

EFFICACY OF NIGELLA SATIVA OIL EXTRACT ON PNEUMOCYSTIS CARINII IN IMMUNOSUPPRESSED RATS

WAHBA A.A.

Animal Health Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt

(Manuscript received 26 May 2001)

Abstract

Nigella sativa (black seed) is a plant used on large scale for many purposes. The prophylactic and treatment effects of the oil extracted from this plant were studied on *Pneumocystis carinii*. For this purpose, 48 male albino rats weighing each 100 - 120 g were used. The induced experimental infection with *Pneumocystis carinii* was carried out by immunosuppression of rats with 2 mg/l dexamethasone sodium phosphate together with 500 mg/l tetracycline hydrochloride in drinking water. Rats acquired the infection with *P. carinii* 5 - 6 weeks post-immunosuppression. Macroscopical examination of lungs was carried out, and stained smears with Giemsa stain were also examined. The results were analysed statistically. The immune enhancing effect was clear in both prophylactic and treated rats. In case of prophylactic and treatment studies, the percentages of both survival prophylactic-infected rats (40%) and survival infected-treated ones (73.3%) were compared to the survival infected-control rats (0%) and survival control non-infected non-treated rats (100%). The percentage of the survived rats in both prophylactic and treated groups that showed no infection with *P. carinii* developmental stages in Giemsa stained lung smears, were 66.7% and 27.3% respectively, although, the percentage of other survived rats in the same 2 groups that showed moderate infection were 33.3% and 72.7% respectively.

INTRODUCTION

Nigella sativa (black seed) is a herb, native to the Mediterranean, that has been used for thousands of years in the Middle East, Far East and Asia as an invaluable health tonic. It is popularly called "the seed of blessing".

Experiments reported that *N. sativa* has an anthelmintic and protozoal effects, as well as, it has an immune enhancing effect (Khaled *et al.*, 1998). Among zoonotic parasites associated with immune deficiency states, is *Pneumocystis carinii* (Eckert, 1989). Presently, the importance of certain zoonoses induced by the acquired immunodeficiency syndrome (AIDS) of man is increased. *Pneumocystis carinii* is a protozoan parasite of such zoonoses (Soulsby, 1982). It is an opportunistic air-borne pathogen which produces diffuse interstitial pneumonia in both children and adults and is secondary to some predisposing disease as AIDS and cancer (El-Gebaly *et al.*, 1996). Moreo-

ver, *Pneumocystis* was recorded in man and numerous mammals, including guinea pigs, mice, rats, rabbits and goats (Hughes, 1987). In addition, the infection is latent, and dog is considered to be an important reservoir host (Soulsby, 1982).

This study has been focused on the possible use of *N. sativa* as supporting agent against *P. carinii* infection in rats.

MATERIALS AND METHODS

The prophylactic and the treatment effects of *N. sativa* oil extract were studied. The oil was obtained from a private seller and administered orally by a stomach tube at a dose of 1 ml/100 gm body weight of rat daily.

Forty-eight male albino rats weighing 100 - 120 gm each, obtained from laboratory colony in Animal Health Research Institute, were used. Induction of *P. carinii* infection was carried out by immunosuppression of these rats using a combination of 2 mg/1 dexamethasone sodium phosphate (Amriya for Pharmaceutical Industries, Alexandria, Egypt) with 500 mg/1 tetracycline hydrochloride (CID Laboratories, Giza, Egypt) added daily in drinking fresh already boiled water (Oz *et al.*, 1996). Each rat was expected to consume 30 - 50 ml of water/day (Hughes, 1982). After immunosuppression, rats had acquired the infection.

These rats were divided into 4 separate groups (I, II, III and IV), each one contained 15, 15, 12 and 6 rats respectively. Each group was completely isolated individually from each other in separate cages. The cages and containers of ration and water were daily changed to avoid any contamination. The rats were maintained on autoclaved ration.

Rats of group I were used for the prophylactic purpose. They were given *N. sativa* oil extract daily for a period of 3 weeks before immunosuppression.

