European Journal of Medicinal Plants
13(4): 1-7, 2016, Article no.EJMP.24773
ISSN: 2231-0894, NLM ID: 101583475

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Efficacy of Black Seeds Oil (*Nigella sativa*) against *Hymenolepis nana* in Infected Mice

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/EJMP/2016/24773
Editor(s):
(1) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Aditi Singh, Amity University, Lucknow, India.
(2) Heethal Jaiprakash, Mahsa University, Malaysia.
Complete Peer review History: http://sciencedomain.org/review-history/14013

Short Communication

Received 31st January 2016
Accepted 24th March 2016
Published 5th April 2016

ABSTRACT

Recently, many biological activities (e.g., antioxidant, anti-inflammatory, anticancer, antimicrobial, antifungal and antiparasitic) of *Nigella sativa* seeds have been reported. We carried out this study to investigate the therapeutic potential of *Nigella sativa* oil as an alternative and safe treatment against *Hymenolepis nana* based on an experimental study of white laboratory mice. Twenty-eight Swiss albino mice naturally infected with *H. nana* were divided into three groups; one group functioned as the control, and the remaining two groups were fed daily doses of black seed oil (2.5 and 5 ml/kg, respectively). We found that the efficacy of the 5 ml/kg *Nigella sativa* oil dose against *H. nana* attained 100% 14 days after treatment; the efficacy of the 2.5 ml/kg *Nigella sativa* oil dose attained an efficacy of 100% 21 days after treatment. Our results indicate that *N. sativa* oil exhibits significant efficacy against *H. nana* in infected mice.

Keywords: Nigella sativa oil; Hymenolepis nana; efficacy; treatment.

1. INTRODUCTION

Hymenolepis nana, generally known as the dwarf tapeworm is a globally widespread zoonosis disease. Also, it is one of the most common cause of cestode infections, commonly infects rodents as well as human beings [1-3]. Mostly, *H. nana* infection has a cosmopolitan distribution

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with the highest prevalence and heaviest parasite burden among children in warm, arid climates with poor sanitation conditions [4,5].

Synthetic anthelmintics are the most effective way of controlling parasitic infections. However, these medicines are costly and sometimes unavailable to smallholder farmers and pastoralists in developing countries. Moreover, there are cases of increased resistance to anthelmintics worldwide in animals [6-8].

Medicinal plants could be a provenance of new antiparasitic medicine with high efficacy, low toxicity and lower price [9]. At present, about 80% of the drugs which used around the world produced from natural products or some derivatives inspired by natural precursors [10]. Consequently, plants form a very rich source of bioactive chemical compounds against many diseases [11]. There are many studies on several plants to test their anthelmintic efficacy [12-15].

Nigella sativa (Family: Ranunculacea), generally recognized as black seed or black cumin or kalonji seed also renowned as habat ul Barakah, is an annual plant growing in Mediterranean countries. It has been traditionally used in Arabian countries, Europe and the Indian subcontinent for dietary and medicinal purposes as a natural remedy for a various of cases and illnesses that include inflammation, diabetes, hypertension, headache, cough, bronchitis, asthma, eczema, influenza, fever and dizziness [16]. Moreover, black seed (*N. sativa*) was used traditionally for increasing the milk production, as diuretic, appetizing, regulation of menstruation and becoming healthy. In addition, it is added to homemade pastry for decoration and booster of taste [17] also, it could be used as a natural growth promoter [18]. Furthermore, black cumin seeds and its essential oil have been widely used in nutraceuticals, functional foods and pharmaceutical products [19]. Recently, many biological activities of *N. sativa* seeds have been reported, including: antioxidant, anti-inflammatory, anticancer, antimicrobial, antifungal and antiparasitic activities [20-21]. Abdel Daim and Ghazy [22] have revealed the preventive role of *N. sativa* oil against the toxic effects and it potent antioxidant activities. Also, Hassanien et al. [19] found that *N. sativa* oil showed stronger antioxidant potential in comparison with synthetic antioxidants. *Nigella sativa* seeds were reported to contain fixed and volatile oils [23]. Hassanein et al. [24] found that *N. sativa* seed oil to be rich in oleic and linoleic acids. Al-Naqeep et al. [25] reported *N. sativa* seeds to contain high amount of oil (30–48%) and the major unsaturated fatty acids were linoleic acid.

