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The Effect of Rubia Tinctorium Extract on Cutaneous Leishmaniasis in BALB/c Mice

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Abstract: Leishmaniasis is one of the most important infectious diseases in the world. There are wide differences in the clinical features of Leishmaniasis. Cutaneous leishmaniasis is self-healing while visceral leishmaniasis is fatal. Efforts have been made since more than a century ago to control and treat the disease, but appropriate vaccines, drugs and pesticides have not yet been prepared. While in these conditions, alternative medicines and herbal medicines were approved by WHO and other reliable worldwide references, Rubia tinctorum has been used traditionally in medicine throughout history. The purpose of this study was to examine the effect of Rubia tinctorum extract on cutaneous leishmaniasis in BALB/c Mice.Rubia tinctorum extracts with 40, 60 and 80% concentrations were prepared. Then, the BALB/c mice were infected with leishmania (L) major [MRHO/IR/75/ER]. Soon after the ulcer started to appear in the early stage, one dose of the herbal medicine with 40, 60 and 80% concentration on honey base was used to treat the ulcer and dosing continued till the death of the mice of negative control group occurred. The mean weight of the mice that received 40% concentration of Rubia. tinctorum extract showed a statistically significant difference compared to the mean weight of the mice receiving 60% concentration of Rubia. tinctorum extract (P=0.001), but showed no statistically significant difference compared to the mean weight of the mice receiving 80% concentration of Rubia. tinctorum extract (P>0.05)The mean weight of the mice that received 40, 60 and 80% concentrations of Rubia tinctorum extracts showed a statistically significant difference compared to the mean weight of the mice in control group (P=0.000).The mean of lesion size of the mice that received 40, 60 and 80% R. tinctorum extracts showed no statistically significant difference compared to the mean of the lesion size of the mice in control group (P>0.05). When the mice infected with Rural Cutaneous Leishmaniasis {Leishmania (L) major [MRHO/IR/75/ER]} were not treated, they develop Visceral Leishmaniasis. When the weight decreased and lesion size of the Cutaneous Leishmaniasis widened, the animal would die. As a whole, in the all of the time,the mice that received 40, 60 and 80% Rubia tinctorum extract their lesion were wet, without secondary infection and without necrosis.

Key words: Cutaneous leishmaniasis . rubia tinctorum . extract . BALB/c mice

INTRODUCTION

Madder (Rubia tinctorum) is a foregoes plant from Rubiaceae family that historically originated from Ghafghaz and Near East. This plant was traditionally cultivated in the central and western regions of Iran. Cultivation of madder is prevailed in the central regions of Iran like Yazd province for dye industry and extracting the drug components. Nowadays, industrial color is used instead of madder extracted color [1,2,3,4].The main parts of madder used for mentioned goals are roots and rhizomes, which contain Alizarin, rubestric acid and pourpourines. The red color of madder is due to the Alizarin component.

Drugs synthesized from products of madder are used as diuretics, laxatives and also to parry the kidney stones.In India, it has been used to redden lips and cheeks. It also has a 2000 year history as a medicinal herb in China, India and ancient Greece for breaking kidney stones (it's a diuretic), to promote the flow of menses, cure jaundice and because of its high tannin content, for various intestinal problems. In Europe, it was used to dye urine and bones for medicinal purposes. It is antibiotic and anti-inflammatory [5-10].