Rats of group II were used as treated ones. They were immunosuppressed by dexamethasone and tetracycline hydrochloride added in the drinking water for 5 weeks, after which, they were supplied with *N. sativa* oil extract daily and water without any additives till sacrifice or death.

Rats of group III were left as control infected animals and were given dexamethasone and tetracycline hydrochloride in water at the same time with rats of both group I and II.

Rats of group IV were left as control non-infected and non-treated.

All groups were observed daily and the percentage of survival rats were calculated till the termination of the experiment (14th week post-immunosuppression).

Macroscopical examination of lungs of dead or sacrificed rats was carried out. Three smears taken from each lung at different levels and stained with Giemsa were examined. Lesions observed on lung surface or any developmental stages due to *P. carinii* in smears were identified and illustrated. Lungs of rats in group I, II, III and IV were compared with each other macroscopically and in stained smears. The results were analysed statistically according to Nikos and Armen (1998).

RESULTS

Figure 1 represents the percentage of survival of both prophylactic, treated, control infected and control non-infected non-treated rats in the four groups. In group I (prophylactic group), 1, 2 and 6 rats died on 4th, 5th and 6th weeks post-immunosuppression, respectively. This showed that the percentage of survival prophylactic was 93.3%, 80% and 40% on 4th, 5th, and 6th week post-immunosuppression, respectively, however, the 40% survivals still survived till they were sacrificed at the termination of experiment.

In group II (treated group), 3 and 1 rat died on 7th and 8th week post-immunosuppression (2nd and 3rd weeks post-treatment), respectively. This showed that the percentage of survival treated was 80% and 73.3% on 7th and 8th week post-immunosuppression (2nd and 3rd week post-treatment), respectively, however, the 73.3% survivals still survived till they were sacrificed at the termination of experiment.

In group III (control infected group), 2, 3, and 7 rats died on 5, 6 and 7th week post-immunosuppression, respectively. This showed that the percentage of survival control infected was 83.3%, 58.3% and 0% on 5th, 6th and 7 week post-immunosuppression, respectively.

In group IV (control non-infected, non-treated group), no rats died till the termination of the experiment, where the survival percentage was 100%.

Macroscopical examination of lung surface of control infected rats with *P. carinii* showed severe congestion and a lot of greyish white nodules (Fig. 2). The prophylactic and treated groups showed slight congestion and scarce or no nodules (Fig. 3).

Lung smears of infected rats stained with Giemsa stain showed different developmental stages of *P. carinii*, as trophozoites measuring 1 - 2.5 μ m in diameter (Fig. 4), and cystic stages measuring 3.75 μ m in diameter with 8 intracystic bodies (Fig. 5). Table 1 showed the number and percentage of the survived rats with no or moderate infection with *P. carinii* in Giemsa stained lung smears, in different groups. The number and percentage of rats with no and moderate infection of the 6 survived prophylactic rats were 4 (66.7%) and 2 (33.3%), respectively. The number and percentage of rats with no and moderate infection of the 11 survived treated rats were 3 (27.3%) and 8 (72.7%), respectively. The number and percentage of rats with no and moderate infection of the 6 survived control non-infected, non-treated rats were 6 (100%) and 0 (0%), respectively. There was no survived rats in the control infected group.

DISCUSSION

Pneumocystis carinii is a protozoan parasite of zoonotic importance. It is an airborne parasite being acquired after immunosuppression (Gattel *et al.*, 1996). In this study, *Nigella sativa* oil extract was used as an immune enhancing factor in both prophylactic and treated rats infected with *P. carinii*.

Oz *et al.* (1996), recorded that *P. carinii* invaded the lungs of rats (200 - 250 g weight), 7 - 8 weeks after starting immunosuppression with dexamethasone and tetracycline hydrochloride. In the present study, the early appearance (5 - 6 weeks post-immunosuppression) of *P. carinii* in rats may be due to the different susceptibility associated with weight.

In case of prophylactic study (group I), it was evident that *N. sativa* gave a great support for some infected rats to tolerate the infection with *P. carinii* (40% survival), while, the dead rats (60%) were not able to tolerate the infection. The low percentage of survival than the percentage of dead rats may be ascribed to the small dose of *N. sativa* as a prophylactic remedy, or may be due to the insufficient period of 3 weeks in which *N. sativa* oil extract had been given to rats before immunosuppression.

