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SOME STUDIES ON THE EFFICACY OF TRIQUIN ON *TRYPANOSOMA EVANSI*

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Abstract

Two experiments were carried out on 48 experimentally infected rats with *Trypanosoma evansi*. Each experiment included 24 rats. In the first experiment, 16 rats were divided into 4 subgroups each of 4 according to time of examination. Each rat was injected with triquin subcutaneously at a single dose of 4.16 mg/kg body weight. Eight rats were left as untreated control. Fresh blood samples, Giemsa stained blood films, impression smears from different tissues were obtained at 6, 9, 12 and 24 hours post-treatment and examined microscopically. In case of the second experiment, the rats were divided into 3 groups each of 8. The first group was supplied with 25% glucose solution orally, while, in the second group, each rat was injected with 2.5 ml of 25% glucose solution intra-peritoneally daily for 3 successive days. The third group was left as untreated control. Giemsa stained blood films from rats were examined one day post-administration of glucose, and then, weekly. Detection, as well as, morphological changes of trypanosomes in the 2 experiments were studied. Some morphological changes of trypanosomes in experimentally infected rats with *T.evansi* after being supplied with glucose solution only (either orally or I/P), were nearly similar to those occurring in trypanosomes in rats treated with triquin. The morphological changes included deformity, structural swelling of the poterior part with thinness of the anterior end, ill-defined cell-wall, lightly stained cytoplasm, damage of cytoplasmic membrane, disappearance of flagellum and undulating membrane and degrenerative changes of nucleus. The kinetoplast disappeared or still be found with a vacuole behind it.

INTRODUCTION

Trypanosoma evansi is one of the most economically important diseases affecting camels in Egypt, and it is a major threat to their health. This protozoon causes surra, which is a chronic wasting disease characterized by fever, anaemia and fluctuating parasitaemia (Syakalima, 1992). Also, the infected animal suffers from hypoglycaemia (Igbokwe, 1994).

Several varieties of drugs have been used to control the disease, among which is triquin; a combination of quinapyramine sulphate and chloride salts. From the available literature, many studies were carried out on its therapeutic and prophylactic action (Gill, 1972 & Prassad and Laha, 1990).

The aim of the present work deals with the morphological changes of *T.evansi* during the curative effect of triquin in experimentally infected rats. Meanwhile, due to the fact that trypanosomes have a high glucose metabolism (Levine, 1985), glucose solution has been tried to the infected rats; a trial which may be beneficial to infected animals.

MATERIALS AND METHODS

Blood infected with T.evansi was obtained from camels slaughtered at Cairo abattoir. Forty-eight rats of both sexes (each about 200 gm body weight) obtained from a colony in Animal Health Research Institute, were chosen and injected intra-peritoneally (I/P) each with 8 x 10^6 trypanosomes diluted with saline. They were grouped for 2 experiments, each of 24 and kept in clean wire cages for the study and supplied with clean dry food and water ad libidum.

After the appearance of infection, the two experiments have been carried out.

First experiment

Twenty-four experimentally infected rats were divided into 2 groups. The first group included 16 rats and was subdivided into 4 subgroups each of 4 according to time of examination. Each rat was injected subcutaneously (S/C) at the peak of parasitaemia (14 days postinfection) with a single dose of triquin (a product of Wockhardt limited, Dublin, Ireland), at a dosage of 4.16 mg/kg body weight. The second group involved 8 rats and was left untreated as infected control ones. From the 4 subgroups of rats, fresh blood samples diluted with saline and Giemsa stained blood films from tail veins were obtained at 6, 9, 12 and 24 hours post-treatment (H.P.T.), then, each rat was sacrificed on each occasion for impression smears examination of tissues from lungs, spleen, liver, heart, brain and kidney. The detection of the parasite, as well as, its morphological changes were studied.

