SOME STUDIES ON THE EFFICACY OF *NIGELLA SATIVA* OIL EXTRACT ON *TRYPANOSOMA EVANSI* IN EXPERIMENTALLY INFECTED RATS

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Abstract

*Trypanosoma evansi* is a widely distributed hemoflagellate causing severe economical losses in farm animals. The present study is a trial to control *Trypanosoma evansi* using *Nigella sativa* oil extract as a medicinal plant. For this purpose, thirty-seven male albino rats weighing 100-150 g each were divided into 5 groups. Rats of groups I and II were infected experimentally with *Trypanosoma evansi* obtained from naturally infected camels and treated with *Nigella sativa* oil extract on 3 and 12 days post-infection (d.p.i.), respectively, while rats of group III were used as prophylactic. Group IV was the control infected non-treated rats, while group V was the control non-infected non-treated one. The parasitaemia level was reduced in both treated and prophylactic groups in comparison with the control infected non-treated one. Some morphological changes appeared in *Trypanosoma* individuals in both treated and prophylactic groups post-treatment with *Nigella sativa* oil extract. These changes included lightly stained cytoplasm, degeneration of nucleus, disappearance of kinetoplast, flagellum and undulating membrane, swelling of the trypanosomes, appearance of vacuoles behind kinetoplast and oval-shaped trypanosomes. *Nigella sativa* decreased the lethal effect of *Trypanosoma evansi* through prolonging the survival time in treated and prophylactic rats.

INTRODUCTION

*Trypanosoma evansi* is a widely distributed hemoflagellate, causing severe economical losses in farm animals. During the acute phase, infected animals suffer much from fever, emaciation, anemia and may end in sudden death in some cases, while, the chronic phase is characterized by progressive weakness, emaciation, a notable decrease in productive capacity and may terminate fatally in untreated cases (Soulsby, 1986). In spite of the susceptibility of almost all species of domesticated livestock to *T. evansi*, the main host of this protozoan in Africa is camels, which are highly susceptible to the infection (Dla, 1997).

Many of the anti-trypanosomal drugs are applicable in the field, but most of them are either associated with severe side effects to animals or the parasite may
rapidly become resistant to these drugs. So, there is an urgent need to control trypanosomosis with encountering the least of such side effects. Many natural medicinal plants have been investigated in search to evaluate their trypanocidal effect (Dwivedi, 1999). Nigella sativa is a herb of family Ranunculaceae (Heywood, 1967) commonly found in Mediterranean area. El-Sarha et al. (1997) found that Nigella sativa had an immunostimulatory activity and plays an important role in combating some pathogens. Nigella sativa was selected on the basis of possessing an antiprotozoal activity.

In Egypt, El-Sayed and El-Hashem (2000) found a significant decrease of Eimeria scores in the intestinal tract and mortality of native chickens fed ration containing Nigella sativa crushed seeds. As well, The prophylactic and therapeutic effects of Nigella sativa oil extract were studied on Pneumocystis carinii infected rats, the percentage of survived rats in both prophylactic and treated groups were 40% and 73.3%, respectively (Wahba, 2002). In addition, El-Refai (2003), showed a reduction of oocysts count in mice experimentally infected with Cryptosporidium parvum and treated with Nigella sativa oil extract.

Therefore, this study was carried out to determine the therapeutic and prophylactic effects of Nigella sativa oil extract on Trypanosoma evansi in experimentally infected rats.

**MATERIALS AND METHODS**

Trypanosoma evansi was obtained from the blood of naturally infected canrels at El-Bassatine abattoir. The protozoon was propagated and maintained in susceptible rats obtained from a colony in Animal Health Research Institute. Each rat was injected intraperitoneally with $10^4$ trypanosomes. Nigella sativa oil extract was administered orally to experimentally infected rats at a dose of 1 ml/100 g body weight daily.