Giemsa stained lung smears of prophylactic survived rats proved that 66.7% did not acquire the infection, while, 33.3% appeared to have a moderate infection.

In case of treatment study (group II), it was evident that *N. sativa* gave a great support to most infected rats enabling them to tolerate the infection with *P. carinii* (73.3% survival), while, the dead ones (26.7%) failed.

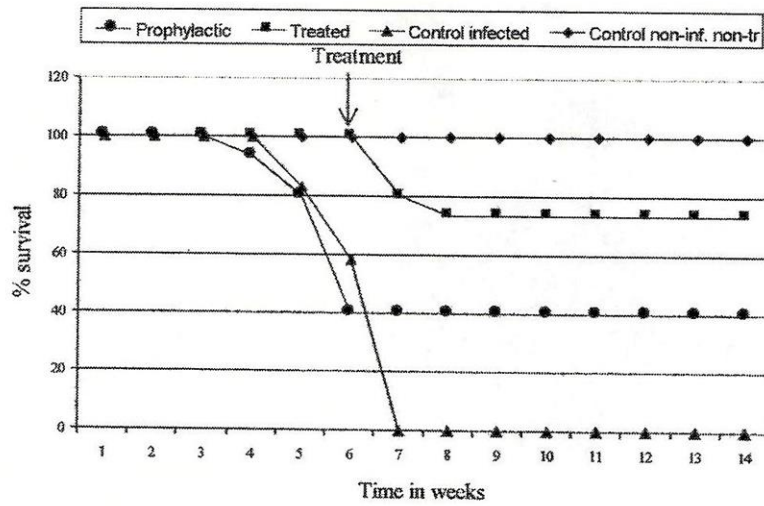


Fig. 1. Survival in the 4 studied groups.

Table 1. Survived rats without or with moderate infection according to lung stained smears.

Groups	Total number of rats	Survived rats		
		Number & % of survived rats	Number & % of survived rats without infection	Number & % of survived rats with moderate infection
Prophylactic group I	15	6 (40%)	4 (66.7%)	2 (33.3%)
Treated group II	15	11 (73.3%)	3 (27.3%)	8 (72.7%)
Control infected group III	12	0 (0%)	--	--
Control non-infected non-treated group IV	6	6 (100%)	6 (100%)	0 (0%)



Fig. 2. Lungs of control infected rat with *P. carinii* 6 weeks post-immunosuppression, showing severe congestion and many scattered nodules.

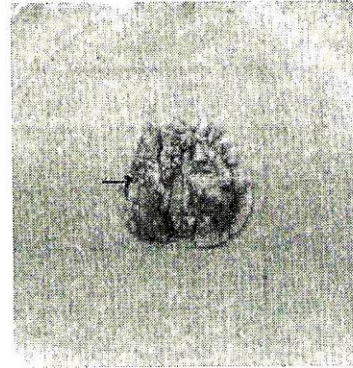


Fig. 3. Lungs of treated rats with *Nigella sativa* oil extract 14th week post-immunosuppression, showing slight congestion and scarce nodules

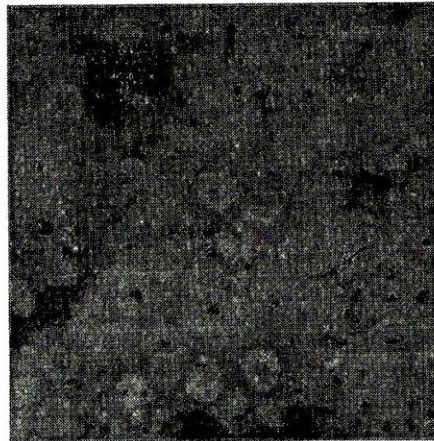


Fig. 4. Lung smear of infected rat 5 weeks post-immunosuppression, showing *p. carinii* trophozoites. Giemsa, X 1250.