Second experiment

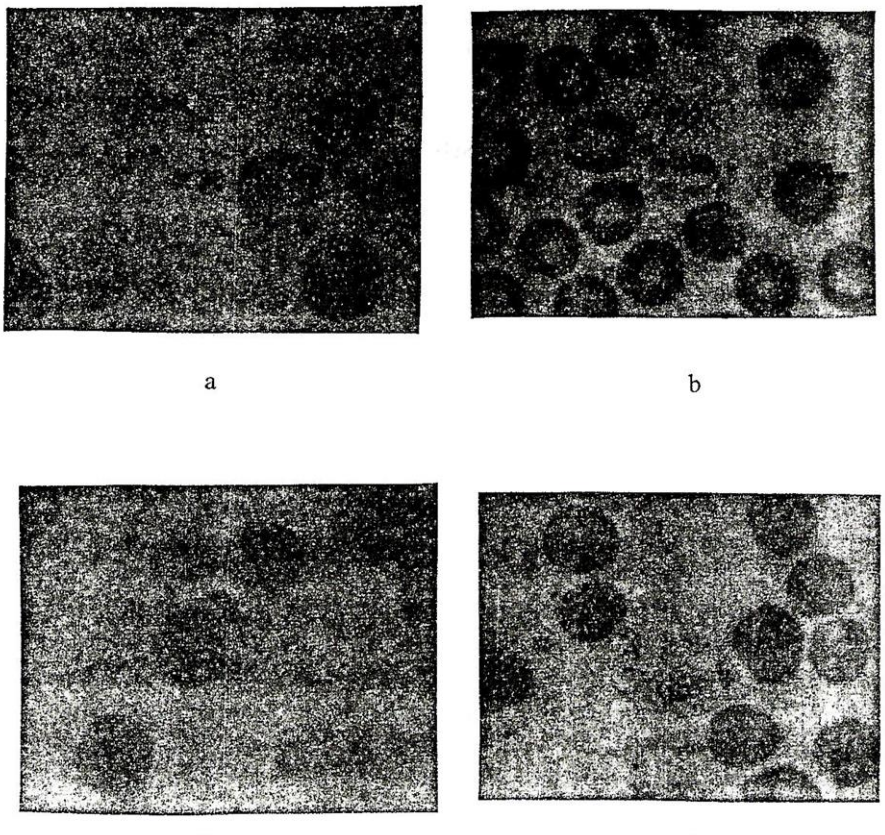
This experiment was carried out on experimentally infected 24 rats grouped into 3 groups, each of 8, to know the effect of glucose on the *T.evansi* at the peak of

infection (14 days post-infection). The first group was supplied with 25% glucose solution orally (the amount of glucose solution was recorded daily for each rat). In the second group, each rat was injected with 2.5 ml of 25% glucose I/P daily for 3 successive days. The third group was left as infected untreated control ones. Giemsa stained blood films from each rat were examined one day postadministration of glucose, and then, weekly, for the detection, as well as, the morphological changes of trypanosomes.

RESULTS

In the first experiment, after injection of a single dose of triquin S/C to experimentally infected rats with *T.evansi*, there appeared a gradual decrease of motility of trypanosomes at 6 and 9 H.P.T. The motility ceased completely at 12 H.P.T. At H.P.T., the mean length of trypanosmes ± SD was 25.94±2.09 μm, while, it was 19.97 ±2.49 μm in control ones. Different changes of trypanosomes appeared at 12 H.P.T. (figure 1) in the form of deformity, structural swelling of the posterior part with thinness of the anterior end, ill-defined cell wall, lightly stained cytoplasm and disappearance of flagellum and undulating membrane. There were also degenerative changes of the nucleus, while, the kinetoplast disappeared or still be noticed with a vacuole behind it. The trypanosomes disappeared 24 H.P.T. with triquin. By examination of impression smears from lungs, spleen, liver, heart and kidney, no trypansomes could be detected at 12 H.P.T., nor at 9 H.P.T. in the brain smear. At 6 and 9 H.P.T., some morphological changes of trypanosomes were observed in smears from tissues (figure 2 a, b, c. e & F). These changes appeared in the form of deformity of the parasite, disappearance of flagellum and undulating membrane, degenerative changes of nucleus, lightly stained cytoplasm and damage of cytoplasmic membrane. At 6 H.P.T. with triquin, some trypanosomes showed only nuclei with remnants of cytoplasm, while, this picture was much more apparent in smears from kidney at 9 H.P.T. (fig. 2 d).

