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Research Article

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Antileishmanial activity of *Nigella sativa* extract against *Leishmania major*: An *in vitro* study

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ABSTRACT

Leishmaniasis is wide range, worldwide, without drug, vaccine, secticide and has not sterile immunity and efforts in this field have not been successful. Herbaceous plants tomentose perennial official Siah Daneh and scientific name Nigella sativa. Nigella sativa L. is an annual dicotyledonous of Ranunculaceae family known commonly as "Siah Daneh" (Persian) and "Black Cumin" (English). Many therapeutic effects of Nigella sativa, such as: antibacterial, antifungal, anti- helminthes, antiprotozoan, anti- inflammatory, antioxidant is reported. The present study was carried out to antileishmanial activity of Nigella sativa extract against Leishmania major: An In vitro study. Amastigote was 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. A fixed initial density of the parasites was transferred to screw-capped vials containing 5 ml of RPMI1640 media to which different concentrations of 0.8, 4, 20 and 100 µg NSE 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. After 72 hour, the percent inhibition was supper in all doses in stationary and logarithmic phases. As shown, after 48hour, viability of parasites in PMs significantly decreased in NSE 0.8, 4, 20 and 100 µg treatment compare to control group. The results show that NSE could prevent from growth and viability of parasites and this can be useful for treatment of Leishmaniasis.

Keywords: Cutaneous Leishmaniasis, Nigella sativa extract, *Leishmania major*, in vitro.

INTRODUCTION

Nigella sativa (NS) is a grassy plant with green to blue flowers and small black seeds which grows in temperate and cold climates. Thymoquinone, monoterpens such as *p*-cymene and "-pinene1, nigellidine, nigellidine and a saponin is containing the seeds of NS. Several therapeutic effects including antiasthma and dyspenea have been described for the seeds of NS in Iranian ancient medical books. In Arabian folk medicine, the whole black seeds alone or in combination with honey are also prompted for treatment of bronchial asthma. There is evidence of relaxant effects of the volatile oil from this plant on different smooth muscle preparations including many therapeutic Effects of *NS*, such as: Antibacterial, antifungal, antihelmenthic, antiprotozoan, anti-inflammatory, and antioxidant is reported too (1, 2, 3 and 4). In the former studies suggested antileishmanial activity and cytotoxicity of the extracts of Berberis vulgaris and Nigella sativa against Leishmania tropica, especially Leishmanicidal and cytotoxic activities of Nigella sativa and its active principle, Thymoquinone (5 and 6). Leishmaniasis is a complicated disease that caused by the protozoan *Leishmania* parasites which are transmitted by the bite of infected sand flies. The disease affects the poorest people on the planet, and is associated with malnutrition, population displacement, poor housing, a weak immune system and lack of resources. Leishmaniasis is linked to environmental changes such as deforestation, building of dams, irrigation schemes and urbanization. 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 occur worldwide annually. No vaccines or drugs to prevent infection are available. Preventive measures are aimed at reducing contact with sand flies by using personal protective measures (7, 8, 9 and 10). 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 (11, 12 and 13). The present study was carried out to antileishmanial activity of NS extract against *Leishmania major*: An in vitro study.

EXPERIMENTAL SECTION

Preparation of *Nigella sativa* seeds

Sufficient NS seed were minced and they were dissolved in Ethanol 80. Then they were placed into the stirrer with magnets in room temperature for 24 hours. Next, they were softened. This compound was, first, sterilized and prepared as tropica with concentrations of 0.8, 4, 20 and 100 μg. The extracts were filtered and the solvents were evaporated in vacuum with a rotator evaporator that yielded a blackish-brown concentrates and kept at 4°C prior to use (14).

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 med sciences. *Leishmania major* strain (MRHO/IR/75/ER) was maintained in BALB/c mice. Amastigote was isolated from mice spleens, and then transformed to promastigotes in Novy-Nicolle-Mac Neal (NNN). Subsequently, the Third passage promastigotes (both logarithmic and stationary phases) from NNN medium were progressively adapted to RPMI 1640 media (Gibco) supplemented 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 (15).