In recent years, *N. sativa* was reported to possess significant efficacy against parasitic worms in several studies [17,20,26-29]. Additionally, El Wakil [30] recorded that It's antiparasitic effect was related to its stimulating immune system. Also, the study confirmed that *N. sativa* aqueous extract could be useful in the treatment of protozoan parasite *Blastocystis hominis* [30], *Trichomonas vaginalis* [31,32], *Plasmodium yoelli nigeriensis* [33] and *Toxoplasma gondii*, [34]. The aim of the present work was to investigate the therapeutic potential of *N. sativa* oil as an alternative and safe treatment against *Hymenolepis nana* through experimental study on the white laboratory mice.

2. MATERIALS AND METHODS

2.1 Black Seed Oil

Amazing Herbs™ Premium Black Seed Oil (100% Pure Cold-Pressed Black Seed Oil).

2.2 Animals

Twenty eight Swiss albino mice of aged 2 months and weight between 25–35 gm each, proven to be naturally infected with *Hymenolepis nana* (by detection of eggs in fecal samples smeared on microscopic slides), were obtained from the animal facilities of King Saud University, Riyadh, Saudi Arabia. The mice were bred under specified pathogen-free conditions and fed with feed (P 684) of the General Organization for Grain Silos and Flour Mills production in Riyadh, Kingdom of Saudi Arabia. The experiments were approved and followed Saudi Arabian rules for animal protection.

2.3 Experimental Design

Animals were divided into three groups, with seven animals in each group.

2.3.1 Control groups

One group functioned as the control group which have animals infected with *H. nana* untreated.

2.3.2 Experimental group

Two groups functioned as the experimental groups.

Groups 2 and 3 were fed daily by single oral gavage with black seed oil (*N. sativa* oil) (2.5 and 5 ml/kg) respectively [27], for twenty one days throughout the experimental period [35].

2.4 Parasitological Studies

Fresh faecal samples of mice were collected from cages on a day (pre-treatment period) and on day 1, 7, 14, and 21 (post-treatment period). Fecal egg counts were estimated using a modified Mc Master technique, and counts were expressed as numbers of eggs per gram (EPG). All animals were then sacrificed under chloroform anesthesia on day 21 and their intestines were opened and washed with a physiologic solution. The content of intestines were examined under a binocular microscope for the presence of worms.

Calculation of the percent of reduction on EPG according to the equation:

$$=100\ ((a-b)/a)$$

a = Mean number of EPG pre-treatment.
b = Mean number of EPG post-treatment on day 21.

2.5 Statistical Analysis

Results were reported as mean ± SD for each group. Statistical analysis was performed with student's t-test using a Microsoft Excel 2010. All $P<0.05$ was considered as significant for all statistical analysis in this study.

3. RESULTS AND DISCUSSION

The present study was constructed to evaluate the effect of *N. sativa* oil against *H. nana* in infected mice. The results displayed in Table 1 showing the effects of *N. sativa* oil against *H. nana* in infected mice. It is shown the effects of treatment with *N. sativa* oil on the number of eggs output in fecal pellets of treated infected groups comparing with control group. The results revealed that *N. sativa* oil has lead to significant decline the mean number of eggs per gram faeces from 583±37.16 to 65±18.7 by 7^{th} day after treatment with 5 ml/kg with efficacy of 88.85% and reached 100% by 14^{th} day after treatment. However, treatment with 2.5 ml/kg contribute to decrease of eggs in faeces with efficacy 100% on day 21 after treatment. Moreover, both doses 5 and 2.5 ml/kg lead to disappearance of worms from the intestines of mice by day 14 and 21, respectively. However, that the adverse effects were not noted.

Some herbals and plants can be a new source of medicines to treatment parasitic infections with low toxicity and high efficacy [9]. In addition, most drugs that used over the world create from natural materials specially plants due to contain chemical compounds which have bio-activity against several illness [10-11].