Regarding the second experiment, and in case of the first group of rats, which were supplied with glucose in drinking water, each one drank about 30 ml/day. By examination of Giemsa stained blood films from those rats, trypanosomes were rare on the first day post-administration, and some morphological changes of trypanosomes were observed as loss of flagellum and undulating membrane, ill-defined cell wall, and lightly stained cytoplasm and presence of vacuole behind kineoplast (figure 3). In the first week post oral administration of glucose, there was slight increase in number of trypanosomes and the rats died between 2 nd and



a b c d

Fig. 1. Giemsa stained blood films from experimentally infected rats with T.evansi after treatment with triquin, showing deformity (a, c), structural swelling of the posterior part with thinness of anterior part (b, c), ill-defined cell wall and lightly stained cytoplasm (a, b, c, d), loss of flagellum (c) and undulating membrane (a, b, c), degenerative changes of nucleus (d), disappearance of kinetoplast (c) or still be noticed with a vacuole behind it (b). (X 2000).

3rd weeks post-administration.

In case of the second group where rats were injected I/P with 25% glucose for 3 successive days, 4 rats (50%) were still harbouring the infection till they died on 3rd week post-glucose injection. However, in case of the other 4 rats (50%), trypansomes were rare in the examined blood films on the first day post-glucose injection, then, disappeared in the 1st week till the 12th week post-glucose injection. Trypanosomes appeared again at the 13th week post-glucose injection and the rats were still surviving. The third group of rats (infected untreated control) died between 16 and 19 days post-infection. Some morphological changes of trypanosomes appeared in case of I/P injection of glucose as deformity, loss of undulating membrane, ill-defined cell wall, lightly stained cytoplasm and presence of vacuole behind kinetoplast (figure 4).

DISCUSSION

In the present study, after injection of a single dose of triquin S/C in experimentally infected rats with *Trypanosoma evansi*, the motility of the parasite decreased at 6 and 9 H.P.T., while, it ceased at 12 H.P.T. Minelli et al. (1981), recorded the decrease in virulene of *T.venezuelense* in mice treated with suramine. The length of trypanosomes in present study increased at 6 H.P.T. with triquin and this may be due to the paralysis and relaxation of the parasite. Page and Lagnado (1995) found an increase in length of T.brucei after exposure to phenothiazine in vitro.

Different morphological and degenerative changes of trypanosomes appeared in tissue smears at 6 and 9 hours post-treatment with triquin. In blood films these changes appeared at 12 hours post-treatment with the drug. These changes included deformity of trypanosomes, disappearance of flagellum and undulating membrane, lightly-stained cytoplasm and damage of cytoplasmic membrane. Trypanosomes showed only the nuclei with remnants of cytoplasm (appeared especially in kidney). Also, there was structural swelling of the posterior part with thinness of the anterior end, ill-defined cell wall and degenerative changes of nucleus, while, the kinetoplast disappeared or still be noticed with a vacuole behind it. Minelli et al. (1981), found morphological variations, vacuolation and swelling in T.venezuelense after treatment of mice with suramine. Bacchi et al. (1983), when injected experimentally infected rats with T.brucei with 4% alpha-DI-difluoro methyl ornithin, they noticed that the parasites were shorter and broader than those in the control ones. Page and Lagnado (1995), when studied the effect of phenothiazine on

