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TRIALS ON THERAPEUTIC EFFECT OF *NIGELLA SATIVA* OIL EXTRACT ON *CRYPTOSPORIDIUM PARVUM* IN EXPERIMENTALLY INFECTED MICE

MAGDA A.H. EL-REFAII

Animal Health Research Institute, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt

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Abstract

This study was carried out to determine the therapeutic effect of *Nigella sativa* oil extract on mice experimentally infected with *Cryptosporidium parvum*. For this purpose, 25 susceptible swiss mice (2-3 weeks old) were divided into 3 groups. Group I included infected 11 treated mice, group II included 10 infected non-treated ones, and group III composed of 4 non-infected non-treated mice. One week before experimental infection, all mice were immunosupressed by a mixture of 2 mg/L dexamethasone sodium phosphate and 500 mg/L tetracycline hydrochloride in daily fresh drinking water. Experimental infection of mice was carried out with *cryptosporidium pawum* oocysts isolated from naturally infected calves. One week later, mice of group I were orally treated with *Nigella* oil at a dose of 1 ml/100g body weight for each mouse. Faecal pellets from rectum of each mouse in all groups were examined every other day post-treatment by the Modified Ziehl-Neelsen technique. Oocysts intensities were scored in 100 randomly selected fields.

The results revealed that mice of group III were refractory, while, mice of group I showed a reduction in oocysts count when compared to those of group II. On day 35th, all mice were sacrificed and parts of their ilea were fixed in 10% formalin solution for histopathological examination, as well as, smears from ilea were taken and stained with the Modified Ziehl-Neelsen technique. Villi of ileum of mice in group I showed normal appearance without any changes, while, those of mice of group II showed apparent villus atrophy; shortening and blunting with desquamation of most of villi. Villi of group III were normal. Data were statistically analysed and discussed.

INTRODUCTION

Cryptosporidium parvum is an intracellular protozoan parasite that replicates within the microvillus region of epithelial cells lining the small intestine of mammals as well as man. It is transmitted via the fecal-oral pathway (Soulsby, 1982). It causes a zoonotic disease of mammals producing a short-term acute diarrhoeal illness in immunocompetent individuals and having a prolonged life-threatening disease in the immuno-

compromised. This protozoon becomes one of the AIDS-related parasitic zoonozes as its severity depends upon the immune status of the host. Young animals are generally more susceptible to infection with *C. parvum* than adults (Unger, 1990). Under natural conditions, clinically infected animals excrete large number of oocysts into the surrounding environment, which is therefore the most common source of infection in young farmed ruminants. It has been suggested that asymtomatic adult animals may also transmit the infection to newborn animals or even man through oocysts shedding (Scott *et al.*,1995).

Up till now, immune mechanisms responsible for acquired resistance to *C. parvum* are not well defined, thus, the development of effective preventive measures or treatment regiments available to combat the infection are still under experiment (Zu *et al.*, 1992).

Therefore, this study was conducted as a trial to determine the therapeutic effect of *Nigella sativa* oil extract on *C. parvum* infection in experimentally infected mice.

MATERIALS AND METHODS

Forty-four faecal samples from cattle calves were collected separately from El-Bassatine abattoir in clean plastic bags. They were examined by the Modified Ziehl-Neelsen staining technique (Henricksen and Pohlenz, 1981). Oocysts were collected by Sheather's solution from positive faecal samples and kept in 2.5% Potassium dichromate solution at 4°C until use within one month for experimental infection of mice (Kathleen *et al.*, 1995).

Twenty-five susceptible swiss male mice aging 2-3 weeks old and weighing 12.5 – 13 g each, were obtained from a colony maintained at the Animal Health Research Institute, offered feed and water *ad lipitum* and were used in experimental infection with *cryptosporidium pawum* oocysts. They were divided into 3 groups: group I, consisted of 11 mice, experimentally infected, then, treated by oral administration of *N. sativa* oil extract. Group II consisted of 10 mice used as infected non-treated control ones. Group III consisted of 4 mice and were used as non-infected non-treated control ones.