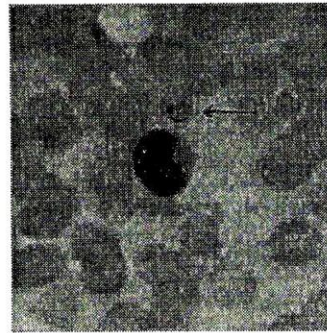


Fig. 5. Lung smear of infected rat 6 weeks post-immunosuppression, showing *p. carinii* mature cyst with 8 intracystic bodies. Giemsa stained, X 1250.

Giemsa stained lung smears of treated survived rats proved that 27.3% had no infection, while, 72.7% appeared to have a moderate infection.

In case of control infected group III, all rats died (100%) with 0% survivals. Macroscopical examination of lung surface showed severe congestion and greyish white nodules. The Giemsa stained lung smears revealed the infection of *P. carinii* developmental stages. On the other hand, the control non-infected non-treated group IV proved 100% survival with no macroscopical lesions or infection being appeared in Giemsa stained lung smears.

From the previous results, it is obvious that *N. sativa* oil extract has a prophylactic and curative effect against *P. carinii* infection in rats. This may be due to its stimulatory effect on the immune system (Haq *et al.*, 1995).

El-Kadery *et al.* (1997), explained the protective effect of *N. sativa* against *Schistosoma mansoni* infection by increasing both humoral and cell mediated immunity. Also, Khaled *et al.* (1998), used *N. sativa* seeds as a prophylactic and curative agent against *Hymenolepis nana* infection in experimental mice, and they stated that it enhanced both humoral and cell mediated immune responses.

In Egypt, Taha (1997), focused on the possible use of *N. sativa* as a remedy for today major health problems as immune deficiency. He proved that *N. sativa* had an immunopotentiating effect, and was effective in the treatment of a specific respiratory disease as *Mycoplasma gallisepticum* infection in chicks.

In addition, El-Sayed and El-Hashem (2000) studied the effect of *N. sativa* on the immune response of native chicks vaccinated with *Eimeria* vaccine. They found a significant lowered infection of *Eimeria* in the intestinal tract and decreased mortality of chicks.

Akhtar and Rifaat (1991) reported that *N. sativa* seeds given at a dose of 40 mg/kg body weight of children infected naturally with cestode, reduced the eggs per gram counts without producing any adverse side effects in the dose tested.

It can be concluded that, the immunestimulatory effect of *N. sativa* was obvious especially in case of immunosuppression infections as *P. carinii*. Thus, the addition of this cheap seeds or its oil extract to the ration of animals is very important as an immune enhancing factor, thence, to be used successfully and effectively in human and animals of low immunity against certain diseases.

REFERENCES

1. Akhtar, M.S. and S. Rifaat. 1991. Field trial of *Saussurea lappa* roots against nematodes and *Nigella sativa* seeds against cestodes in children. J. Pakistan Medical Assoc., 41 (8): 185-187.
2. Eckert, J. 1989. New aspects of parasitic zoonoses. Vet. Parasitol., 32 : 37-55.
3. El-Gebaly, W.M., S.M.F. El-Nassery and H.A.H. Sadaka. 1996. Serum IgG antibody response to *P. carinii* among immunosuppressed malnourished and healthy rats. J. Egypt. Soc. Parasitol., 26 (1): 63-71.
4. El-Kadery, A.A., H.M. Hussein, M.M. Emara and T.H. El-Khashaab. 1997. Prophylactic and therapeutic effect of *Nigella sativa* seeds (black cummin) on experimental infection with *Schistosoma mansoni*. JIMA, 29: 3038.
5. El-Sayed, M. and M. EL-Hashem. 2000. Effect of *N. sativa* on the immune response to *Eimeria* vaccination in chickens. Egypt. J. Agric. Res., 78 (1): 231-239.
6. Gattel, J.M., R. Marrades, M. El-Ebiary and A. Torres. 1996. Severe pulmonary infections in AIDS patient. Semin. Resp. Inf. Jun., 11 (2): 119-128.
7. Haq, A.M., M. Abdullatif, P.I. Lobo, K.S. Khabar, K.V. Sheth and S.T. Al-Sedairy. 1995. *Nigella sativa*: effect on human lymphocytes and polymorphnuclear leukocyte phagocytic activity. Immunopharmacology, 30 (2): 147-155.
8. Hughes, W.T. 1982. Natural mode of acquisition for de novo infection with *Pneumocystis carinii*. J. Infect. Dis., 145 (6): 842-848.
9. Hughes, W.T. 1987. *Pneumocystis carinii* pneumonitis. CRC Press, Boca Raton, Florida, Vol. I & II.
10. Khaled, M.L.M., A. El-Kadery, B. El-Gozy and N. Abdel-Fatah. 1998. Effect of *N. sativa* seeds (black cummin) on experimental *Hymenolepis nana* infection. Egypt. J. Med. Sci., 19 (1): 47-56.
11. Nikos, M.L. and K. Armen. 1998. Biostatistics and epidemiology. McGram-Hill, Health Professions Division.

