The effect of Nigella sativa oil against *Aspiculuris tetraptera* and *Hymenolepis nana* in naturally infected mice

Erol Ayaz, PhD, Hasan Yilmaz, PhD, Hanefi Ozbek, PhD, Zeynep Tas, PhD, Ozlem Orunc, PhD.

ABSTRACT

Objectives: To investigate the antiparasitic effect of Nigella sativa oil (NSO) on *Aspiculuris tetraptera* (*A. tetraptera)* and *Hymenolepis nana* (*H.nana)* in mice in January 2005.

Methods: Mice were obtained from the animal house facility of the Faculty of Medicine, Yuzuncu Yil University, Van, Turkey. The natural infections were determined by the cellophane tape method and the centrifugal flotation method of stool samples. The infected mice with *A. tetraptera* and *H.nana* were divided into 4 groups; 2 treatment and 2 control groups. Nigella sativa oil was given at the dose of 250 µl/kg body weight orally for 2 consecutive days in the 2 treatment groups. All the mice were sacrificed on the seventh day after the last treatment. Gastrointestinal tract of the sacrificed animals was opened and washed with a serum physiologic. The contents were examined under a stereo microscope for counting and identifying of the parasites. The treatment and the control groups were compared using Mann-Whitney U-Test.

Results: Nigella sativa oil reduced both *A. tetraptera* and its eggs. The difference was significant between Group 1 and Group 3 ($p<0.05$). Nigella sativa oil reduced *H.nana* eggs starting from second day of the treatment until necropsy day during 5 days, but it was not significant between Group 2 and Group 4 ($p>0.05$).

Conclusions: Antiparasitic effect of NSO is related to its stimulating immune system.

Saudi Med J 2007; Vol. 28 (11): 1654-1657

From the Yenisehir Ibrahim Orhan Vocational School (Ayaz), Uludag University, Bursa, Department of Parasitology (Yilmaz, Tas, Orunc), and the Department of Pharmacology (Ozbek), Medical School of Yuzuncu Yil University, Van, Turkey.

Received 1st January 2007. Accepted 9th June 2007.

Address correspondence and reprint request to: Dr. Erol Ayaz, Yenisehir Ibrahim Orhan Vocational School, Uludag University, 16900-Bursa, Turkey. Tel. +90 (224) 7736042 Ext. 13. Fax. +90 (224) 7736041. E-mail: eayaz@yyu.edu.tr/ erolayaz68@hotmail.com/eayaz@uludag.edu.tr

In Turkey, some plants such as garlic (*Allium sativum*), onion (*Allium cepa*), blackseed (*Nigella sativa* [*N. sativa*]), nettle (*Urtica diocia*) and ribwort (*Plantago lanceolata*) are commonly and traditionally used to cure some diseases such as parasitic infections, diabetes regulation of blood pressure and cancer. The seeds of *N. sativa* are especially used for regulation of blood pressure, adjustment of sexual potency, treatment of icterus, diabetes, cancer and intestinal parasitic infections.[1] *Nigella sativa L.* belongs to the plant family *Ranunculaceae*, with a height of 20-30 cm. The seeds of *N. sativa* sometimes known as black cumin or habatul Barakah, have been used for a long time in the Middle East as a traditional medicine for a variety of complaints such as headache, cough, flatulence, as a choleretic, antispasmodic, and uricosuric.[2,3] It was reported that the extracts of *N. sativa* seeds have many therapeutic effects such as antibacterial,[4] antifungal,[5] and choleretic.[2] It has also an enhancing effect on the immune system.[6] It was also shown to possess bronchodilatator, spasmolytic and calcium antagonistic activities.[7,8] Similarly, its active principle, nigellone was also detected to have bronchodilatator activity.[9] In recent years, the seeds have been subjected to a range of pharmacological investigations. It has been shown that the essential oils of *N. sativa* have anthelmintic activity against cestodes, nematodes, and trematodes.[3,10-12] Constitutes of *N. sativa* seed extract are thymol, thymoquinone, dithymoquinone, thymohydroquinone, and they are believed to be pharmacologically active.[12,13] It was also demonstrated that both *N. sativa* extract and thymoquinone were considered as protective agents against the chromosomal aberrations induced as a result of schistosomiasis.[12] In another study,[3] the effect of Nigella sativa oil (NSO) on *Schistosoma mansoni* (*S. mansoni*) in infected mice was examined. The results of the study suggest