One week before experimental infection, all mice groups were immunosuppressed using a combination of 2 mg/L dexamethasone sodium phosphate (Amriya for Pharmaceutical Industries, Alexandria, Egypt) and 500 mg/L tetracycline hydrochloride (CID Laboratories, Giza, Egypt) added in daily fresh drinking water to facilitate infection with *cryptosporidium*, then, by experimental infection, these compounds were stopped.

Each mouse of group I and II was experimentally infected with 106 oocysts orally using an orogastric intubation (Kathleen *et al.*,1995). One week later, faecal pellets from rectum of each mouse were collected and faecal smears were examined by the Modified Ziehl-Neelsen staining technique.

After being infected, mice of group I were treated with *N. sativa* oil extract at a dose of 1 ml/100 g body weight daily for each mouse till the termination of the experiment. The oil was obtained from a private seller.

Faecal smears from each mouse in the three groups were examined by the Modified Ziehl-Neelsen technique every other day post-treatment to monitor oocyst shedding intensities. Oocyst shedding of each smear was scored according to the average number of oocysts in 100 randomly selected fields at X 100 magnification. The number of oocysts every time in infected treated mice and control infected non-treated ones was compared with each other. All mice were sacrificed 35 days post-treatment and parts of their ilea separately were fixed in 10% formalin solution , embedded in paraffin, sectioned at 5 microns and stained with haematoxylin and eosin for examination of the morphological appearance of villi of ileum of each mouse in the three groups, then, they were compared with each other. As well, smears from ilea of mice were obtained and stained with the Modified Ziehl-Neelsen technique.

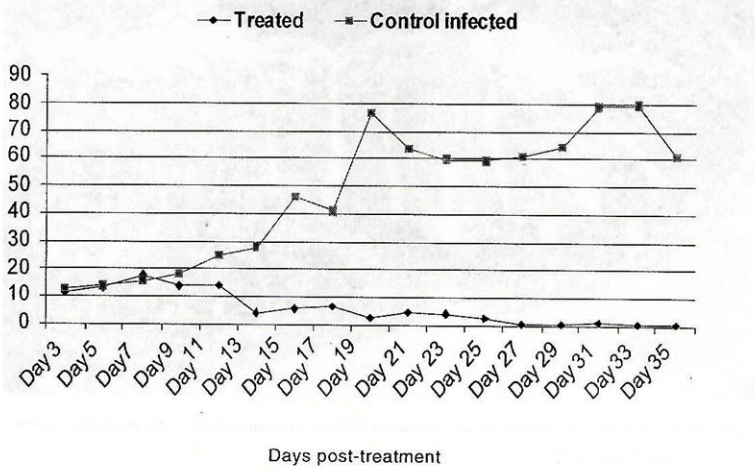
Differences between the number of oocyst shedding among treated and control groups were analysed statistically with the aid of SPSS Wind. 2000 by using of ANOVA test. Significance level for all analysis was set at $P<0.05$.

Table 1. Means of oocysts count in infected treated and infected non- treated mice.

Days post-treatment	Infected treated mice (mean ±SD)	Infected non-treated mice (mean ±SD)	P value
D3	11.50 ± 3.89	12.80 ± 5.96	0.57
D5	13.30 ± 4.00	13.60 ± 5.02	0.88
D7	17.70 ± 3.92	15.70 ± 4.47	1.00
D9	13.60 ± 5.44	18.00 ± 5.31	0.08
D11	11.40 ± 6.42	24.90 ± 14.53	0.02
D13	4.10 ± 4.61	27.60 ± 17.33	0.00
D15	5.60 ± 4.67	45.80 ± 23.75	0.00
D17	6.60 ± 4.60	40.80 ± 19.44	0.00
D19	2.60 ± 2.07	76.40 ± 62.57	0.00
D21	4.30 ± 3.20	63.80 ± 18.28	0.00
D23	4.10 ± 2.10	59.70 ± 14.26	0.00
D25	2.30 ± 1.77	59.30 ± 17.62	0.00
D27	0.60 ± 0.84	60.50 ± 17.97	0.00
D29	0.60 ± 0.59	64.20 ± 23.67	0.00
D31	0.90 ± 0.99	78.70 ± 26.82	0.00
D33	0.60 ± 0.52	79.50 ± 27.99	0.00
D35	0.60 ± 0.52	60.80 ± 26.47	0.00

Significance at $P<0.05$ using ANOVA test.

