, anti inflammation, anti rheumatism, improving intelligence [4-5]. Many pharmacological investigations are performed to detect new drugs for the treatment of diseases such as cancer[6]. Parts used of BTG is gum resin and medicinal uses of BTG is anti- inflammation, anti- oxidant, aromatherapy, arthritis, bronchitis, Christmas, cuts & wounds, facial care, pain relief and rheumatoid arthritis[7]. BTG Properties is vulnerary , uterine tonic, meditation, sedative, diuretic , Cicatrisant , astringent , analgesic anti-inflammatory, anticancer, Antirheumatic, aromatic and BTG constituents is resins (65%), volatile oil (6%), water soluble gum (20%), basso in (6-8%), and plant residue (2-4%). the resins contain boswellic acid and alibanoresin[8-11]. Many pharmacological investigations are performed to detect new drugs for the treatment of diseases such as tumor, analgesia, inflammation, rheumatism, improving intelligence, and cancer12[-13].

Fig a. Yemenian BTG b. Structure of β-boswellic acid, one of the main active components of BTG

118 years ago (Since 1898) that the first explanation of the perfect clinical course of *Leishmania tropica* infection by Peter Borovsky[14]. Leishmaniasis is wide range, worldwide, without drug, vaccine, and has not sterile immunity and efforts in this field have not been successful and The World Health Organization has identified it as a major public health problem. 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 being aimed at reducing contact with sand flies by using personal protective measures[15-19]. 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, and the disease prevalence is increasing and new foci of CL emerging in Iran. Although for more than 60 years, Meglumine Antimonite (MA) was 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[20-21]. The present study was carried out to Effect of Boswellia thurifera oleo gum resin watery extract on the viability of *Leishmania (L) major* promastigotes an *in vitro* study.

MATERIALS AND METHODS

A- Preparing of Boswellia thurifera Resin:

Boswellia resin was ground and 20g of powder was extracted with 150 ml petroleum ether (three times). The extract was washed with 200 ml NaOH 5% solution (three times) and acidified with several drops of HCl (1M). The extract was then washed with saturated NaCl solution and finally dehydrated with MgSO4 and prepared to screw-capped vials containing 5 ml of liquid medium to which different concentrations of 2.5, 5, 10 and 20µg/ml[22].

B- 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-Nicolle-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[23].

C- Cell proliferation ELISA, Nrdu (Chemiluminescent) method

The cell proliferation ELISA, Nrdu (Chemiluminescent) was performed as described by Roche Diagnostics GmbH Roche Applied Science 68298 Mannheim Germany (Version march 2016, Cat. No. 1027640). The assay is based on the detection of BadU incorporated into the genomic DNA of proliferating cells. A fixed initial density of the parasites was transferred to screw-capped vials containing 5 ml of liquid medium to which different concentrations of 0.1, 0.5, 1 and 5 and 10 µg of BTG and MT were added. Each concentration was done and each run included control Cells grown in 96-well tissue-culture micro plates are labeled by the addition of BrdU for 2 - 24 hours. During this labeling period, BrdU is incorporated in place of thymidine into the DNA of cycling cells. After removing the labeling medium, the cells are fixed, and the DNA is denatured in one step by adding FixDenat. After removing FixDenat, the anti-BrdU-POD antibody is added, and then bound to the BrdU incorporated into the newly synthesized cellular DNA. The immune complexes are detected by the subsequent substrate reaction. The reaction product is quantified by measuring the light emission using a scanning multi-well luminometer (luminescence ELISA reader, synergy x HTX, USA).

Statistical analysis

The results were expressed as mean ± 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

Lethal Dose50 (LD50) FAG and MA against logarithmic and stationary phase's promastigotes calculated. Furthermore, the sensitivities of *Leishmania (L) major* strain *[MRHO/IR/75/ER]* were tested using a simple slide method and compared to results of the standard method the in vitro sensitivities of promastigotes *Leishmania (L) major* strain *[MRHO/IR/75/ER]* (Table 1).

Table 1: Calculation of the LC50 of BTG and MA against promastigotes *Leishmania* (L) *major* [MRHO/IR/75/ER].