In present study *N. sativa* oil was used as alternative drug to treatment to *H. nana* in infected mice. The result show that treatment with *N. sativa* oil lead to highly reduce eggs passed per gram of faeces from one day until 14 day after treatment with efficacy14.24%, 88.85% and 100% respectively, by using does 5ml/kg. However, using dose 2.5ml/kg show efficacy reached to 100% by day 21. Our finding is in agreement with Ayaz et al. [17] who found *N. sativa* oil has reduced *H. nana* eggs starting from second day of treatment. Furthermore, the present results were supported by recent studies which reported that *N. sativa* has a significant efficacy against parasitic worms. Aboul-Ela [26] reported that *N. sativa* crushed seeds stimulate an oxidative stress against adult worms which was indicated by a reduction in the activities of both antioxidant enzymes and enzymes of glucose metabolism. This perturbation of such enzyme activities in adult worms could render the parasite vulnerable to damage by the host and may play a role in the anti-schistosomal strength of *N. sativa* .In addition, the extract of *N. sativa* was reported to work as protective agents against the chromosomal aberrations produce in mouse cells as a result of schistosomiasis. Moreover, Mahmoud *et al.* [27] found that *N. sativa* oil has decreased the number of *Schistosoma mansoni* worms in the liver and reduce the total number of ova stabilized in both the liver and the intestine of infected mice. Furthermore, *N. sativa* seeds demonstrated an inhibitory effect on egglaying of adult female worms and also exerted active biocidal effects against miracidia, cercariae, and adult worm stages of *Schistosoma mansoni* [28]. Additionally, *N. sativa* oil prevented most of the hematological and biochemical changes caused by schistomiasis and significantly improved the antioxidant capacity of *Schistosoma mansoni*-infected mice [29]. In another study, Kalonji (Black seed) was reported to possess significant efficacy against fascioliasis in buffalos. Kailani et al. [36] found that treatment with kalonji exerted lead to decreasing eggs per gram faeces by 88.2%. In addition, other studies found that *N. sativa* oil had anthelmintic effect in the rats

Table 1. Effects of *N. sativa* oil against *Hymenolepis nana* in infected mice

Groups	EPG count (mean ± SD)					Worms at necropsy (mean ± SD)
	(Pre-treatment)	(Post-treatment period) days				
		1st	7th	14th	21st	
Control group	560.7±25.3	565.3±19.85	562.3±13.3	550.7±17.04*	534.3±14.5	25.1±2.2
N. sativa oil (2.5 ml/kg)	607±22.27	567±28.47	232.67±44.54*	131±39.34	0	0
EPG reduction (%)	-	6.59	61.7	78.4	100	-
N. sativa oil (5 ml/kg)	583±37.16	500±23.06*	65±18.7*	0	0	0
EPG reduction (%)	-	14.24	88.85	100	100	-

** P< 0.05 compared with control group (t test)*

infected with *Trichinella spiralis* infection and increased the production of antibodies generated during life cycle of *T. spiralis* [20]. Moreover, *N. sativa* oil reduce infection of *Aspiculuris tetraptera* and its eggs significantly in mice [17]. On the other hand, El Wakil [30] reported that antiparasitic effect of *N. sativa* as result to its activating immune system. Studies confirm that *N. sativa* aqueous extract could be useful in the treatment of intestinal protozoan parasite *Blastocystis hominis*. In addition, *N. sativa* aqueous extract has demonstrated a potential therapeutic effect against *Trichomonas vaginalis* [31]. Moreover, Okeola et al. [33] reported that treatment with methanolic extract of *N. sativa* seeds significantly attenuated the serum and hepatic malondialdehyde levels in *Plasmodium yoelli nigeriensis* -infected mice. Additionally, they suggested that *N. sativa* seeds had strong antioxidant property. In other studies, Aminou *et al*. [32] reported that *N. sativa* oil showed high toxic effect on *Trichomonas vaginalis* as evidenced by severe cell damage with cytoplasmic and nuclear destruction. The remarkable effect of *N. sativa* oil may be attributed to the fact that the active principles extracted from *N. sativa* seeds are mostly from its essential oil (omega 3, 6, 9 as well as 7 fatty acids) [37]. In another study on *Toxoplasma gondii*, although *N. sativa* oil, if administered alone, has significant immunostimulant and antioxidant properties, it failed to decrease tachyzoite counts. Combination of *N. sativa* oil and pyrimethamine had synergistic effect in treatment of toxoplasmosis [34].

Recently, several *in-vivo* studies have been done to find new natural compounds which treat cestoda infections. The natural products were used to explore their antiparasitic prospective effects. Moreover, there are many recent studies which recorded the positive effect of several natural products against *H. nana* such as *Carica papaya* seeds [38], *Zingiber officinale* [39], *Artemisia abrotanum* and *Salvia officinalis* [40] and *Coriandrum sativum* seeds [35].

4. CONCLUSION

In conclusion, the results of the present study re-inforced the effectiveness of *N. sativa* oil as effective treatment against *H. nana* through experimental study on the white laboratory mice.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Author hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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DOI: 10.1016/S2222-1808(14)60449-7

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14013