Fig 1. Difference between oocysts count in infected treated and infected non-treated mice



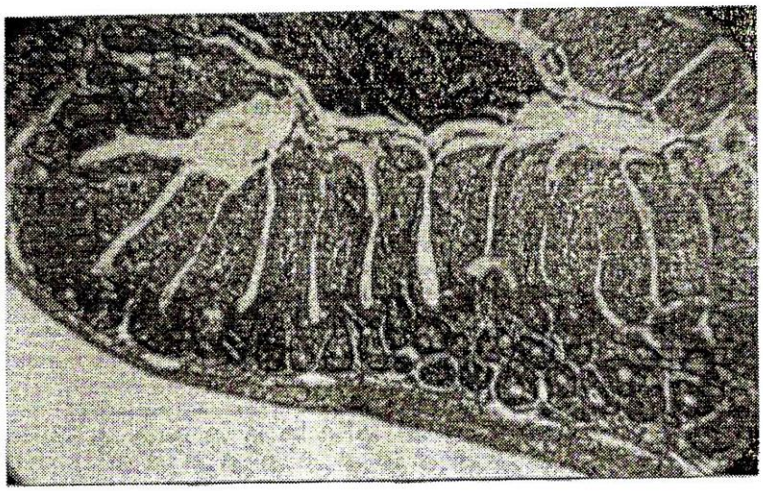


Fig 2. Ileum of infected treated mouse showing normal appearance of Villi (Haematoxylin and Eosin, x 125)

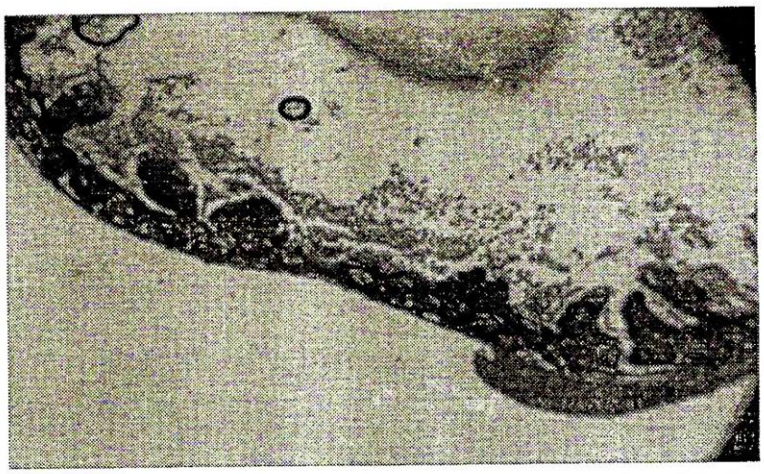


Fig 3. Ileum of infected non-treated mouse showing villus shortening, atrophy, and blunting with desquamation of most of villi (Haematoxylin and Eosin, x 125)

Fig 4. Ileum of non-infected non-treated mouse showing normal appearance of villi (Haematoxylin and Eosin, x 125)

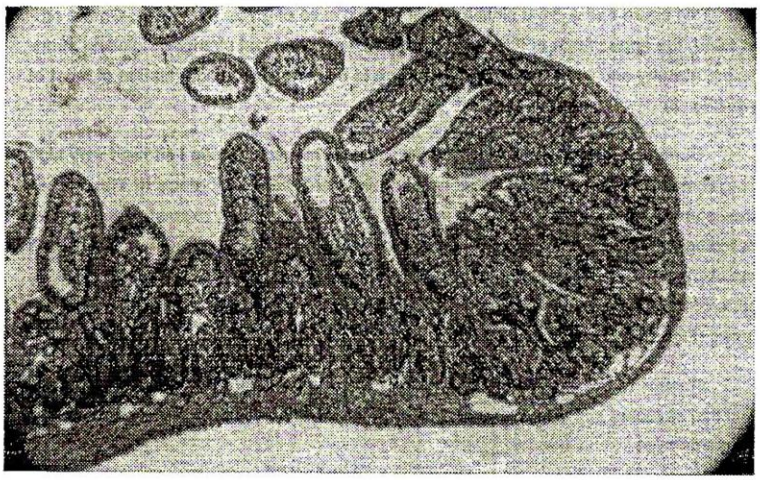


Fig 5. Ileum of infected treated mouse showing normal appearance of villi in spite of the presence of high oocyst intensities (Haematoxylin and Eosin, x 125)

