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Full Length Research Paper

The *in vivo* antileishmanial activity of alcoholic extract from *Nigella sativa* seeds

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The effect of an alcoholic extract of *Nigella sativa* seeds was surveyed on cutaneous leishmaniasis in BALB/c mice. A subcutaneous inoculation of *Leishmania* (L) major at the dorsal base of the tail produced swelling, inflammation, stiffness, redness and sore. As soon as symptoms appeared, the ointment was used on the wound of mice as a treatment (the seeds were dried and crushed into coarse powder. Five hundred grams of the powder were extracted with ethanol 95% v/v. The extract was concentrated under reduced pressure of 22 to 26 mmHg at 45°C, then 40, 60 and 80% of *Nigella sativa* seed extract were prepared in ointment base). As the animals were weighed by the scales, lesion development was monitored every other day with a direct-reading vernier caliper gauge. This monitoring continued to the death of the last mice in the control group. Then the spleens of mice in each group were measured and controlled. Weight loss or stoppage was not observed in any of the case group mice. But the wound diameter in all case groups was smaller, compared to the control group. Swelling, inflammation, stiffness, redness, necrosis and secondary infection were less in the case group mice compared to the control group mice. So, these results indicate that the alcoholic extract of *Nigella sativa*, showed a significant anti-cutaneous leishmanial activity and this validates the traditional use of the plant in fungal infections.

Key words: *Leishmania* (L) major, alcoholic extract, antileishmania, BALB/c mice.

INTRODUCTION

Leishmaniasis is a complicated disease induced by an obligate intracellular parasite from the genus *Leishmania*. It is one of the major infectious diseases affecting the low economic populations throughout the world and there are two million annual new reports in 88 countries. Where the disease is endemic,the parasite is transmitted via the bite of a sandfly, and leads to visceral, cutaneous, or mucocutaneous leishmaniasis. The global burden of this disease has remained stable for some years, causing 2.4 million disability adjusted life years and 59 000 deaths in 2001 (Raza et al., 1999; Khan, 1999). For more than 60 years, pentavalent antimonials(sb^v), 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 antileishmanial drug (Mehta et al., 2008; Salman et al., 2008; Zuridah et al., 2008; Hannan et al., 2008). Although experimental 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

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sight, its control relies mostly on chemotherapy, so, there is an urgent need to develop new and better drugs to combat this infectious disease (Nazrul et al., 1987; Khan et al., 2003; Tandon et al., 1997).

Nigella sativa L. is an annual dicotyledon of Ranunculaceae family known commonly as “Siah Daneh” (Persian) and “Black Cumin” (English). it is a herbaceous plant that grows in Iran and other middle east countries and has been widely used as condiment in pickles, bread and other foods. As a common indigenous plant, it has been used for the treatment of many diseases, including parasitic disease.

Figures 1 and 2 Major constituents of its seed are nigellin, metarbin, melanthin, anthraqui-nones, glycosides, saponines, volatile oils, fixed oil, albominous proteins, tannin, glucose, mucilage resins etc., (Mohammad et al., 2005; Abdulelah and Zal-Abidin, 2009; Abdulkader and Tonkal, 2009). Many therepeuthic effects of *Nigella sativa*, such as: Antibacterial (Zuridah et al., 2008), antifungal (Rathee et el., 1982), antihelmenthic (Desjeux, 2001; Bryceson, 2001; Shyam, 2003), antiprotozoan (Khaled et al., 2005; Fattahi et al., 2009; Chherti et al., 2010), anti-inflamatory (Musa et al., 2004; Bukhari, 1985; Ghaznavi, 1988), antioxidant (Al Jawziyya and Al Akili, 1993; Salem and Hossain, 2000; Morsi, 2000) is reported. The present study was carried out to investigate the effect of *Nigella sativa* extract (NSE) on cutaneous leishmaniasis in BALA/c mice.