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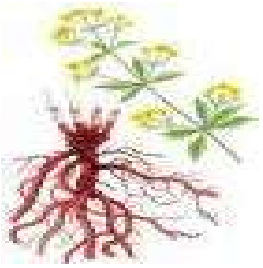


Leishmaniasis is endemic in 88 countries in the world and 350 million people are considered at risk. An estimated 14 million people are infected and each year about two million new cases occur. The disease contributes significantly to the propagation of poverty, because treatment is expensive and hence either unaffordable or of a substantial economic burden. Leishmaniasis with HIV coinfection is an emerging condition that demands urgent attention. Even when coinfected patients receive proper treatment, they relapse repeatedly and the outcome frequently is fatal. Each year, there are some 500 000 cases of visceral leishmaniasis (90% in Bangladesh, Brazil, India, Nepal and Sudan), with an estimated rate of more than 50 000 deaths and 1 500 000 cases of cutaneous disease (90% in Afghanistan, Algeria, Brazil, Islamic Republic of Iran, Peru, Saudi Arabia and Sudan). The global mortality from visceral leishmaniasis can only be estimated, because in many countries the disease is not notifiable or is frequently undiagnosed, especially where there is no access to medication. In some cases, for cultural reasons and lack of access to treatment, the case-fatality rate is three times higher in women than in men. The disease burden is calculated at 2090000 disability adjusted life years (1 249 000 in men and 840 000 in women), a significantly high rank among communicable diseases [11-15].

MATERIALS AND METHODS

To carry the study, 1) a sufficient amount of the roots of R. tinctorum was prepared and ground to powder and then dissolved in 80% Ethanol alcohol. Next, the erlen containing the solution was placed on the magnetic stirrer and kept in room temperature for 48 hours. After filtering, the solution was put in 37° centigrade to obtain a dry extract without water and alcohol. Then, an extract was prepared from the solution using the foluculation method. Finally, 40, 60 and 80% concentration extracts were prepared on honey base. 2) Leishmania (L) major [MRHO/IR /75/ER] obtained from the Iranian Pasteur Institute was cultured in NNN media and transferred to the enriched medium of $RPMI_{1640}$ for mass reproduction. It was given passage four times. Then, Leptomonades in the static form wrere elevated to the concentration of $1x10^{7}$. 3) Leishmania (L) major [MRHO/IR/75/ER] was injected subcutaneously at the base of the tail of 40 inbred (BALB/c) female mice simultaneously at the age of 8 weeks. The mice were obtained from Razi Serum and Vaccine Production Institute. 4) The mice were assigned to the following groups: Control group without receiving any extract and three experimental groups receiving 40, 60 and 80% concentration extracts of R. tinctorum, respectively. 5. after the appearance of the nodule at the site of parasite injection, the every-other-day use of the 40, 60 and 80% extracts for the groups initiated and the lesion size and mice weight were monitored weekly using vernieh colise(Metller Switzerland), respectively. This trend continued till the death of the last mouse in the 15^{th} week. 6. The data were collected and analyzed using the SPSS through the statistics of anova.

RESULTS

The mean weight of the mice that received 40% concentration of Rubia. tinctorum extract showed a statistically significant difference compared to the mean weight of the mice receiving 60% concentration of Rubia. tinctorum extract (P=0.001), but showed no statistically significant difference compared to the mean weight of the mice receiving 80% concentration of Rubia. tinctorum extract (P>0.05). The mean weight of the mice that received 40, 60 and 80% concentrations of Rubia. tinctorum extracts showed a statistically significant difference compared to the mean weight of the mice in control group (P>0.05), (P=0.018) (Fig. 1).

The mean of lesion size the mice that received 40% concentration of Rubia. tinctorum extract showed a statistically significant difference compared to the mean of the lesion size of the mice receiving 60 and 80% Rubia. tinctorum (P=0.000). The mean of lesion size of the mice that received 40, 60 and 80% concentrations of Rubia. tinctorum extracts showed no statistically significant difference compared to the mean of the lesion size of the mice in control group (P>0.05) (Fig. 2).