The cell proliferation ELISA, Nrdu (chemiluminescent) method

The cell proliferation of enzyme-linked immunosorbent assay (ELISA), Nrdu (Chemiluminescent) was performed as described by Roche Diagnostics GmbH Roche Applied Science 68298 Mannheim Germany (Version march 2005, Cat. No. 11 669 915 001) 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 bromoorydin was added and it was incubated at 37°C for 8 hours
• Supernatant was removed
• Fixator was added to the permeable membrane
• Anti-oxibromoouridin 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 ± 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 AND DISCUSSION

The percentage inhibition of NSE and stationary and logarithmic phases of *Leishmania major [MRHO/IR/75/ER]* showed that the % inhibition is density in time (hour). After 72 hours, the %inhibition was supper in all doses in stationary and logarithmic phase. As shown, after 48h, viability of parasites in PMs significantly decreased in NSE 0.8, 4, 20 and 100 μg treatment compare to control group.

Figure 1: % inhibition NSE against *Logarithmic* Phase *Leishmania* major *[MRHO/IR/75/ER]* in concentrations of 0.8, 4, 20 and 100 µg. Results are compared µg. To the control on 6-72 hours

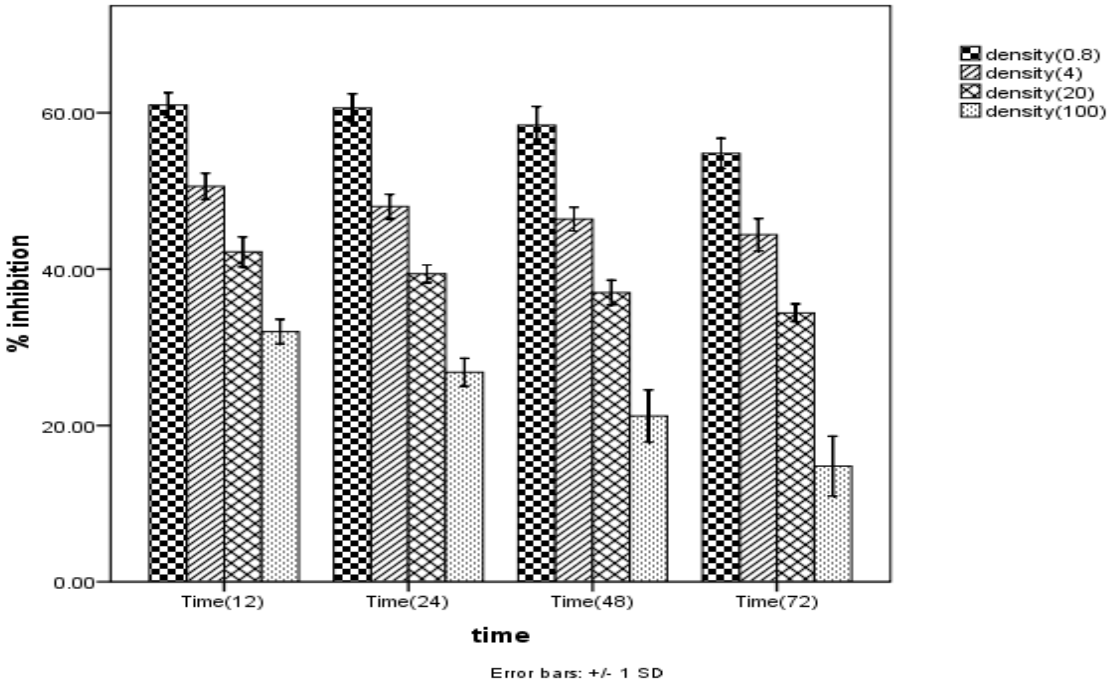
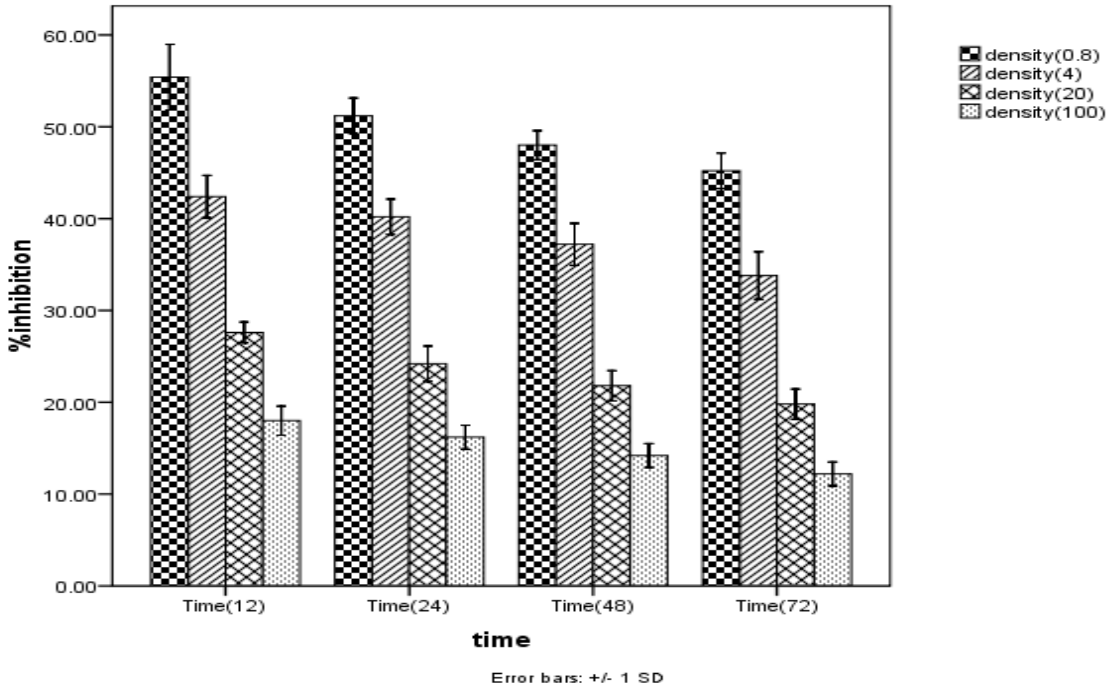