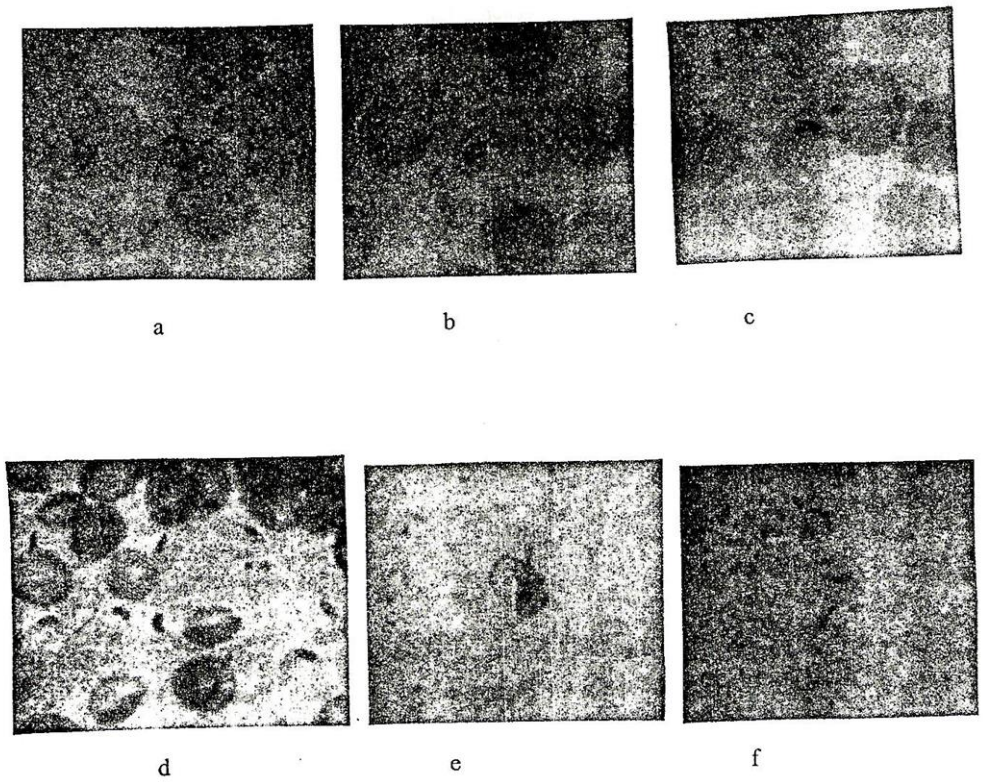
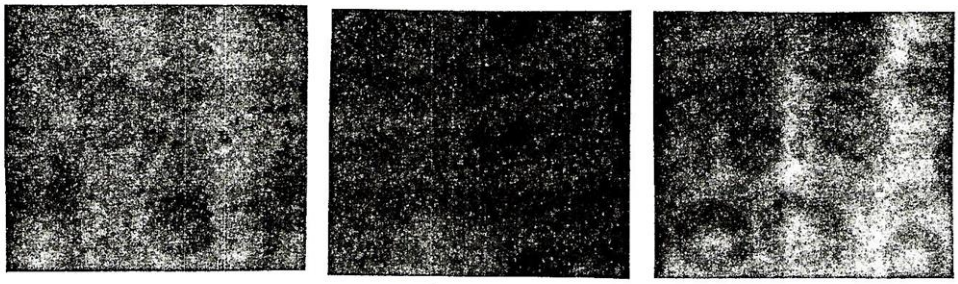


Fig. 2. Giemsa stained impression smears from heart (a, b, c), kidney (d), lung (e) and liver (f) of experimentally infected rats with T.evansi after treatment with triquin, showing deformity (a, c, e, f), disappearance of flagellum (f) and undulating membrane (a, b, c, f), degenerative changes of nucleus (b), lightly stained cytoplasm (a, b, c) and damage of cytoplasmic membrane (c). Some trypanosomes showed only nuclei with remnants of cytoplasm (d). (a, b, c: X 2000 and d, e, f: X 1250) .



a b c

Fig. 3. Giemsa stained blood films from experimentally infected rats with *T.evansi* after oral administration of 25% glucose solution, showing loss of flagellum and undulating membrane (a, b, c), ill-defined cell wall and lightly stained cytoplasm (b, c) and presence of vacuole behind kinetoplast (a). (X 2000).