RESULTS

Examination of faecal smears from examined mice revealed that infected control mice shed more oocysts than those of infected treated group throughout the experiment (35 days post-treatment). Table 1 showed that there was significant reduction in oocyst shedding ($P<0.05$) in treated mice on day 11 post-treatment. This reduction continued till sacrifaction of mice 35 days post-treatment. On the other hand, infected control mice showed higher oocyst shedding intensities till sacrifaction.

Figure 1 showed that there was a clear difference in oocyst intensities among group I, infected treated, and group II, infected non-treated mice. Oocyst shedding intensities in mice of group II might reach 80 oocysts/100, fields, while, they did not exceed 10 oocysts/100 fields till reaching a negligible degree of oocysts count in mice of group I.

Examination of faecal smears of mice of group III, non-infected non-treated, revealed absence of any oocysts all over the period of experiment.

Histopathological examination of status of ileal villi revealed a sharp indicative clear difference in the appearance of villi of mice . Figure 2 showed the status of villi of mice of group I. The villi had normal appearance without showing any atrophy or villi fusion. This figure had a great similarity with that of mice of group III (Fig. 4). On the other hand, the appearance of the villi of group II showed evident changes including shortening, atrophy and villi fused together leading to blunting with desquamation of most of villi (Fig. 3)

During examination of faecal smears, the 11^{th} mouse of group I showed very high oocyst intensities which might exceed oocysts count of mice of group II. This mouse was eliminated from the statistical analysis. After sacrifaction, examination of histopathological section of ileum of such mouse revealed no villus atrophy and the villi still maintained their normal appearance when compared to those of mice of group II, in spite of the presence of cryptosporidium oocysts in faecal and ileal smears at high degrees (Fig. 5).

Results of examination of smears from ilea of mice of the three groups after sacrifaction revealed coincidence in oocyst intensities with faecal smears examination of the same mice.

DISCUSSION

Cryptosporidium parvum is a zoonotic protozoan parasite, located on the brush border of small intestine, especially the ileum, of all mammals (Koudella and Jiri,.1997). So far, up till now, there are no effective preventive measures or treatment to combat the infection (Zu *et al.*, 1992). Cryptosporidia infection was greatly related with the immune status of the host. In immunocompromised individuals, the infection had been reported to be life -threatening (Unger, 1990).

Nigella sativa is a herb that was reported to have an immune stimulant and antiprotozoal effects (Khaled *et al.*,1998). The oil extract of such plant contained several ingredients with potential value.It was composed of more than 100 components that worked synergistically. The oil was a rich source of polyunsaturated fatty acids, which were the building blocks of cells, protein, which sustained body health, as well as, thymohydroquinone which was proved to have a high antimicrobial effect (El-Fatatry, 1975).

El-Sayed and El-Hashem (2000) found a significant decrease of Eimeria scores in the intestinal tract of native chickens fed ration containing *N. sativa* crushed seeds. They also recorded an improvement in serum total protein, globulin, leukocytic count and lymphocyte percentage. This result proved the immunestimulatory effect of nigella. Wahba (2000), studied the prophylactic and therapeutic effects of nigella oil extract on rats infected with the fatal protozoon *Pneumocystis carinii.* He found that rats treated with the oil could tolerate the infection, while, those non-treated did not survive.