Thirty-seven male albino rats, weighing 100-150 g each were obtained from laboratory colony maintained in Animal Health Research Institute. These rats were divided into five groups. Experimentally infected rats of Groups I and II each of 10 rats, were used to evaluate the therapeutic effect of Nigella sativa oil extract for T. evansi infection. Rats of group I were given Nigella sativa oil extract on 3 days post-infection (d.p.i) while, rats of group II were given the oil extract on 12 d.p.i daily till the termination of the experiment. The prophylactic group III, containing 10 rats, were given Nigella sativa oil extract daily for 15 days before the experimental infection.
with *T. evansi*. Group IV containing 4 rats was used as control-infected non-treated, while, the remaining 3 rats of group V were left as control non-infected non-treated ones.

Rats were observed daily till the end of experiment (10 weeks post-infection) to record the survival rate. The tail blood from rats in all groups was examined microscopically using thin blood films stained with Giemsa stain every 5 days to record the pattern of parasitaemia and any morphological changes. Count of trypanosoma organisms was carried out per 10 fields. Comparison between both treated and prophylactic groups with the control one was recorded.

**RESULTS**

The present study revealed that *Nigella sativa* was effective in reducing the level of parasitaemia in both treated and prophylactic groups.

Figure 1 showed that, rats of group I developed parasitaemia after 3 d.p.i and appeared parallel to its control group till reaching its peak on the 5th d.p.i. Thereafter, the peak started to decrease gradually post-treatment with *Nigella sativa* oil extract till the 50th d.p.i. Complete elimination of the *Trypanosoma evansi* was evident on 55th d.p.i till the termination of the experiment.

In group II, parasitaemia level started to decline after 15th d.p.i and the reduction continued till the end of the experiment. This group showed no complete elimination of parasites. There were no marked differences between parasitaemia levels of the two treated groups (I and II) except that, the complete elimination of the parasites was apparent in group I.

Figure 2 showed that rats of the prophylactic group III developed parasitaemia and reached its peak on 10th d.p.i (25 days post-treatment with *Nigella Sativa*) then decreased gradually with complete elimination on 60th d.p.i till the termination of experiment.

The control infected non-treated group IV showed increased parasitaemia ending in death of all rats within 5 weeks post-infection.

Microscopical examination of the stained blood films showed different morphological changes of trypanosomes in treated and prophylactic groups. Figure 3 showed some degenerative changes in trypanosomes of experimentally infected rats of group I after treatment with *Nigella sativa* oil extract, and appeared in the form of lightly stained cytoplasm, degeneration of nucleus and disappearance of flagellum and undulating membrane.
Figure 4 showed some morphological changes observed in Giemsa stained blood film of experimentally infected rats in group II after treatment with Nigella sativa oil extract, remarked by fragmentation of the nucleus and lightly stained cytoplasm. The kinetoplast was still noticed but lightly stained. Deformities of trypanosomes in experimentally infected rats in both treated and prophylactic groups were clear in figure 5. These deformities appeared in the form of swelling of the trypanosome and disappearance of kinetoplast (Fig. 5a). There was also swelling of some trypanosomes and vacuolation behind the kinetoplast (Fig. 5b). Some trypanosomes were oval-shaped with lightly stained cytoplasm and disappearance of the undulating membrane, but flagellum was still noticed (Fig. 5c). Figure 5d showed swelling of trypanosome having a blunt posterior part, disappearance of kinetoplast and loss of flagellum.

Nigella Sativa oil extract had proved to prolong the survival rate of both treated and prophylactic rats compared with the control infected non-treated ones (Table 1).

The survival in group I (treated on 3 d.p.i.) was 100% till the 6th week p.i., then, decreased gradually to reach 80% on the 8th week p.i. and became 20% at the termination of the experiment (10th week p.i.).

The survival in group II (treated on 12 d.p.i.) was 100% at the 8th week post-infection, however, it was 80% at the termination of the experiment (10th week).

In group III (prophylactic group), the survival was 80% at the 6th week p.i. and decreased to reach 60% till the termination of the experiment. Regarding the control infected non-treated (group IV) the survival was 50% at the 4th week p.i and reached 0% at the 5th week p.i.

