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

EL KALSH, EMAN A. M. AND A. A. WAHBA

Animal Health Research Institute, Agricultural Research Center, Ministry of Agriculture, Dokki, Giza.

(Manuscript received 16 March 2005)

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, apperance 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 hemaflagellate, 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 protozoon in Africa is camels, which are highly susceptible to the infection (Dia, 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-Refaii (2003), showed a reduction of oocysts count in mice experimentally infected with *Cryptosporidum 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 camels 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 trypanosma 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 5^{th} d.p.i. Thereafter,t the peak started to decrease gradually post-treatment with *Nigella sativa* oil extract till the 50^{th} d.p.i. Complete elimination of the *Trypanosoma evansi* was evident on 55^{th} d.p.i till the termination of the experiment.

In group II, parasitaemia level started to decline after 15^{th} 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 10^{th} d.p.i (25 days post-treatment with *Nigella Sativa*) then decreased gradually with complete elimination on 60^{th} d.p.i till the termination of experiment.

The control infected non-treated group IV showed increased parasiraemia 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 be noticed but lightly stained. Deformities of trypansomes 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.

Rats of group V were still alive (control non-infected non-treated group) till the termination of the experiment (100% survival).

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

Groups	Total No. of rats	Survival percent in different weeks				
		2nd wk	4th wk	6th wk	8th wk	10th wk
GI (treated 3 d.p.i)	10	100	100	100	80	20
GII (treated 12 d.p.i)	10	100	100	100	100	80
GIII (prophylactic)	10	100	100	80	60	60
GIV (control infected non-treated)	4	100	50	0	–	–
GV (control non-infected non-treated)	3	100	100	100	100	100

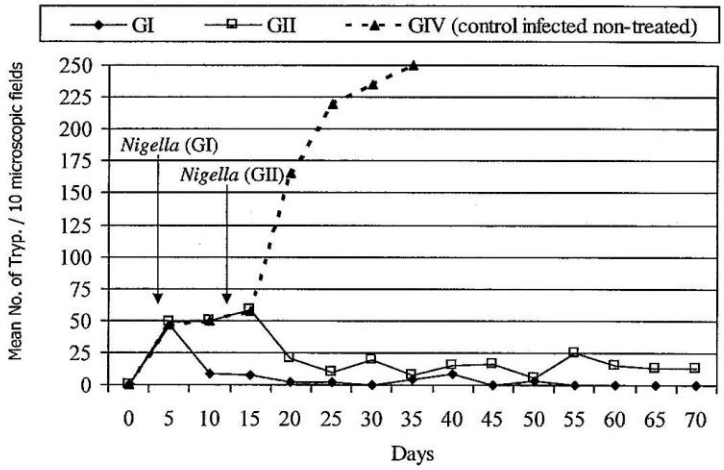


Fig. 1. The mean values of Trypanosomes in Giemsa stained thin blood films of rats in treated groups (I and II) compared with the infected non-treated control (group IV).

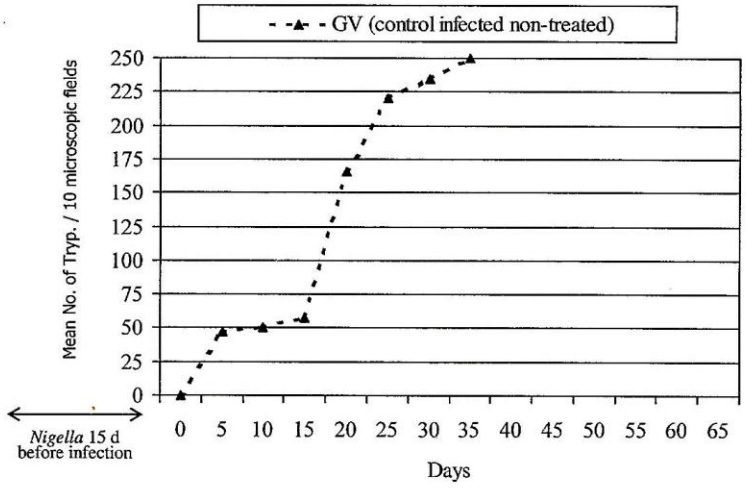


Fig. 2. The mean values of Trypanosomes in Giemsa stained thin blood films of rats in prophylactic group III compared with the infected non-treated control (group IV).

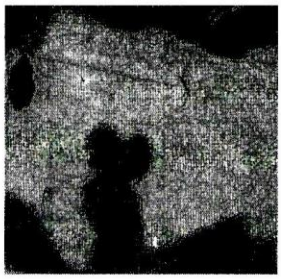


Fig. 3. *Trypanosoma* organism showing some degenerative changes appearing in the form of lightly stained cytoplasm, degeneration of nucleus and disappearance of flagellum and undulating membrane.

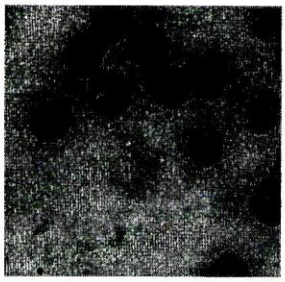
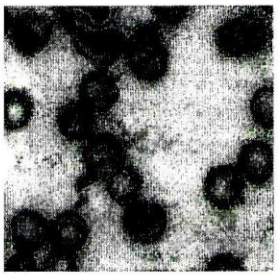
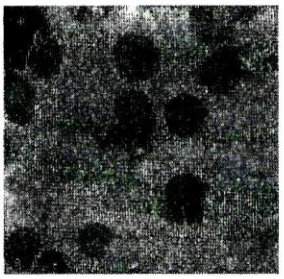


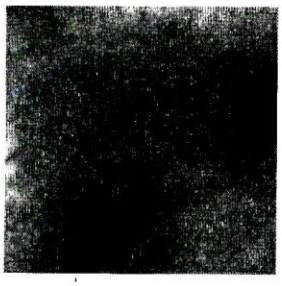
Fig. 4. *Trypanosoma* organism showing fragmentation of nucleus and lightly stained cytoplasm.