MATERALS AND METHODS

Preparation of plant extract

Nigella sativa seeds of Iranian origin were 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) (Khaled et al., 2005; El-Dakhakhny, 2000). 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 yieled 40, 60 and 80% of *Nigella sativa* seed 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. Amostigotes were isolated from mice spleens, and then transformed to promastigotes in Novy-Nicolle-Mac Neal (NNN) medium supplemented with penicilin (100 U/ml), streptomycin (100 µg/ml) and 20% heat-inactivated fetal calf serum (FCS) at 25°C. Subsequently, the third passage promastigotes from NNN medium were progressively adopted 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 Nigella sativa extract) and 1 control (receiving the ointment base) groups. Mice were subcutaneously inoculated with 0.1 ml = 10^6 infective promastigotes at the dorsal base of the tail. As soon as the sypmtopm 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.

Statistical analysis

Data are expressed as Mean ± SEM of lesion’s diameter and animal’s weight, and were statistically analysed by multivariate test for analysing the effect of time within-subjects and multiple comparisons test for comparing the variables between groups. In this study, $p < 0.05$ 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% NSE was the same as those receiving 60, 80% NSE and control mice ($p > 0.05$). (Figure 3) Also, there was no significant difference between the average weight of mice receiving 60 and 80% NSE (>0.05) (Figure 4).There was a significant difference between the average weight of mice receiving 60 and 80% NSE as compared with the average weight of control group ($p > 0.05$.

Average diameter of ulcer mice receiving 40% of *Nigella sativa* with a mean diameter of ulcer mice receiving extracts 60, 80% of *Nigella sativa* extracts and the control group mice showed a significant difference($p < 0.05$). Average diameter ulcer mice receiving 60% *Nigella sativa* with a mean diameter of ulcer mice receiving extracts 40, 80% of *Nigella sativa* extracts and the control group mice showed a significant difference ($p < 0.05$). Average diameter ulcer mice receiving 80% of *Nigella sativa* with a mean diameter of ulcer mice receiving extracts 40, 60% of *Nigella sativa* 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 *Nigella sativa* showed significant difference ($p < 0.05$). At the end of experiments, average diameter of ulcer in mice receiving 40% NSE showed a significant difference as compared to those receiving 60 and 80% NSE ($p > 0.05$). Ulcer's diameter in animals receiving 80% NSE was significantly less than those receiving 60% NSE ($p > 0.05$). Ulcer's diameter in all test groups was significantly less than the control group ($p > 0.05$, which received 40, 60 and 80% NSE.

Spleen size in test groups was smaller than the control

Figure 1. Flower of *Nigella sativa* seeds.

Figure 2. *Nigella sativa* seeds.

group. It showed that the more the concentration of the extract was higher, the more the spleen size was smaller. Our findings also revealed that there is a negative relation between the NSE percentage and the spleen size. Wounds in mice receiving different concentrations of NSE also showed less secondary infection, necrosis, stiffness, redness, swelling and inflammation as compared with the control group (Figures 5 to 8).

DISCUSSION

Antimony-containing compounds that are the main drugs used to treat leishmaniasis include: Meglumine

