Some Studies on The Efficiency of Allium *Sativum* for *Control* of *Trypanosoma Evansi* in Rabbits

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Abstract

The present study was conducted to evaluate the efficacy of *Allium sativum* on *Trypanosoma evansi.* Eight New Zealand white rabbits were inoculated intra veinously with 10^5 *T. evansi.* Eight rabbits were assigned into two groups (gp I, gp II) each contain four rabbits. *Allium sativum* oil extract was administrated orally to gpI at dose 1ml daily from day 0 till end of experiment (60DPI). Group II was considered as infected non treated control. Blood smear were examined daily from all rabbits for estimation of parasitaemia and for detection of any morphological changes in the parasite. Sera from both groups were collected once/week for detection the antigenic variation by serological examination using enzyme immunoblot technique. Parasitaemia was reduced dramatically in gp I compared with the infected control one (gp II). Great deformities in shape of *T. evansi* were reported during microscopical examination of the stained blood smears in treated group as trypanosomes appeared with lightly stained cytoplasm, marked fragmentation of the nucleus, absence of flagellum and undulating membrane and vacuole behind the kinetoplast. While examination of stained blood smears of non treated control revealed no morphological alteration of *T. evansi.* The analysis of EITB technique of antigen of gp I revealed there common polypeptide bands of 273.3, 144.8 and 101.3 KDa appeared in all lanes. While that bands of 62.0, 30 and 13.6 KDa appeared only during early infection (2nd WPI). Finally the band of 15.1 KD was appeared on the 4th WPI while in late stage (8th WPI) no specific epitopes were detected. While EITB technique of antigen of gp II revealed no antigenic changes along experimental period. There are antigenical changes in *Trypanosoma evansi* due to treatment with garlic oil in compared with non-treated group especially at early stages (2-4WPI).

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Introduction

Trypanosoma evansi is widely distributed hemoflagellate causing severe economic losses in farm animals. About 25 million cattle in Africa are at risk of infection with pathogenic trypanosomes **(16)**. In spite of the susceptibility of almost all animal species to *T. evansi*, the main susceptible hosts to infection with it in Africa are camel and horse **(7)**.

The chemotherapy of trypanosomosis to animals faced several problems due to its side effects and / or the parasite may rapidly become resistant to theses drugs. These factors emphasize the need for safe and cheaper source has trypanocidal effect. Many natural medicinal plants have been evaluated as it has trypanocidal effect **(8)**.

Garlic (*Allium sativum*) is bulb popularly used for more than 4000 years in traditional medicine **(3)**. Garlic oil has broad spectrum activity against *Trypanosoma* spp., **(1)**. Diallyle trisulfide one of the major components of *Allium sativum* inhibits growth of *T. brucei, T. rhodesiense, T. congolense, T. equiperdum* by 50% in practices **(11)**. In this study we examined the trypanocidal effect of garlic oil extracts against *T. evansi*.

Materials and Methods:

1-propagation of the *T. evansi* strain:

Trypanosoma evansi was isolated from naturally infected camels at Cairo abattoir. The isolate is propagated by subpassaging in mice. Preparation of the inoculum to infect rabbits was done according to **(6)**.

2- Experimental animal inoculation:

Eight New Zealand white rabbits were inoculated intraveinously with 10^5*T. evansi*. The rabbits were assigned into two groups (gp I, gp II) each includes four rabbits. *Allium sativum* oil extract was administrated orally to gpI at dose 1ml (150 mg) daily from day 0 till end of experiment (60DPI). GroupII was considered as infected non treated control. The animals of both groups were monitored daily and thin blood smears were obtained from the ear vein from all rabbits. The smears were stained with Geimsa and examined microscopically for estimation of parasitaemia (no of parasites /20 HPF) and for observing morphological changes of *T. evansi* in both groups. Also, sera were collected from each infected rabbit of both groups once/ week from day zero till end of experiment (60 DPI) for studying the effect

of *Allium sativum* on the antigenic structure of the *T. evansi* during the experimental period by using immunoblot technique.