that NSO may play a role against the alterations caused by *S.mansoni* infection, an effect that may be induced partly by improving the immunological host system and to some extent with its antioxidant effect. It was found that it reduced *S.mansoni* worms in the liver and decreased the total number of ova deposited in both the liver and the intestine; it increased dead ova in the intestinal wall. The cestode *H.nana* and the nematode *A.tetrapter*a occur widespread in the intestine of wild and laboratory rodent. At the same time, *H.nana* is the most common tapeworm in the tropics and subtropics. The aim of this study was to determine the possible antinematodal and anticestodal effect of NSO in mice infected with *A.tetraptera* and *H.nana*.

Methods. The ground dried fruits of *N.sativa* were boiled in Clevenger device (Ildam, Istanbul, Turkey). Collected essential oil was kept in tubes and the yield was determined as 0.1%. Male Swiss albino mice (20-24 gms) were used in this experimental study. They were obtained from the animal house facility of the Faculty of Medicine, Yuzuncu Yil University, Turkey. The animals were housed at room temperature (20 ± 2ºC) in standard cages with food (Van Animal Feed Factory, Van-Turkey) and water ad libitum, in rooms lightened in a rhythm of 12 hours light, 12 hours dark, 45% relative humidity, and kept under controlled environment following the standard operating procedures of the animal house of the Faculty of Medicine. The approval from the Animal Ethics Committee was obtained. The stool samples of 100 mice were examined for detecting naturally infected animals. Infective animals were identified by cellophane tape method on the anal region, and by stool examination, which was examined by the technique of centrifugal flotation in saturated zinc sulphate solution. The infected mice were divided into 4 groups: 2 treatment groups (Group 1 infected with *A. tetraptera* and Group 2 infected with *H. nana*) and 2 control groups (Group 3 had *A. tetraptera* but not treated and Group 4 had *H. nana* but not treated). Each treatment group had 9 mice, and each control group had 7 mice. Mice from Group 1 and Group 2 treated with NSO at a dose of 250 µl/kg body weight orally for 2 consecutive days.[3] Mice in Group 3 and Group 4 received the same amount of serum physiologic orally. The stool samples from the mice were examined one day before the treatment, on day of the treatment and daily for 7 days after treatment using cellophane tape method and centrifugal flotation technique in saturated zinc sulphate. The necropsy was humanely applied (by deep ether anesthesia) to both treatment groups and control groups on the seventh day after the last treatment. Gastrointestinal tract of the sacrificed animals was opened and washed with a serum physiologic. The contents were examined under a stereo microscope for counting and identifying of the parasites. The treatment and the control groups were compared using Mann-Whitney U-Test.

Results. The eggs of *A. tetraptera* from the stool samples of Group 1 were detected in 3 mice (second day), 4 mice (third day), and one mouse (fifth day). The eggs of *H. Nana* were detected in 4 mice (second day), 6 mice (third day), and 5 mice (fifth day). Animals excreting eggs were then increased in both treatment groups up to necropsy day (fifth, sixth and seventh days) (**Table 1**). But, eggs were detected lower in treatment groups than control groups from the second day of treatment to the necropsy day. It is necessary to take 3 gm of stool sample from each rat in order to make faecel egg counts of the parasite eggs by the McMaster methods. However, this is not possible, so we counted it using microscope. The difference was significant between Group 1 and Group 3 (p=0.0397), but it was not significant between Group 2 and Group 4 (p=0.0736) for the parasites detected after the necropsy due to wide variation of parasites (**Table 2**). No side-effect was observed depending on the application of the NSO in the mice.

Discussion. Blackseed (*N. sativa*) is traditionally used by people living especially in the middle, eastern, and southeastern region of Turkey for increasing the milk production, as diuretic, appetizing, regulation of

Table 1 - The effect of Nigella sativa oil (NSO) on the *Aspiculuris tetraptera (A. tetraptera)* and *Hymenolepis Nana (H. nana)* according to stool examination, and anal tape (n=32).