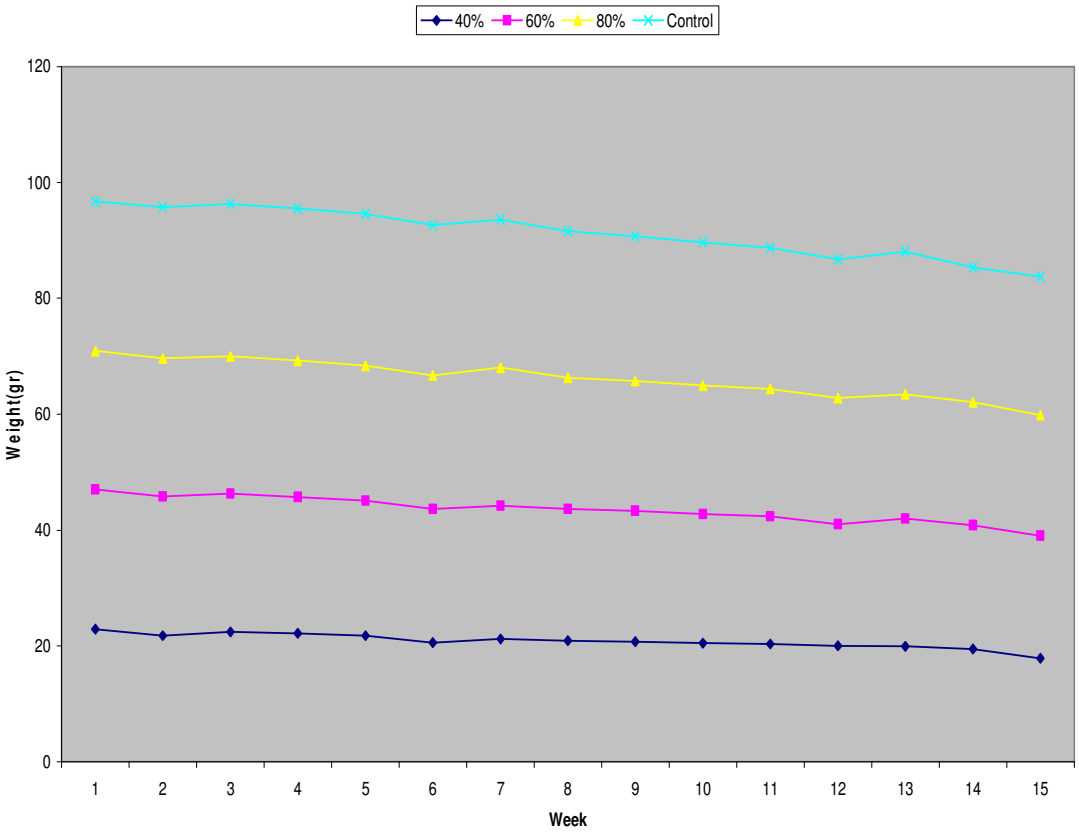

Figure 3. Effect of *Nigella sativa* extract on average weight of BALB/c mice receiving 40, 60, 80% of and the control group.

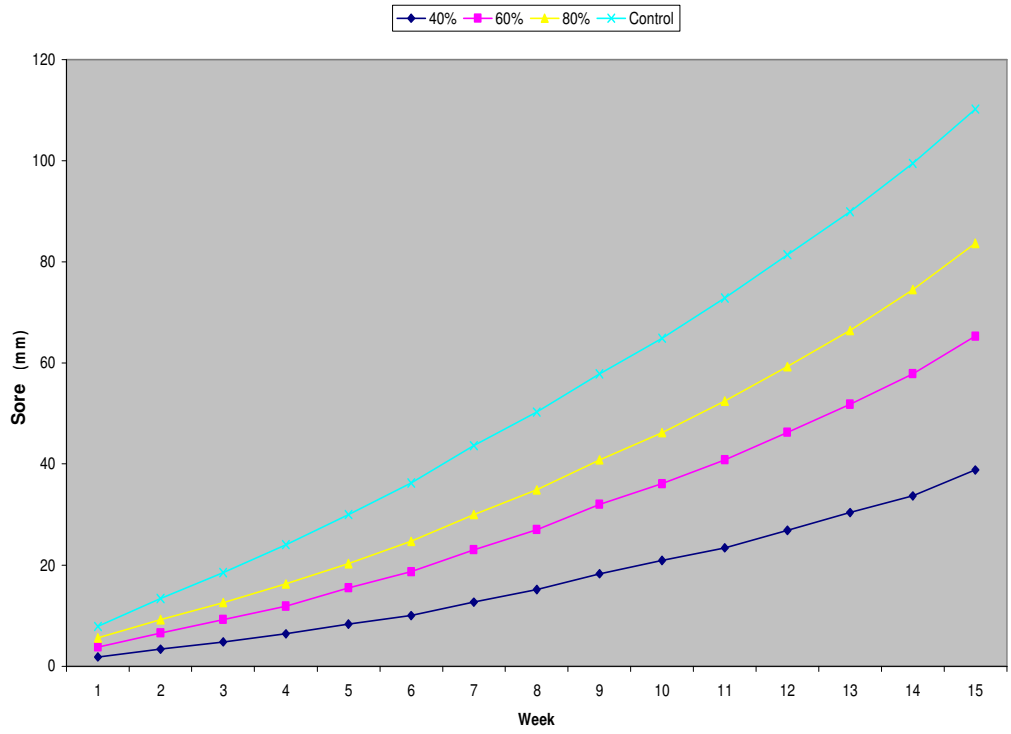

Figure 4. Effect of *Nigella sativa* extract on average diameter of ulcer BALB/c mice receiving 40, 60, 80% of and the control group.