3- Preparation of *T. evansi* antigen:

Whole cell lysate (WCL) antigen was prepared from *T. evansi* camel strain according to method described by **(2)**. *Trypanosoma* were separated from blood of the infected mice by freezing and thawing 5 times, the suspension in phosphate buffer saline (PBS) was sonicated for 5 times / 2 min with 1 min interval each in ice box. The suspension was centrifuged at 14000 rpm for 45 min at 4°C and supernatant with protein content adjusted to 1.0 mg/ml. A drop of merthiolate was added and the sample was stored at -20 C until used.

4-Enzyme immunoblot (EITB):

Trypanosoma evansi antigen (10 µg / well) were electrophoresed using 10% SDS-PAGE under reducing conditions **(10)**. The fractionated antigen was electrically transferred on to nitrocellulose (NC) membrane. NC sheets were cut into 0.5 cm strips followed by blocking in 5% bovine serum albumin (BSA) in PBS for 2 hrs on a rocker platform. Rabbit sera diluted at 1:100 in 5% BSA/PBS-T were reacted with fractionated NC strips for 2hrs on a rocker platform, followed by washing by PBS-T. Horse reddish peroxidase labeled ant-rabbit IgG diluted at 1:1000 (Bio-Rad co) in PBS-T was added to NC strips for 1hr on a rocker platform. The chromogen AEC (Sigma) was added to NC strips and allowed to develop for 30 min. The reaction was visualized by the naked eyes.

Results

The present study showed that the used dose of *Allium sativum* oil has effective trypanocidal activities since it reduces the level of parasitaemia in treated group when compared with infected control one. As shown in fig. 1 *T .evansi* started to appear on the day10 PI in gpI (treated group). Parasitaemia with *T .evansi* started to appear on the day 5 PI in gpII (non treated infected control group). The maximum level of parasitaemia was recorded in gpI on the 28th DPI (29/20HPF) and in gpII on the 38th DPI (60/20HPF). The parasitaemia in two groups were fluctuated along the experimental period but it was reduced dramatically in treated group than the control group.

Microscopical examination of the stained blood smears showed variable deformities in the shape of *T .evansi* in the group treated with *Allium sativum* differed along the experimental period. Some trypanosomes

appeared with vacuoles behind the kinetoplast on the 14th DPI the vacuoles increased gradually along the experimental period (fig. 2a). Lightly stained cytoplasm (fig. 2b), marked fragmentation of the nucleus appeared in experimentally treated rabbits with *Allium sativum* on the 23rd DPI and remained till end of the experiment (fig. 3a). On the 25th DPI flagellum and undulating membrane were absent in the treated rabbit and then remained along the experimental period (fig. 3b). Microscpical examination of stained blood smears of gpII revealed no morphological alteration of *T. evansi* (fig. 4).

The sera from experimentally inoculated rabbits of both groups (2nd, 4th and 8th week post infection (WPI)) reacted with *T. evansi* antigen and fractionated by EITB into several bands. In gpI the bands ranged from 273.3 KDa to 13.6 KDa (fig.5). We noticed that bands of 273.3, 144.8 and 101.3 KDa were appeared in all lanes indicating that these bands are common bands of *T. evansi*. While the bands of 62.0, 30 and 13.6 KDa were appeared only in early infection (2nd WPI). The 15.1 KD band appeared in the *T. evansi* antigen after 4th WPI while the antigen obtained on the 8th WPI showed no specific bands.

In the sera from experimentally inoculated non treated rabbits the bands appeared in all lanes are the same (common bands beside 62, 30 and 13.6K.Da) in the 2nd, 4th and 8th week post infection (WPI) (Fig.5).

Discussion

In this study the appearance of parasitaemia in the treated group (gp I) was delayed in appearance (10th DPI) than in the infected and non treated control group (gp II, 5th DPI).

These results indicated the active immune system due to administration of *Allium sativum* enabled rabbits to tolerate infection and decrease parasitaemia. These is may be due to broad spectrum activity of garlic oil against parasites as it modify host parasite immunobiology and offer alternative transcutanous delivery routes **(1).** The low level of parasitaemia (29/20 HPF) in gp I was attributed to trypanocidal effect of garlic oil as mentioned by **11; 13 and 1** initially through reducing level of parasitaemia and inducing some morphological changes in trypanosome.