Table 1. Survived rats in therapeutic, prophylactic and control groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total No. of rats</th>
<th>Survival percent in different weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI (treated 3 d.p.i.)</td>
<td>10</td>
<td>100 100 100 100 80 70</td>
</tr>
<tr>
<td>GII (treated 12 d.p.i.)</td>
<td>10</td>
<td>100 100 100 100 80 80</td>
</tr>
<tr>
<td>GIII (prophylactic)</td>
<td>10</td>
<td>100 100 100 80 60 60</td>
</tr>
<tr>
<td>GIV (control infected non-treated)</td>
<td>4</td>
<td>100 50 0 0 0 0</td>
</tr>
<tr>
<td>GV (control non-infected non-treated)</td>
<td>3</td>
<td>100 100 100 100 100 100</td>
</tr>
</tbody>
</table>
Fig. 1. The mean values of Trypanosomes in Giemsa stained thin blood films of rats in treated groups (I and II) compared with the infected non-treated control (group IV).

Fig. 2. The mean values of Trypanosomes in Giemsa stained thin blood films of rats in prophylactic group III compared with the infected non-treated control (group IV).
Fig. 3. *Trypanosoma* organism showing some degenerative changes appearing in the form of lightly stained cytoplasm, degeneration of nucleus and disappearance of flagellum and undulating membrane.

Fig. 4. *Trypanosoma* organism showing fragmentation of nucleus and lightly stained cytoplasm.

(a)  

(b)  

Fig. 5. *Trypanosoma* organism showing deformity appearing in the form of (a) swelling of the parasite and disappearance of kinetoplast, (b) swelling of the parasite and appearance of vacuoles behind kinetoplast, (c) oval-shaped *Trypanosoma* with lightly stained cytoplasm and disappearance of undulating membrane, (d) swelling of trypanosome with blunt posterior part, disappearance of kinetoplast and loss of flagellum.
DISCUSSION

The present study is a trial to control Trypanosoma evansi in experimentally infected rats by using Nigella sativa oil extract as a medicinal plant in order to avoid the disadvantages of chemotherapeutic drugs.

Nigella sativa is a herb that had been reported to have an immune stimulatory (Khaled et al., 1996) and anti-protozoal effects (El-Refaie, 2003).

The present study demonstrated that Nigella sativa was considered one of the effective anti-trypanosomal herbs which gave great support for the experimentally infected rats to tolerate the infection with T. evansi through reducing the level of parasitaemia, inducing some morphological changes, their survival time rather being prolonged.

The parasitaemia in both treated and prophylactic rats decreased gradually till the termination of the experiment. This indicated that, the active immune system, due to the administration of Nigella enabled rats to tolerate the infection, then, damaging T. evansi. In the present study, it can be noticed that, there was no difference in parasitemia levels of both treated groups I and II except that complete elimination of trypanosomes was clear in group I. So, it was better to administer Nigella sativa at the beginning of infection than at the peak, as such plant appeared to be more effective during this period. In case of prophylactic group, it was evident that parasitaemia level was low in comparison to its control group. These results were in agreement with El-Refaie (2003) who found that Nigella sativa oil extract had induced a significant reduction in Cryptosporidium parvum oocysts count starting from 11 days post-treatment till sacrifice of mice on 35th day post infection.

Different morphological and degenerative changes of trypanosomes appeared in both treated and prophylactic groups. These changes included lightly stained cytoplasm, degeneration of nucleus, disappearance of kinetoplasts, flagella and undulating membranes and swelling of trypanosomes. The kinetoplast may be still present with vacuoles behind it. Such changes were nearly similar to those occurring in trypanosomes in animals treated with suramine (Minelli et al., 1981) and triquin (Wahba, 1999).