Groups	Number of mice with parasite at the days of the feces and anal tape examination							Number of mice with parasites at necropsy
	1st	2nd	3rd	4th	5th	6th	7th	8th
Group 1 (*A.tetraptera*; n=9)	9	3	4	1	5	3	3	7/9
Group 2 (*H.nana*; n=9)	9	5	6	5	5	4	6	3/9
Group 3 (as control of Group 1; n=7)	7	5	6	4	5	6	6	7/7
Group 4 (as control of Group 2; n=7)	7	6	7	5	6	7	5	6/7

Table 2 - Number of detected parasites in the intestines of mice at necropsy.

Group 1 n=9*	Group 3 (as control of Group 1) n=7	Group 2 n=9†	Group 4 (as control of group 2) n=7
87	105	9	8
42	86	3	3
18	44	1	2
4	15	0	1
3	11	0	1
3	10	0	1
3	4	0	0
0		0	
0		0	

*p=0.0397, †p=0.0736

menstruation and becoming healthy. Moreover, it is added to homemade pastry such as bread, mince-pie, and kombe (kind of traditionally home-made pastry) for decoration and booster of taste. According to our observations and investigations, garlic (*Allium sativum*), pumpkin seeds (*Cucurbita pepo*) and blackseed (*Nigella sativa*) are often used in combination with honey as paste to cure gastrointestinal parasitic infections.[1,13] The most effective way of controlling parasitic infections is synthetic anthelmintics. However, these drugs are expensive and sometimes unavailable to smallholder farmers and pastoralists in developing countries. There are also cases of increased resistance to anthelmintics worldwide in ruminants,[14] and in monogastric animals such as horses.[15,16] Studies were performed on alternative treatment method with some plants extract due to the side-effect of the synthetic anthelmintics drugs, its food accumulation and cases of increased resistance to the drugs, but these studies are insufficient.[17,18] *Nigella sativa* has been used for a long time and its great number of therapeutic effects is known in traditional medicine, but it has still not been used exactly in modern medicine. In recent years, anthelmentic effect has been discovered but this efficacy could not be exploited for both human and animals. Nowadays, immune modulating effect of NSO is also a new and interesting research area. It has been reported that 2 gram blackseed intake per day enhanced the ratio between helper T (T4) and suppressor T cells (T8) by 72% and increased the natural killer cell activity by 74%.[6] Furthermore, in advanced cancer patients, it was observed that enhancement of T4:T8 ratio and NK activity were in the range of 200-300%.[19] Nigella sativa oil has been used in the treatment of schistosomiasis, and it has been reported that the drug decreased both adult parasites in liver and eggs in liver and intestine. It has been thought that this effect is related to stimulating of NSO immune system.[6,20] Ezzat[12] reported that NSO may play a role against the alterations caused by *S. mansoni* infection, an effect which may be induced partly by improving the immunological host system. The difference was significant between Group 1 and Group 3 (p=0.0397), but it was not significant between Group 2 and Group 4 (p=0.0736) in terms of number of parasites detected in the mice. Mice excreting parasite eggs in their stool samples were observed to reduce starting from second day of the treatment, and then it increased during 5 days after the second treatment, but parasite ova in each mouse reduced in both treatment groups from second treatment day to the necropsy day. These results shows that NSO probably stimulated the immune system of the hosts,[6] therefore, it might cause reduction in parasite eggs and partially shortened the life-span of the parasites. Such speculation may be considered obscure. Therefore, controlled infections could be better to understand the effect of NSO on parasite infection. If NSO was really effective in the preimmunisation against nematode and cestode infections, the drug would be very important in combating parasitic diseases in animals by adding *N. sativa* seeds to the food. In addition, it may use against human parasites. If these seeds will be added to foods it can constantly stimulate the living parasites in its body and the seeds can partly prevent increasing of the parasites in the organism. It has also been reported that when NSO was administered in combination with praziquantel, the most prominent effect was a further lowering in the dead ova number over that produced by praziquantel alone in *Schistosoma mansoni* infection in mice.[3] We think that if both NSO and antiparasitic drugs are used together, the antiparasitic effect may increase in combating cestodes and nematodes, and the side affects of the drugs may be induced.

Finally, this study shows that NSO reduced both *A. tetraptera* and its eggs. Therefore, this effect might cause

shortened the lifespan of the parasites. The difference was significant between Group 1 and Group 3 (p<0,05) in terms of number of parasites detected in the mice. Nigella sativa oil reduced *H. nana* eggs starting from second day of the treatment up to the necropsy day during 5 days, but it was not significant between Group 2 and Group 4 (p>0.05) in terms of number of parasites detected in the mice.