Figure 2: % inhibition NSE against *Stationary* Phase *Leishmania major [MRHO/IR/75/ER]* in concentrations of 0.8, 4, 20 and 100 µg. Results are compared µg to the control on 6-72 hours



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. Leishmaniases present a DALYs (Disability Adjusted Life Years). Therefore, new drugs are constantly being tested for their potential Leishmanicidal activity (16 and 17).

Medical plants are clearly a potential source of new anti-protozoal drugs. The biological activity of plant extracts has been attributed to compounds belonging to diverse chemical groups including alkaloids, flavonoids, phenylpropanoids, steroids, and terpenoids (18, 19). In this study, we investigated the antileishmanial activity of

assafoetida on Leishmania major parasites in vitro. Our result indicated that NSE could increase mortality of *L. 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 Leishmania body with increase in exposure period or it may be possible that the active component (s) could change into more toxic forms in the Leishmania body by the action of different enzymes. Cytotoxicity activity of NSE was researched in several different studies. Recently, antiviral activity of assafoetida was assessed against some human rhinovirus serotypes (18). Recently, many biological activities of NS have been reported, including antifungal, Antibacterial, antiviral, anti-inflammatory, antioxidant, antihelmenthic, etc (21, 20, 23, 24). Antiparasitic activity of NSE was studied and researchers found that NSE possesses moderate Antiparasitic properties against *Entamoeba histolytica* (25). Tonkal also showed that Ns aquatic extract have *Trichomonas vaginalis* activity against (24). Antidiabetic, antibacterial, anticancer and antihepatotoxicity of NSE was studied too (27). Evidence indicates that N. sativa seeds have a potential medicinal value and are relatively safe to consume. Future research should focus on the mechanisms by which N. sativa seeds medically and understand its mechanism of bioactivity and diagnostic the active components that have medicinal effectiveness (28 and 29).