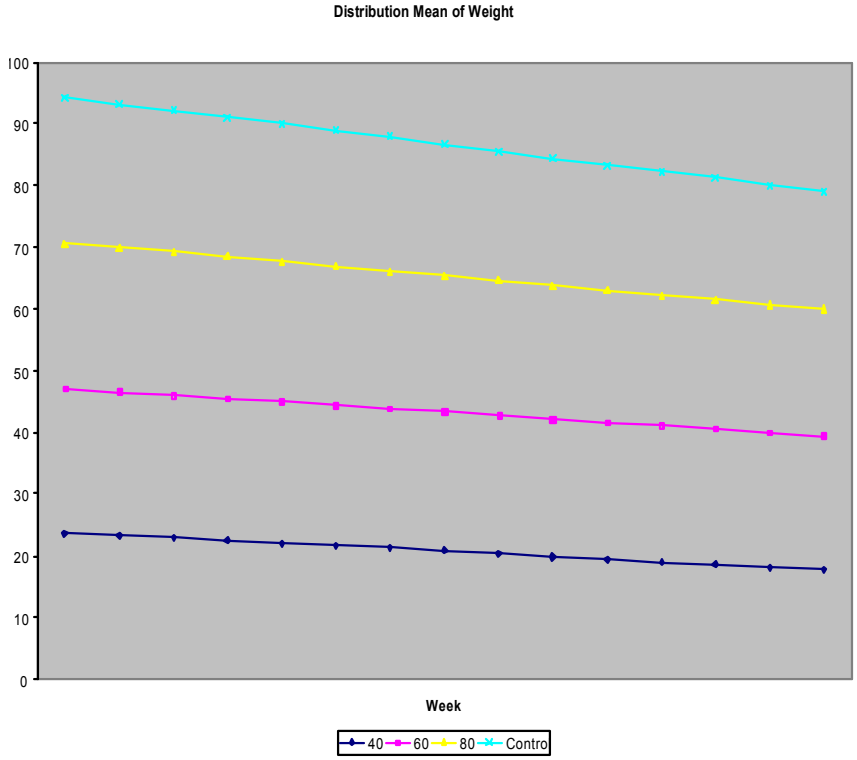
Distribution Mean of Weight
100
90
80
70
60
50
40
30
20
10
0
Week
40
60
80
Contro

Fig. 1:

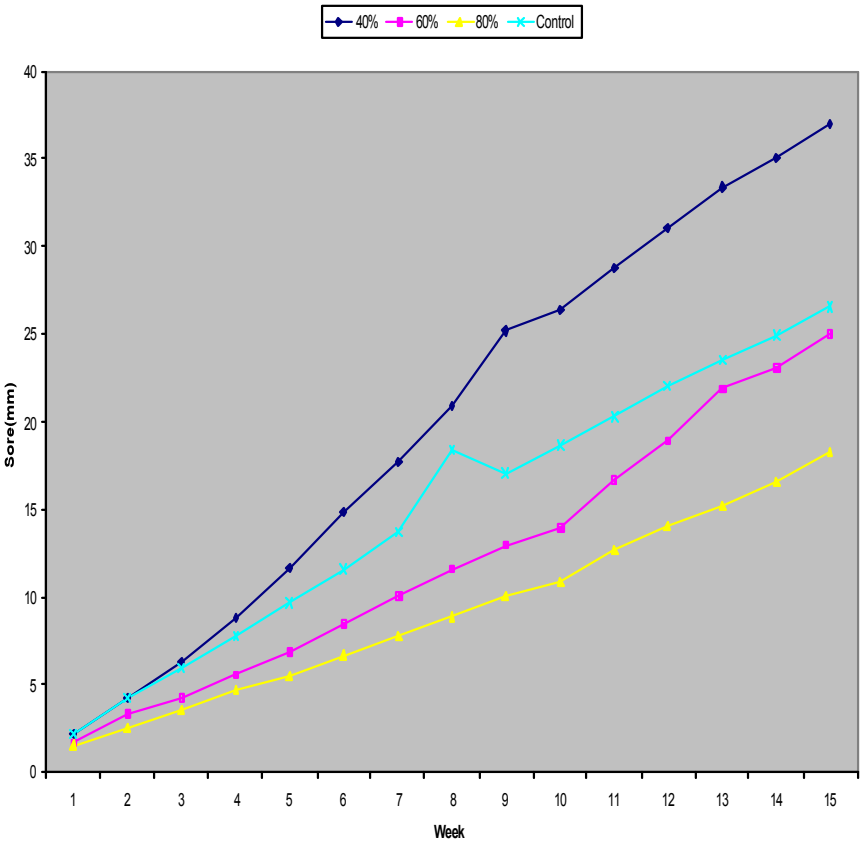
40%
60%
80%
Control
40
35
30
25
20
15
10
5
0
Sore(mm)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Week