The morphological deformities in trypanosome of treated group were similar to those described by **(9)** in experimentally infected mice with Trypanosoma *evansi* and treated by *Nigella sativum* and by **(18)** in rats infected with *Trypanosoma evansi* treated with triquin. in Also such changes were similar to that appeared in mice infected with *Trypanosoma Venezuelense* and treated with suramine **(12)**.

The previous results explained by **(17)** who reported that Ajoene compound which derived from garlic oil showed alteration of intracellular membrane structure particularly the mitochondrion and endoplasmic reticulum of trypanosomal cell due to it interfere with phospholipids composition. Also **(13)**, explain these changes as the garlic oil extracts interfere with synthesis of sterol and inhibit the phospholipase A2 so that lead to inhibition of lipid metabolism in trypanosomes.

The sera from experimentally inoculated rabbits treated with garlic oil (2^{nd}, 4^{th} and 8^{th} WPI) reacted with *T. evansi* antigen and fractionated by ELIB into several bands ranged from 273.3KDa to 13.6KDa. In our study we used ELIB to declare presence of antigenic changes correlated to antigenic deformity and to identify the stage of infection to adjust the protocol of treatment and control consequently.

This study revealed that polypeptides bands of 273.3, 144.8 and 101.3 KDa are common epitopes for trypanosomes appeared in all lanes in the treated group. Similar results were reported previously by **(5)**, they recognized two antigens approximately of 102-104 KDa by using immunoblot reacted with rabbit antitrypanosomal antibodies.

The bands 62, 30 and 13.6 KDa were appeared only at early infection (2^{nd} WPI) in gp I. Similarly **(15)** reported that a molecule of 61 KD was recognized in the anterior variant surface glycoprotein of *T. brucei* using immunoblot technique. Also, **(14)** detected monoclonal reagent bound with protein of 31 KD, this antigen apparent to be located in the plasma membrane of *T. congolense*. **(4)** detected that bands of 28-32KDa in immunoblot analysis of the whole procyclic lysat of *T. congolense* were specific for carbohydrate epitopes. Finally the present study detected that the band of 15.1 appeared in the 4^{th} WPI while in the 8^{th} WPI no specific band were appeared. These results nearly similar to that reported by **(19)**, they detected antigenically related molecule of 58 and 15.5 KDa were

present in whole blood stream and procyclic form of *T. brucei* by using immunoblot test. This epitope was detected on membrane of endosome and lysosome like structure, suggesting it has an important role in parasite membrane organization and function.

Our results obtained by ELIB coincided with the results of microscopical examination which recorded the disappearance of undulating membrane and free flagellum after 25DPI, so we can suggested that the late stage reveal no specific bands because the *Allium sativum* stimulate immune system to affect parasite lead to interference and alteration of membrane structure of trypanosome which consequently lead to disappearance of the specific band present in the plasma membrane.

The results of ELIB of gp II indicated that the bands appeared in all lanes are the same (common bands beside 62, 30 and 13.6KDa) in the 2nd, 4th and 8th week post infection (WPI). Our results indicated that no changes of antigen in infected non treated control group when compared with infected and treated group (gp I) so that declared the antigenic changes due to treatment with garlic oil.