Organism	BTG (mg/ml)	MA (mg/ml)
Logarithmic Phase Promastigotes	31.4	334.6
Stationary Phase Promastigotes	37.2	339.0

2. Frequency average of Viability PM of *Leishmania (L) major* strain *[MRHO/IR/75/ER]* in culture according to BTG gradients in comparing with MA group and control by the Cell proliferation ELISA, Nrdu (Chemiluminescent) method:

Effect of BTG against *Leishmania (L) major* strain *[MRHO/IR/75/ER]* of stationary phase PMs shows the results of different concentrations of BTG on stationary phase PMs of *Leishmania (L) major* strain *[MRHO/IR/75/ER]*. In geometrically increasing concentrations, dose dependently inhibited the growth of *Leishmania (L) major* strain *[MRHO/IR/75/ER]* PMs. The results show strain of *Leishmania (L) major* strain *[MRHO/IR/75/ER]* was sensitive to BTG, increasing concentrations; dose dependently inhibited the growth of parasite. BTG concentrations 0.1, 0.5, 1, 5 and 10 mg/ml was added to cultured parasite and counted PMs with the Cell proliferation ELISA, Nrdu (Chemiluminescent) method. There was statistically significant difference between BTG and MA groups and Control (P=0.000) (Figure 1).

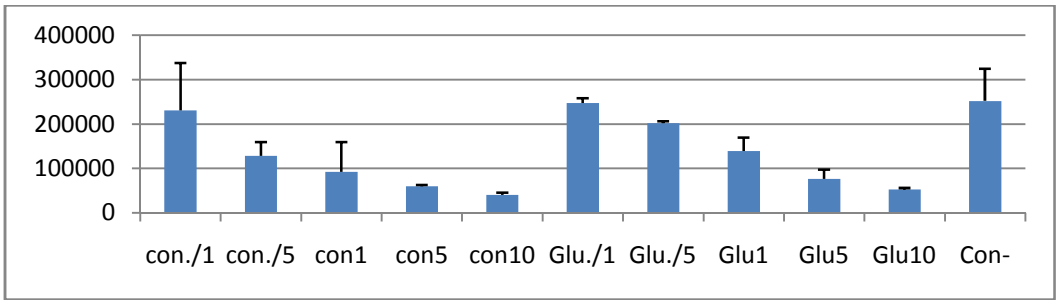

Figure1. Average number of viability PM *Leishmania (L) major* strain *[MRHO/IR/75/ER]* in concentrations of 0.1, 0.5, 1, 5 1nd 10 mg of BTG and MA and control (P=0.000).

3. Frequency average of growth PM of *Leishmania (L) tropica* [MHOM/IR/NADIM3] in culture according to BTG and MA gradients in comparing with control group by the Cell proliferation ELISA, Nrdu (Chemiluminescent) method:

Effect of BTG against *Leishmania (L) major* strain *[MRHO/IR/75/ER]* of stationary phase PMs shows the results of different concentrations of BTG and MA on stationary phase PMs of *Leishmania (L) major* strain *[MRHO/IR/75/ER]*. In geometrically increasing concentrations, dose dependently inhibited the growth of *Leishmania (L) major* strain *[MRHO/IR/75/ER] PMs*. The results show strain of *Leishmania (L) major* strain *[MRHO/IR/75/ER]* was sensitive to BTG and MA, increasing concentrations; dose dependently inhibited the growth of parasite. BTG concentrations 0.1, 0.5, 1, 4 and 10 mg/ml was added to cultured parasite and counted PMs with the Cell proliferation ELISA, Nrdu (Chemiluminescent) method. There was statistically significant difference between BTG, Ma groups and control (P=0.000) (Figure 2 and 3).