References

1. Baytop T. Treatment of plants. Turkey: Nobel Tip Bookstore; 1999.
2. Mahfouz M, El-Dakhakhny M, Gemed A, Moussa H. Choleretic action of Nigella sativa seeds oil. ***Pharmacol Bull*** 1962; 44: 225-227.
3. Mahmoud MR, El-Abhar HS, Saleh S. The effect of Nigella sativa oil against the liver damage induced by Schistosoma mansoni infection in mice. ***J Ethnopharmacol*** 2002; 79: 1-11.
4. Hanafy MS, Hatem ME. Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). ***J Ethnopharmacol*** 1991; 34: 275-278.
5. Khan MA, Ashfaq MK, Zuberi HS, Mahmood MS, Gilani AH. The in vivo antifungal activity of the aqueous extract from Nigella sativa seeds. ***Phytother Res*** 2003; 17: 183-186.
6. El-Qadi A, Qandil O. The black seed (Nigella sativa) as a natural immune enhancer. First International Conference on Scientific Miracles of Quran and Sunnah. Islamabad (Pakistan); 1987.
7. Mahmoud BA. Effects of Nigella sativa seeds on intestinal smooth muscle. ***Int J Pharmacol*** 1993; 31: 55-60.
8. Gilani AH, Aziz N, Khurram IM, Chaudhary KS, Iqbal A. Bronchodilator, spasmolytic and calcium antagonist activities of Nigella sativa seeds (Kalonji): a traditional herbal product with multiple medicinal uses. ***J Pak Med Assoc*** 2001; 51: 115-120.
9. Mahfouz M. El-Dakhakhny M. Some chemical and pharmacological properties of the new antiasthmatic drug "Nigellone". ***Pharmacol Bull*** 1960; 42: 411-415.
10. Agarwal R, Kharya MD, Shrivastava R. Antimicrobial & anthelmintic activities of the essential oil of Nigella sativa Linn. ***Indian J Exp Biol*** 1979; 17: 1264-1265.
11. Akhtar MS, Riffat S. Field trial of Saussurea lappa roots against nematodes and Nigella sativa seeds against cestodes in children. ***J Pak Med Assoc*** 1991; 41: 185-187.
12. Ezzat IAE. Cytogenetic studies on Nigella sativa seeds extract and thymoquinone on mouse cells infected with schistosomiasis using karyotyping. ***Mutation Research*** 2002; 516: 11-17.
13. Levent T. Bayarak A. Çörekotu (Nigella sativa L.)' nun sabit ve uçucu yağ kompozisyonunun araştırılması. ***Standart*** 1997; 430:128-137.
14. Waller PJ. Sustainable helminth control of ruminants in developing countries. Vet Parasitol 1997; 71: 195-207. Review.
15. Kaplan RM. Anthelmintic resistance in nematodes of horses. ***Vet Res*** 2002; 33: 491-507. Review.
16. Tarigo-Martinie JL, Wyatt AR, Kaplan RM. Prevalence and clinical implications of anthelmintic resistance in cyathostomes of horses. ***J Am Vet Med Assoc*** 2001; 218: 1957-1960.
17. Githiori JB, Hoglund J, Waller PJ, Baker RL. The anthelmintic efficacy of the plant, Albizia anthelmintica, against the nematode parasites Haemonchus contortus of sheep and Heligmosomoides polygyrus of mice. ***Vet Parasitol*** 2003; 116: 23-34.
18. Shalaby NM, Maghraby AS, el-Hagrassy AM. Effect of Daucus carota var. boissieri extracts on immune response of Schistosoma mansoni infected mice. ***Folia Microbiol (Praha)*** 1999; 44: 441-448. Erratum in: Folia Microbiol (Praha) 2000; 45: 191.
19. Houghton PJ, Zarka R, de las Heras B, Hoult JR. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. ***Planta Med*** 1995; 61: 33-36.
20. Haq A, Lobo PI, Al-Tufail M, Rama NR, Al-Sedairy ST. Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. ***Int J Immunopharmacol*** 1999; 21: 283-295.