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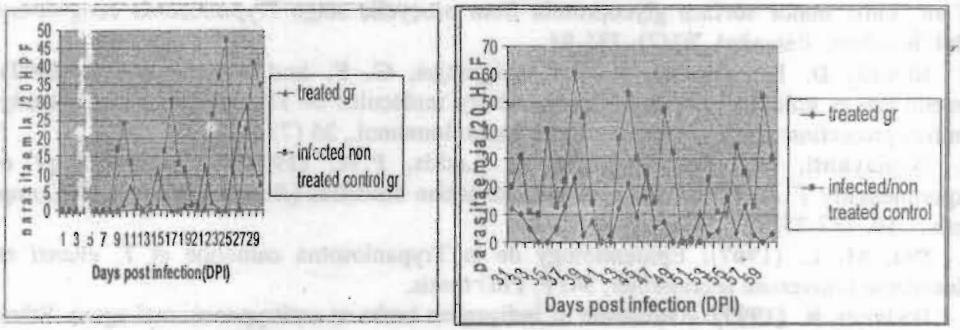
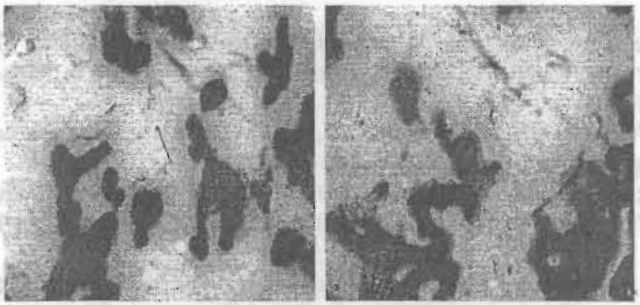


Fig.1Parasitaemia/2OHPF of experimentally inoculated rabbits with T. evansi gpI (treated group with *Allium sativum*) and gpII (infected non treated control group)

Fig.2: *Trypanosoma evansi* of experimentally infected rabbits treated with *Allium Sativum*. a,
Showing vacuole behind the kinetoplast.
b, showing disappearance of the flagellum and undulating membrane.



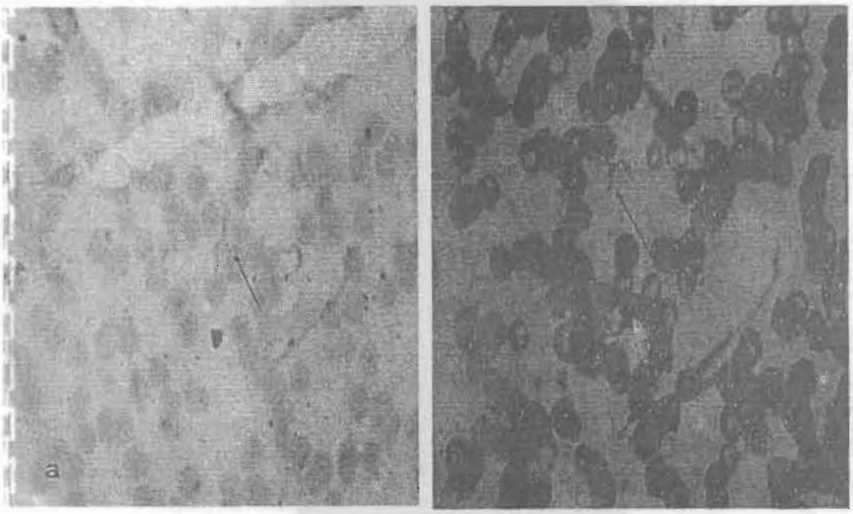


Fig.3: ***Trypanosoma evansi*** **of experimentally infected rabbits treated with** ***Allium Sativum*** **a, Showing degeneration of the nucleus. b, showing slightly stained cytoplasm.**