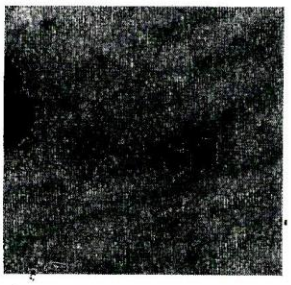
(a)



(b)



(c)



(d)

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 apperance 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-Refaii, 2003).

The present study demonstrated that *Nigella sativa* was considered one of the effective anti-trypanosomal herbs which gave a 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-Refaii (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 sacrification 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 stimuolatory 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 Wahba (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

1. Dia, M. L. 1997. Epidemiologie de la *Trypanosmosa cameline* et *T. evansi* en Mauritanie. Universite Montpellier, 34 pp Ph.D. Thesis.
2. Dwivedi, K. 1999. Evaluation of indigenous herbs as antitrypanosomal agents. Ethnoveterinary Medicinal plants, 9 (3): 9-14.
3. El-Refaii, M. A. H. 2003. Trials on therapeutic effect of *Nigella sativa* oil extract on *Cryptosporidium parvum* in experimentally infected mice. Egypt. J. Agric. Res., 81 (2): 805-816.
4. El-Sarha, A. I., H.Y. Hassan and K. M. Said. 1997. Haemato-biochemical changes induced by oral administration of *Nigella sativa* (balck seed) to goats. J. Egypt. Ger. Soc. Zool., 22A: 69-83.

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. Heywood, V. H. 1967. Plant taxonomy. English book published by Edward Arnold. London.

7. Khaled, M. L., A. El-Kadery, B. El-Gozamy and N. Abdel Fattah. 1998. Effect of *Nigella sativa* seeds on experimental *Hymenolepis nana* infection. Egypt. J. Med. Sci., 19 (1): 47-56.

8. Minelli, E. B., G. Ludice and N. Ercoli. 1981. Chemotherapy of *Trypanosoma Venezuelense*. Activity of metal-free organic trypanocides in mice and in vitro. Ann. Trop. Med. Parasit., 75 (4): 383-392.

9. Soulsby, E. J. L. 1986. Helminths, Arthropods and Protozoa of Domesticated Animals 7th Ed. The English Language Book Society and Bailliere Tindall. London.

10. Wahba, A. A. 1999. Some studies on the efficacy of triquin on *T. evansi*. Egypt. J. Agric. Res., 77 (2): 401-410.

11. Wahba, A. A. 2002. Efficacy of *Nigella sativa* oil extract on *Pneumocystis carinii* in immunosuppressed rats. Egypt. J. Agric. Res., 80 (1): 367-376.

بعض الدراسات على تأثير مستخلص زيت حبة البركة على التريبانوسوما إيفانزاى فى الفئران المعدية تجريبياً

إيمـــان أحمد محمد القلش ، أحمـــد أنـــور وهبـــه

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

نظراً لانتشار طفيل التريبانوسوما إيفانزاى وما يسببه من خسارة اقتصادية كبيرة خاصة في الجمال فقد أجريت هذه الدراسة فى محاولة للسيطرة على التريبانوسوما إيفانزاى باستخدام مستخلص زيت حبة البركة الذى ثبت أن له تأثيراً مناعياً وموثراً على بعض الطفيليات الأولية. ولهذا الغرض تم استخدام ٣٧ فأراً من الذكور تتراوح أوزان كل منها بين ١٠٠ ،١٥٠ جراما وتم تقسيمها إلى ٥ مجموعات. أجريت العدوى التجريبية لفئران المجموعتين الأولى والثانية بطفيل التريبانوسوما إيفانزاى ثم أعطيت فئران المجموعة الأولى مستخلص زيت حبة البركة من اليوم الثالث للعدوى بينما تم إعطاء المستخلص للمجموعة الثانية عند اليوم الثانى عشر للعدوى. أما فئران المجموعة الثالثة فقد استخدمت كمجموعة وقائية وأعطيت الزيت لمدة ١٥ يوماً قبل العدوى. بالنسبة للمجموعة الرابعة فقد استخدمت كمجموعة ضابطة معدية دون علاج والمجموعة الخامسة استخدمت كمجموعة ضابطة غير معدية وغير معالجة.

اتضح انخفاض مستوى وجود الطفيل بشكل ملحوظ فى كل من المجموعات المعالجة والوقائية بالمقارنة بالمجموعة الضابطة كما لوحظ وجود بعض التغيرات الظاهرية لطفيل التريبانوسوما إيفانزاى في الفئران بعد إعطائها مستخلص زيت حبة البركة وهذه التغيرات ظهرت على هيئة تغير لون السيتوبلازم وتحلل النواة واختفاء مركز الحركة والسوط والغشاء المموج كما ظهر انتفاخ في التريبانوسوما وقد تبين ان زيت حبة البركة يقلل من التأثير المميت للتريبانوسوما إيفانزاى حيث طالت فترة حياة الفئران نوعاً ما فى كل من المجموعتين المعالجة والوقائية.

ومن هذه النتائج نرى أن زيت حبة البركة يمكن استعماله مستقبلاً فى مكافحة تريبانوسوما إيفانزاى فى مجموعات الجمال التى قد تتعرض للعدوى فى بيئة موبوئة بهذا الطفيل وفى علاج الجمال والحيونات المصابة بطفيل التريباتوسوما وكذلك الإنسان.