Fig. 2

DISCUSSION

The first alternative in treating Lishmaniasis includes the 3 and 5 valence compounds of Antimowan as glucontum and pentostam, the first being more commonly used in Iran. The prolonged administration of this drug induces severe complications on kidneys and liver. Further, the intramuscular use of this medicine causes pain and dry joints or arthrosclerosis, thus not being favored by the patients. Presently, there are no effective and appropriate cures for this condition, hence, preparing a suitable drug for this purpose is of utmost importance [16]. In this regard, attention was paid to the effect of R. tictorum extract on the small white rat, BALB/c. When this mouse is infected with the parasite Leishmania (L) major,it develops cutaneous leishmaniasis at first. Then, the lesion size increases gradually, the animal loses weight and if untreated, the lesion develops into visceral leishmaniasis and finally the animal dies [17]. In this experimental study, an attempt was made to survey the effect of R. tinctorum extract on leishmaniasis in BALB/c through monitoring weight and lesion size.

The use of Rubia tinctorium extract slowed down the course of weight decrease in the mice afflicted with Lishmaniasis, so that the swelling, redness of lesion and weight decrease in these mice were statistically different compared to those of the control mice. The greater the extract concentration, the smaller the amount of weight decrease. Yet, the use of 40, 60 and 80% extract concentrations had no effect on the decrease in lesion size of the mice with Lishmaniasis. Although the lesion size of the mice with Lishmaniasis was smaller than that of the control mice, it was not statistically significant, that is, this extract had no effect on lesion size increase in the mice with Lishmaniasis. On the whole, the use of R. tinctorium extract ceased the weight decrease in the mice with Lishmaniasis, yet it showed no perceptible impact on lesion size increase in the mice.

The mice with Lishmaniasis survived 43 days more than the control mice. Meanwhile, their lesion remained moistened, without dryness, necrosis, or secondary infection Kalyoncu F, Cetin B, Saglam H (2006) reported Antimicrobial activity of common madder (Rubia tinctorum L.) [18]. Nakanishi *et al*. (2005) reported the efficacy of lucidine a-beta prime and rocide (LUP) found in the roots of R. tinctorum on the immune system [19]. Jacor and colleagues (2005) demonstrated that antraquinons, a derivative of R. tinctorum, can function as mutagens and carcinogens, also they have antiseptic effects [20]. Manojlovic and others (2005) demonstrated the antifungal activity of R. tinctorum extracts including antraquinon, alizarin and emodin [21]. Ozgon *et al*. (2003) reported that the hydro alcoholic extract of R. tinctorum has an antioxidant activity and an antimicrobial effect on bacilli, escirichiae and staphylococci [22]. Tao and others (2003) reported the releasing effect and nitric acid forming effect and also degranulation in macrophages [23]. Singh R., Geetanjali and Chuhan S M (2004) isolated more than 150 chemical compounds of different types of active compounds including antrakinon, glycosids, trepens, Hexapeptids, iridoids and carbides [24]. Bisswas TK *et al*. (2003) and BiswasTK *et al*. (2004) talk about the use of this extract in the treatment of wounds and injuries in the traditional medicine of India (Ayurveda) [25, 26].In sum, the use of R. tictorum slowed down the weight decrease and lesion size increase in mice BALB/c compared to the control mice. Also, it postponed the death of the infected mice for about 43 days compared the control mice, while during all this time the lesion remained moist without any necrosis or secondary infection.

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