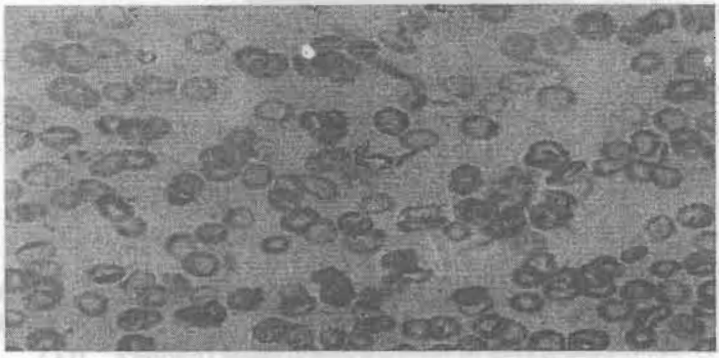


Fig.4: showing normal ***T. evansi*** **of the infected non treated rabbits**

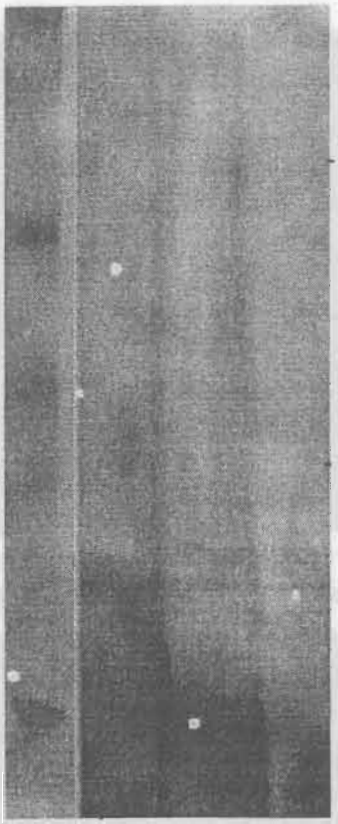


Fig.5: Enzyme immuno blot of 10%SDS-PAGE of *T. evansi* antigen. Lane1, Marker; Lane2, sera of infected and treated rabbits 2nd WPI; Lane3; sera of infected and treated rabbits 4th WPI: Lane4, sera of infected and treated rabbits 8th WPI.

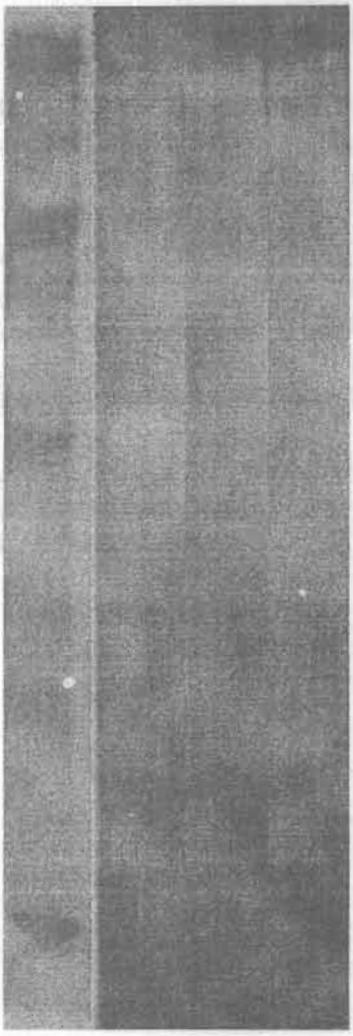


Fig.6: Enzyme immuno blot of 10%SDS-PAGE of *T. evansi* antigen. Lane1, Marker; Lane2, sera of infected non treated rabbits 2nd WPI; Lane3; sera of infected non treated rabbits in 4th WPI; Lane4, sera of infected non treated rabbits 8th WPI.

الملخص العربى

أجريت هذه الدراسة لتقييم تأثير حبة البركة على العدوى بطفيل الترياتوزوما فيفاكس ولقد استخدم فى ذلك عدد ٨ من الارانب النيوزيلاندى البيضاء والتى تم حقنها عن طريق الوريد بتركيز ١٠^٥ من الطفيل . ولقد تم تقسيم الارانب الى مجموعتين تحتوى كل منها على أربع أرانب.

وأعطى مستخلص زيتى من حبة البركة عن طريق الفم للمجموعة الاولى بجرعة ١ مليمتر يوميا من اليوم ٠ وحتى نهاية التجربة. أما المجموعة الثانية فلقد اعتبرت مجموعة قياس ضابطة بها العدوى ولم تتلق العلاج. وتم فحص مسحات من الدم يوميا من كل الارانب وذلك لقياس تواجد الطفيل فى الدم وكذلك لتشخيص التغيرات المورفولوجية فى الطفيل كما تم أخذ عينات مسل من المجموعتين مرة كل اسبوع وذلك لقياس التغيرات المناعية.

وقد وجد أن نسبة تواجد الطفيل فى الدم قد تراجعت بصورة حادة فى المجموعة الاولى بالمقارنة بالمجموعة الثانية. كما لوحظت تغييرات فى الشكل الظاهرى للطفيل فى عينات الدم تشمل مكوناته الداخلية وكذلك فى الدراسات المناعية تم تسجيلها.