At the 6th week p.i the survival in treated groups (I and II) was 100%, while, in prophylactic group (III) it was 80%, although all rats in the control infected non-treated group died (0% survival). Furthermore, the survival at termination of
experiment were 20% and 80% in groups I and II, respectively, although, there was complete elimination of parasitaemia in group I. In group III, the survival percentage was 60% at the termination of experiment.

The previous results indicated that, the immune stimulatory effect of Nigella sativa was apparent as a medicinal herb. It stimulated the immune systems and decreased the lethal effect of Trypanosoma evansi in the infected rats compared to those of control group which died within 5 weeks, as the immune system of such rats was unable to control T. evansi. The obtained results are in agreement with those of Wahlba (2002) who found that, the effect of Nigella sativa oil extract on the survival percentage in both prophylactic and treated experimentally infected rats with Pneumocystis carinii was 40% and 73.3%, respectively. The diversity of results in survival percentage between treated and prophylactic groups may be attributed to that Nigella sativa stimulated immune system to produce specific antibodies against T. evansi in case of treated groups, while, in prophylactic group immune system had produced non-specific antibodies in the period before infection. So, this non-specific antibodies could not have a noticeable effect on T.evansi.

Further studies are needed to adopt the foregoing results in groups of camels subjected to infection with Trypanosoma evansi in an endemic environment. Also, it may be adopted in other Trypanosoma spp. infection in animals or man.

REFERENCES


بعض الدراسات على تأثير ستخلص زيت حبة البركة
على التريبوزوما إفانتازي في الفئران المعدية تجريبياً

أيمن أحمد محمد القندي، أحمد أسور وهبة

نُظَرُأ لانتشار طبل التريبوزوما إفانتازي وما يسبب من خسارة اقتصادية كبيرة خامسة في
الجمال فقد أجريت هذه الدراسة في محاولة للسيطرة على التريبوزوما إفانتازي باستخدام مستخلص
زيت حبة البركة الذي أثبت أن له تأثيراً ماناً وميثاً على بعض الطفيليات الأولية. ولذا، لم يتم
استخدام 37 فكراً من الكحول تحت أوزان كل منها بين 100-150 جراماً وتم تقسيمها إلى 5
مجموعات. أجريت العدوى الحيوية لفرق المجموعتين الأولى والثانية بطول التريبوزوما
إفانتازي ثم أجريت قنار المجموعة الأولى مستخلصًا زيت حبة البركة من اليوم الثالث للعووض
ثم نخلطة المجموعة الثانية عش الزهور قبل العووض. أمراً قنار المجموعة الثالثة فقد
استخدمت كثجكة وقائية وأجريت زيت لفترة لمدة 15 يوماً قبل العووض. بالنسبة للمجموعة الرابعة قُد
استخدمت كثجكة ضاربة ضعيفة دون علاج والمجموعة الخامسة استخدمت كثجكة ضاربة ضعيفة
عديمة وقائية وقائمة.

تضح انخفاض مستوى وجود الطفيل بشكل ملحوظ في كل من المجموعات المعلجة والوقائية
بالمقارنة بالمجموعة الضائعة كما لوحظ وجود بعض التغيرات الطارئة لطول التريبوزوما
إفانتازي في الفئران بعد إعطاءها مستخلصات زيت حبة البركة وهذه التغيرات ظهرت على شكل تغيير
فجائي لون الستيوماز وتغيير في طول الذيل وانخفاض مركز الحركة والنمو وال같ة. كما ظهر انخفاض في
التيروزوما وقد تبين أن زيت حبة البركة يقلل من التأثير المدى التريبوزوما إفانتازي حيث
نُجِلَت نتائج إيجابية لفائدة المفعول على الجمال.

ودِمغ هذه النتائج نرى أن زيت حبة البركة يمكن استعماله مستقبلاً في مكافحة تريبوزومة
إفانتازي في مجموعات الجمال التي قد تمرض لعدوى إفانتازي في بيئات مرتدة بهذا الطفيل والعديم
الجمال والحيوانات المحسية بطول التريبوزوما، وكذلك الإنسان.