Figure 5. Control.

Figure 6. Rceiving 40% of *N. sativa* extract.

antimonate and Sodium stibogluconate. 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 (Hadighi et al., 2007; Singh, 2006). Regarding *Nigella sativa* therapeutic use, Holy Mohammad (pbuh) said " there exist, in the black seed grains, health and cure of all the diseases except death". Avicenna recognized that, black seed grains, act as an expectorant and helps recovery from fatigue, and dispirit, and stimulates the body's energy (Bukhari, 1985; Ghaznavi, 1988; Al Jawziyya and Al Akili, 1993). Recently, many biological activities of *Nigella sativa* have been reported, including antifungal, antibacterial, antiviral, anti-inflamatory, antioxidant, anti-helminthic, etc., (Daoud et al., 2004; Salem and Hossain, 2000; Morsi, 2000; El-Dakhakhny et al., 2000).

Different concentrations of NSE did not show any significant change in weight loss in BALB/c mice. Although, 40% NSE did not show any significant weight loss as compared with control group, 60 and 80% NSE showed a significant weight loss as compared to control mice. This result indicates that the NSE could not prevent the spread of parasite inside the animal's gut and failed

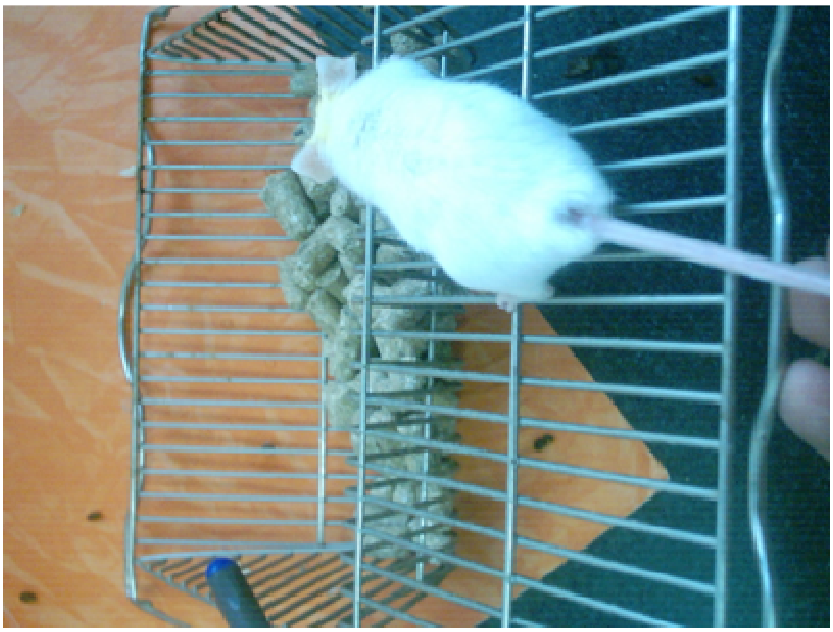

Figure 7. Receiving 60% of *N. sativa* extract.

Figure 8. Receiving 80% of *N. sativa* extract.

to prevent the conversion of cutaneous leishmaniasis to its visceral type .

However, using different concentrations of NSE, 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 *Nigella sativa* extract was more 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 *Nigella sativa* extract 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. This shows an inverse dose dependent effect on

Lishmania induced wound diameter, indicating its effective role in the inhibition of parasite proliferation in macrophages, which is also indicative in less inflammatory responses in wounds treated with NSE.

Overall, it was concluded that a relatively positive activity was shown against cutaneous leishmania, but the conversion of cutaneous leishmaniasis to Visceral leishmaniasis, could not be prevented by this extract. This may be due to insufficient concentration of NSE or the route of its administration,perhaps, because *Nigella sativa* extract concentration used was insufficient. Although, anti-cutneous leishmanial activity with no visible adverse effects on the tissue architecture has been demonstrated for the plant extract, further investigations are needed, prior to be recommended for its use as a safe and effective anti-cutneous leishmanial agent. We also recommend using the more advanced, dense concentration of *Nigella sativa* extract obtained (for example, the oil produced), through a systematic way and not through the topical route. Further investigations using higher concentrations of NSE in a topical and/or systemic routes of administration is recommended.

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