12. Oz, H.S., W.T. Hughes and S.L. Vargas. 1996. Search for extrapulmonary *P. carinii* in an animal model. *J. Parasitol.*, 82 (2): 357-359.
13. Soulsby, E.J.L. 1982. Helminths, Arthropods and Protozoa of domesticated animals. 7th ed. The English Language Book Society and Baillière Tindall. London.
14. Taha, H.S. 1997. Pharmacological studies on *N. sativa* and its interaction with an insecticide. Thesis, Ph.D., Fac. Vet. Med., Cairo Univ.

تأثير مستخلص زيت حبة البركة على الإصابة بطفيل نيموسيسستس كاريني في الفئران المثبطة مناعياً

أحمد أنور وهبه

معهد بحوث صحة الحيوان - مركز البحوث الزراعية - وزارة الزراعة - الدقى - جيزة

نبات حبة البركة أو الحبة السوداء من النباتات الطبية واسعة الانتشار لاستخدامها فى أغراض متعددة. أجريت هذه الدراسة على الزيت المستخلص من حبوب هذا النبات لبيان فاعليته الوقائية والعلاجية على الجرذان المصابة بطفيل نيموسيسستس كاريني. استخدم فى هذه الدراسة ٤٨ جرذاً أبيضاً يزن الواحد من ١٠٠-١٢٠ جرام جميعها ذكور. أجريت عدوى بطفيل نيموسيسستس كاريني عن طريق إنقاص مناعة هذه الجرذان وذلك بوضع ٢ مجم/لتر ديكساميثازون صوديوم فوسفات مع ٥٠٠ مجم/لتر تتراسيكلين هيدروكلوريد فى ماء الشرب يومياً.

تم فحص رئة هذه الجرذان بالعين المجردة وكذلك أخذت مسحات من الرئة وتم صبغها بصبغة الجيمسا لفحصها ميكروسكوبياً.

تم تحليل النتائج إحصائياً ووجد أن هناك تأثير مناعى واضح فى الجرذان فى كل من المجموعة الوقائية والمجموعة العلاجية. فقد كانت نسبة الفئران المستمرة على قيد الحياة فى المجموعات الوقائية، العلاجية، الضابطة المعديّة، الضابطة الغير معدية الغير معالجة هى ٧٣,٣٪، صفر٪، ١٠٠٪ على التوالي.

وبفحص مسحات الرئة المصبوغة بصبغة الجيمسا وجد أن نسبة الفئران المستمرة على قيد الحياة فى كلتا المجموعتين الوقائية والعلاجية والتي ليس بها إصابة بالأطوار النامية للطفيل هى ٦٦,٧٪، ٢٧,٣٪ على التوالي، كما كانت نسبة الفئران التى على قيد الحياة فى نفس المجموعتين وبها إصابة نادرة هى ٣٣,٣٪، ٧٢,٧٪ على التوالي.

يتضح من الدراسة أن مستخلص نبات حبة البركة له تأثير واضح من الناحية الوقائية والعلاجية على طفيل نيموسيسستس كاريني. وعليه ينصح بإضافة حبوب أو مستخلص هذا النبات إلى العليقة المستخدمة فى تغذية الحيوانات. ومن ثم يمكن استخدامه بنجاح وفاعلية لمقاومة وعلاج الأمراض المثبطة للمناعة فى الإنسان والحيوان.