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

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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/kg dexamethasone sodium phosphate together with 500 mg/kg 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 (46%) 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 (Khalaf 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., 1990). 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 Arken (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 6th, 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 μm in diameter (Fig. 4), and cystic stages measuring 3.75 μm 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 (Gatti 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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total number of rats</th>
<th>Number &amp; % of survived rats</th>
<th>Number &amp; % of survived rats without infection</th>
<th>Number &amp; % of survived rats with moderate infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylactic group I</td>
<td>15</td>
<td>6 (40%)</td>
<td>4 (66.7%)</td>
<td>2 (33.3%)</td>
</tr>
<tr>
<td>Treated group II</td>
<td>15</td>
<td>11 (73.3%)</td>
<td>3 (27.3%)</td>
<td>8 (72.7%)</td>
</tr>
<tr>
<td>Control infected group III</td>
<td>12</td>
<td>0 (0%)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Control non-infected, non-treated group IV</td>
<td>6</td>
<td>6 (100%)</td>
<td>6 (100%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>
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. Mac-
roscopical examination of lung surface showed severe congestion and greyish white
nodules. The Giemsa stained lung smears revealed the infection of *P. carinii* develop-
mental 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 prophylact-
ic and curative effect against *P. carinii* infection in rats. This may be due to its stimula-
tory effect on the immune system (Haq et al., 1995).

El-Kadey 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 en-
hanced 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 im-
munopotentianting 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 Rifat (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 im-
mune enhancing factor, thence, to be used successfully and effectively in human and
animals of low immunity against certain diseases.
REFERENCES


تأثير مستخلص زيت حبة البركة على الإصابة بطفيل
نيموسيستس كارتيني في الفئران المثبتة مناعياً

أحمد أنور وهبة

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

نبات حبة البركة أو الحبة السوداء من النباتات الطبية وأسلوب الاستخدام في
أعراض متعددة. أجريت هذه الدراسة على الزيت المستخلص من حبوب هذا النبات لبيان فاعليته
الوقائية والعلاجية على الجرذان المصابين بطفيل نيموسيستس كارتيني.

استخدمت هذه الدراسة 18 جرذًا أتونًا، وزن الواحد من 100-120 جرام جميعها ذكوراً.
أجريت مذروج بطفل نيموسيستس كارتيني عن طريق إضافة مادة هذه الجرذان وذلك بوضع
2 مجم/لتر نتر ديكسيتميثيون سوبيوم فوسفات مع 50 مجم/لتر تتراتيكسيكين هيدروكلافيدين في ماء
شرب يوميًا.

تم فحص رئة هذه الجرذان بالعين المجردة وكذلك أخذت مسحات من الرئة وتم صيغة بعضها بصيغة
الجيسما لفحصها ميكروسكوبياً.

تم تخليص النتائج وإحصاءها ووجد أن هناك تأثير متاح واضح في الجرذان في كل من
الجروهات الوقائية والعلاجية. فقد كانت نسبة الفئران المستمرة على قيد الحياة في
الجروهات الوقائية والعلاجية، الضابطة المحبة، الضابطة الغير متعددة الفيروس معالجة هي 0/10.
صدور 7/20 % على التوالي.

وفيما مات نسبة الرئة الصبغة بصبغة الجيسما وجد أن نسبة الفئران المستمرة على قيد
الحياة في كلا الجروهات الوقائية والعلاجية و센터ها إسيا إلى الأطوار واصلياً لطيف ينفي
0/77 ، 0/77 على التوالي، كما كانت نسبة الفئران التي على قيد الحياة في نفس الجروهات وبها
إسيا ابتداء هي 7/20 ، 7/20 على التوالي.

يشير من الدراسة أن مستخلص نبات حبة البركة له تأثير واضح من الناحية الوقائية
والعلاجية على طفيل نيموسيستس كارتيني، وعليه ينصح بإضافة حبوب أو مستخلص هذا النبات
إلى العلاقة المستخدمة في تخليص الحيوانات، ومن ثم يمكن استخدامه بنجاح وفعالية لمقاومة وعلاج
الأمراض المثبتة للمناعة في الإنسان والحيوان.