In the present study, and from the obtained data, *N. sativa* oil had induced a significant reduction ($P<0.05$) in oocysts count of *Cryptosporidium parvum* starting from the 11^{th} day post-treatment till sacrifaction of mice on day 35^{th}. As infection with cryptosporidia was highly related with the state of immunity of the host and was self-limiting in immunocompetent individuals, so, administration of such effective oil might have helped in stimulating the immune system of the body rendering the intestinal cells less susceptible to infection with cryptosporidia, and consequently, leading to a sharp reduction in oocysts count. Furthermore, this herbal oil proved to have improved the appearance of villi of ileum, where the parasite colonized. The villi in treated mice retained their normal appearance, while, those in non-treated mice suffered from apparent shortening, atrophy, and they fused together leading to blunting with desquamation of most of villi. Therefore, *N. sativa* might compete for or block receptor sites on

the surface of ileum, thus, leading to reduction in parasite colonization (Harp, 1999).

So, it was concluded that administration of *N. sativa* oil proved to be beneficial in protecting susceptible hosts against opportunistic parasites such as *C. parvum* that, in many cases, may be fatal to the host. Thus, it had an efficient therapeutic effect on such parasite. Such result could be adopted in similar infections in other animals or even man.

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محاولات لمعرفة التأثير العلاجى لمستخرج زيت حبة البركة على كريبتوسبوريديم بارفام فى الجرذان المعدية تجريبياً

ماجدة عبد الحى الرفاعى

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

تمت هذه الدراسة لتحديد التأثير العلاجى لمستخلص زيت حبة البركة فى الجرذان المعدية تجريبياً بطفيل كريبتوسبوريدم بارفام . لهذا الغرض تم الإستعانة بخمسة وعشرين جرذا يتراوح أعمارها بين ٢ – ٣ أسابيع . تم تقسيم هذه الجرذان لثلاث مجاميع ، ضمت المجموعة الأولى الجرذان المعدية المعالجة ، المجموعة الثانية شملت الجرذان المعدية غير المعالجة ، أما المجموعة الثالثة فتكونت من الجرذان غير المعدية غير المعالجة قبل إسبوع من بداية العدوى ، تم تثبيط المناعة لجميع الجرذان بإعطائها ٢ مجم / لتر ديكساميثازون صوديوم فوسفات مع ٥٠٠ مجم / لتر تتراسيكلين هيدروكلوريد يومياً فى مياه الشرب تمت عدوى الجرذان بحويصلات الكريبتوسبوريديم المعزولة من العجول المعدية طبيعياً ، بعد أسبوع من العدوى ، تم علاج كل جرذ من جرذان المجموعة الأولى بجرعة ١ مللى / ١٠٠ حجم من وزن الجسم بزيت حبة البركة ، ثم تم فحص براز كل جرذ فى المجاميع كلها بصبغة الزيل نيلسون المعدلة ، كما تم عد حويصلات الكريبتوسبوريديم عشوائياً لكل ١٠٠ مجال ميكروسكوبى .

وقد تبين من نتائج الفحص أن جرذان المجموعة الثالثة كانت خالية تماماً من العدوى ، فى حين أن جرذان المجموعة الأولى أظهرت إنخفاضاً كبيراً فى عدد حويصلات الكريبتوسبوريديم مقارنة بجرذان المجموعة الثانية. عند اليوم الخامس والثلاثين ، تم قتل جميع الجرذان وأخذت أجزاء من الأمعاء الدقيقة وتم تثبيتها فى محلول فورمالين ١٠٪ للفحص الهيستوباثولوجى ، كذلك تم أخذ مسحات من الأمعاء الدقيقة وصبغها بصبغة الزيل نيلسون المعدلة . تبين من الفحص الهيستوباثولوجى أن خملات الأمعاء لجرذان المجموعة الأولى كانت طبيعية فى شكلها ولم تبد أى تغيرات، فى حين أن خملات الأمعاء الدقيقة لجرذان المجموعة الثانية أظهرت ضموراً واضحاً ، إدماجاً وغير حادة ، أما خملات الأمعاء لجرذان المجموعة الثالثة فكانت طبيعية الشكل تم مناقشة النتائج وتحليلها إحصائياً .

ويتضح من هذه النتائج أن مستخلص زيت حبة البركة له تأثير فعال فى علاج الإصابة بطفيل كريبتو سبوريديم بارقم فى الجرذان .

ومن ثم يمكن إستعماله فى الحقل التطبيقى بالنسبة للحالات المماثلة فى الحيوان والإنسان .