Figure 3: Effect of NSE on viability *Leishmania* major in Logarithmic of Phase. Each bar represents means ± SD. promastigotes were cultivated in the presence of different concentrations of the 2.5, 5, 10 and 20 µg and counted after 48 hours

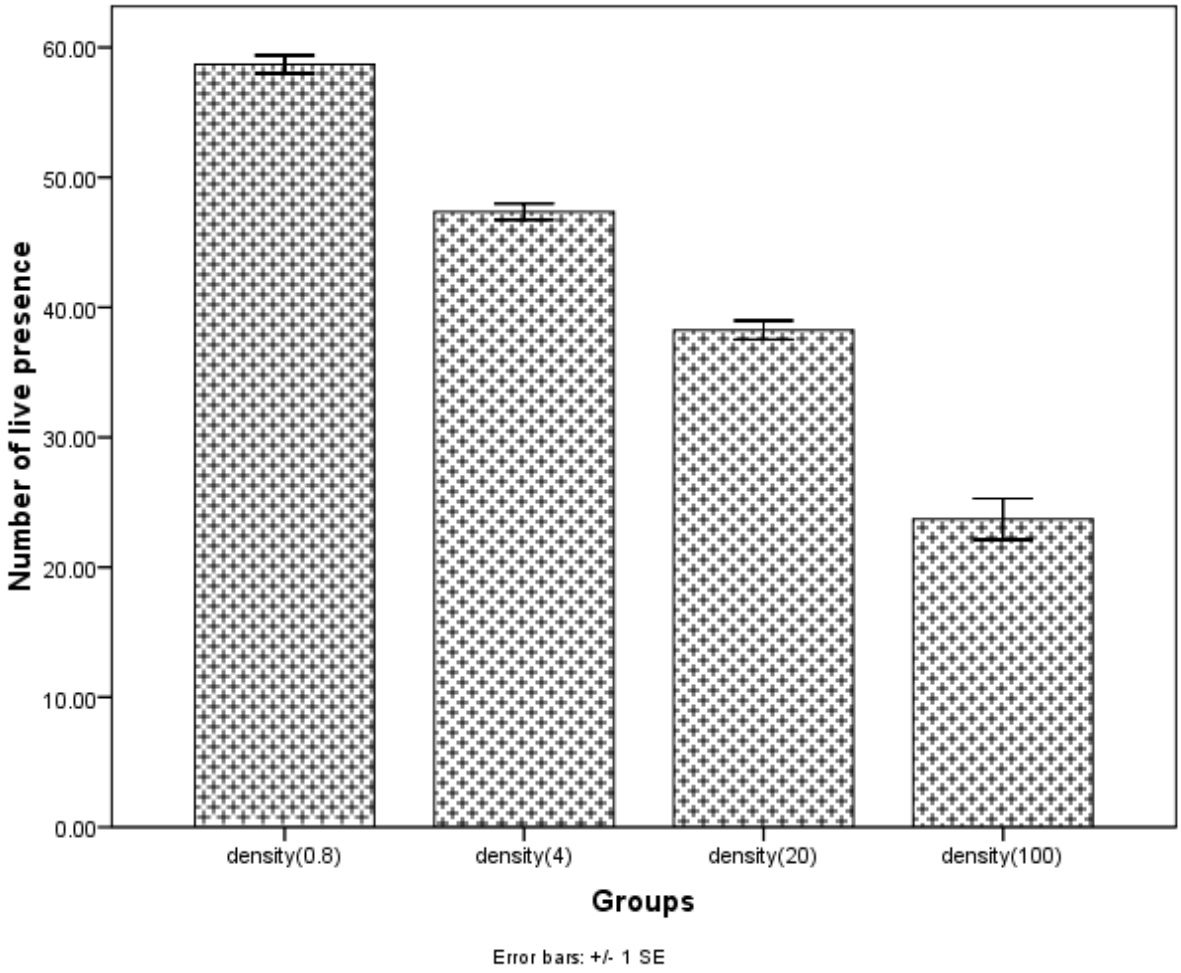
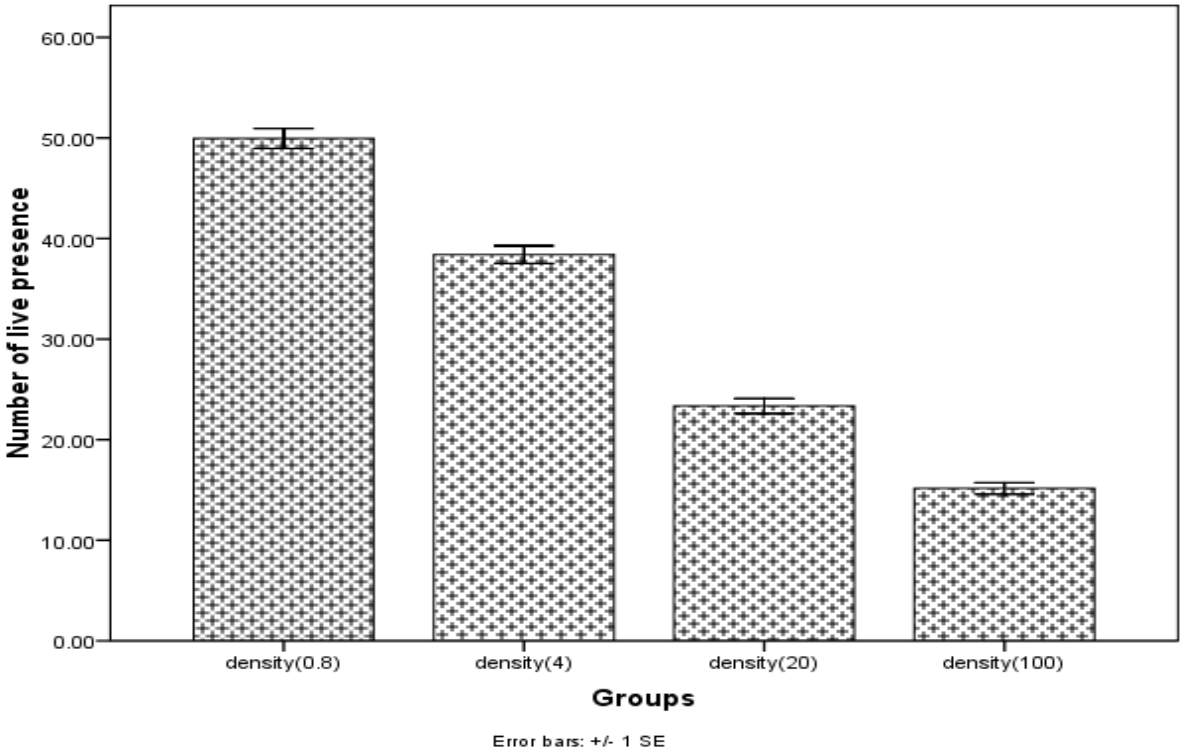


Figure 4: Effect of NSE on viability *Leishmania* major in Stationary of Phase. Each bar represents means ± SD. promastigotes were cultivated in the presence of different concentrations of the 2.5, 5, 10 and 20 µg and counted after 48 hours



CONCLUSION

Overall, it was concluded that a relatively positive activity was shown against CL, but the conversion of CL to Visceral Leishmaniasis, could not be prevented by this extract. This may be due to concentration of NSE or the route of its, perhaps, because NSE concentration used was insufficient. Although, anti-cutaneous Leishmanial activity with no visible adverse effects on the tissue has been demonstrated for the plant extract, further investigations are needed, prior to be recommended for its use as a safe and effective anti-cutaneous agent. We also recommend using the more advanced, dense concentration of NSE obtained (for example, the oil produced), through a systematic way and not the topical route. Further investigations using higher concentrations of NSE 0n a topical and/or systemic route of administration is recommended.

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