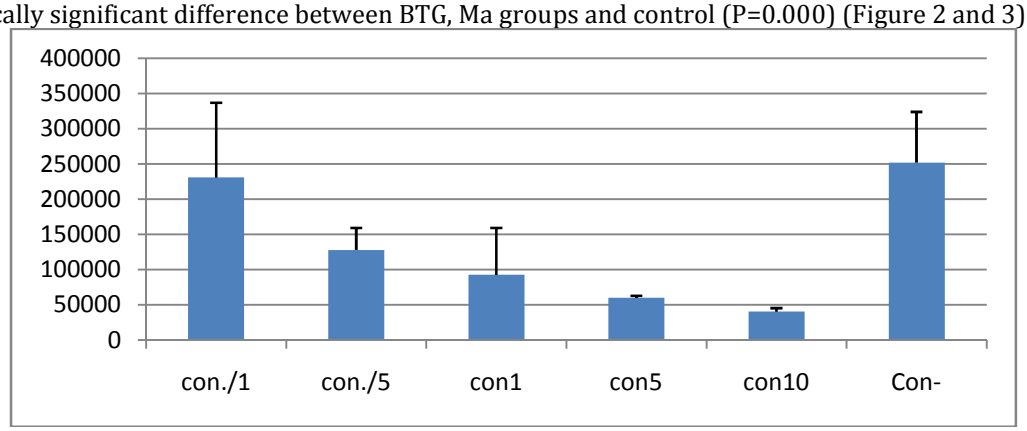

Figure2. Average number of growth PM *Leishmania (L) major* strain *[MRHO/IR/75/ER]*. in concentrations of 0.1, 0.5, 1, 5 1nd 10 mg of BTG and Control P=0.000).

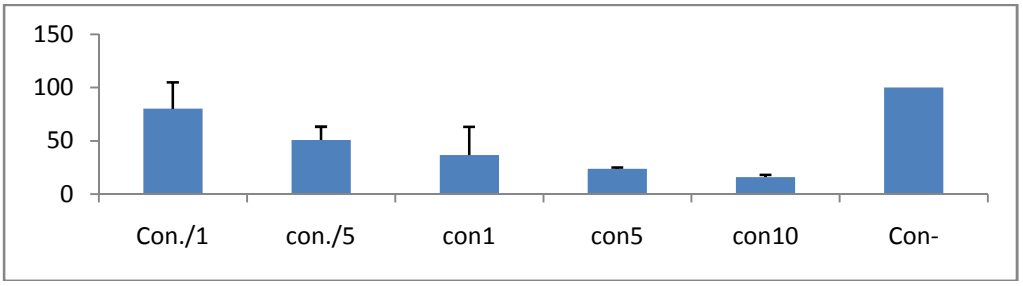

Figure3. Average number of growth PM *Leishmania (L) major* strain *[MRHO/IR/75/ER]*. in concentrations of 0.1, 0.5, 1, 5 1nd 10 mg of MA and Control P=0.000).

DISCUSSION

In the recent past the standard treatment of Leishmaniasis involved the use of pentavalent antimonials Sb (V).Antimony-containing compounds that are the main drugs used to treat Leishmaniasis include: Meglumine antimonite and Sodium stibogluconate. Importance of antimony in the early medicine is well documented, due to the debate created around their utilization in this period. 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. It has been posed since 1940 for systemic treatment. It has been used as the first priority in treatment since 1984. Different methods to cure Leishmaniasis have been recommended that most of them have been topical and systemic treatments [24-26]. Until now, many studies have been carried out regarding the effect of herbal compounds on parasitic infections. Given that Cutaneous Leishmaniasis is limited to the skin typically, so the use of an effective topical medicine in treating this disease is very important. In the present study has suggested in geometrically increasing concentrations, dose dependently inhibited the growth of *Leishmania (L) major* strain *[MRHO/IR/75/ER]* PMs. Studies showed that a number of herbs have moderate to strong anti-Leishmania activity[26]. Recent studies showed that BTG has potent have cytotoxic, probably anti-cancer effects and could induce *P53* gene transcription and toxicity in the cultured breast cancer cell line Cytostatic and apoptosis-inducing activity[27-28]. It has Anti-tumor and ant carcinogenic activities[29], Anti-inflammatory activities[30], Anti- leishmanial effects[31]and Anti- microbes[32]. According to

the results, the anti-Leishmania effect of the BTG may make it possible to use them in the treatment of Cutaneous Leishmaniasis as a complementary or alternative therapy; however, further studies are necessary and should be evaluated in cell culture and in vivo conditions to confirm it.

CONCLUSSION

Based on the result of this study, Considering the made compound BTG comparison with MA, its use possibility in the treatment of species, Leishmania(L)major, is not far to reach**.** Although BTG in vitro was affected, but probes are mandatory in the cases of animal and sick persons, figure out its daily dosage, concentration, time and duration.

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CONFLICTS OF INTEREST

The authors have no conflict of interest.

AUTHORS' CONTRIBUTIONS:

Ali Fattahi Bafghi conceived, designed and is accountable for all aspects of the work.
Approval of the final version of the manuscript and & editing of manuscript.
Ali Mohammad Ranjbar contributed in the design of the work, revising the draft,
Sayyed Sina Montakhab & Banafsheh Goudiani did data collection and manuscript writing.
Arefeh Dehghani did statistical analysis.

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