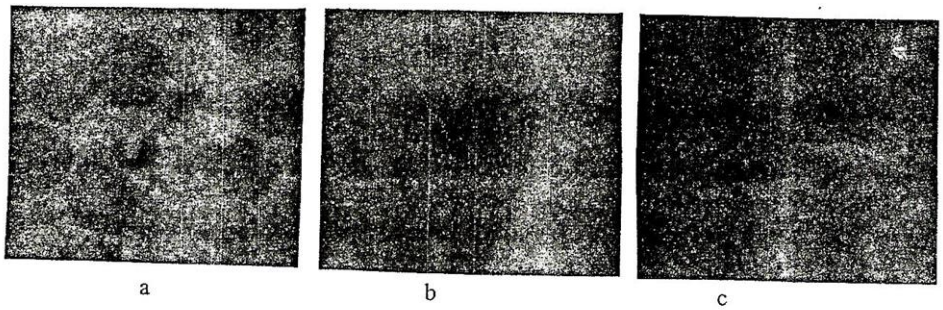


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Fig. 4. Giemsa stained blood films from experimentally infected rats with *T.evansi* after I/P injection of 25% glucose solution, showing deformity (b, c) loss of undulating membrane (a, b), ill-defined cell wall (b,c), lightly stained cytoplasm (c) and presence of vacuole behind kinetoplast (a,b). (X 2000).

T.brucei in-vitro, they found considerable swelling of the posterior end of the parasite associated with thinness towards the anterior end. They recorded the appearance of numberous vacuoles in cytoplasm and nucleus, and also damage of nuclear and cytoplasmic membranes.

In the present study, the parasite could not be detected from tissue smears and blood films at 12 and 24 H.P.T. in rats treated with triquin, respectively, and also from brain tissue at 9 H.P.T. This indicates that the parasite may have been perished by the effect of the drug.

In case of oral administration of glucose to experimentally infected rats, there was a temporary decrease in degree of parasitaemia and also some morphological changes of trypanosomes. After that, slight increase in parasitaemia was noticed till death of rats. This may explain the fluctuating parasitaemia or the slight infection of trypanosoma in case of infected camels, as they may be fed on ration or herbs rich in glucose which enable them to tolerate the infection. In case of deficiency of glucose, trypanosomes may flourish up and affect their victim.

Concerning the I/P injection of glucose in experimentally infected rats with *T.evansi*, 50% of rats showed a decrease in number of trypanosomes which could not be detected in 1st week post-glucose injection, after which, it appeared again in the 13th week post-glucose injection. The longevity of rats (> 13 weeks post glucose injection) was more noticeable than that in case of oral administration of glucose (2-3 weeks). This may be due to the fact that, parenteral administration of glucose is more effective than that of oral administration. Also, there were some morphological changes of trypanosomes in case of I/P glucose injection. This may be due to the effect of glucose on rats that had restored their activity, and consequently affected the parasite. Mallick and Dwivedi (1981) found that cattle and buffaloes infected with *T.evansi* died when treated with berenil only, while, those injected I/V with 25% dextrose besides treatment were saved.

From the present study, it was found that there were some morphological changes in trypanosomes in experimentally infected rats with *T.evansi* when glucose was administered only (either orally or IP). Such changes were nearly similar to those occurring in trypansomes in rats treated with triquin. Ration, herbs additives rich in glucose and offered to animals susceptible to trypanosoma infection may help in controlling the infection.

This study has shown how triquin could kill T.evansi and eliminate it from its host. In addition, it is advisable to give solutions of sugars such as glucose, dextrose

or sucrose in case of mild or acute infections with T.evansi, hence, the application of triquin in the field.

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بعض الدراسات عن فعالية مستحضر تراي كوين علي التريبانوسوما إيفانزي

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أجريت هذه الدراسة علي ٤٨ جزذا معديا بالتريبانوسوما إيفانزاي حيث تم عمل تجربتين، استلزمت كل تجربة ٢٤ جرذا.

في التجربة الأولي: تم تقسيم ١٦ جرذا إلي ٤ مجموعات، كل مجموعة ٤ جرذان حسب مواعيد الفحص. حقن كل جرذ تحت الجلد بجرعة ٤,١٦ مجم/كجم من وزن الجسم بعقار تراي كوين جرعة واحدة. ترك ٨ جرذان بدون علاج كضوابط للتجربة. فحصت عينات دم، شرائح دموية مصبوغة جيمسا ومسحات من الأنسجة بالميكروسكوب بعد ٦ ، ٩، ١٢، ٢٤ ساعة من العلاج.

في التجربة الثانية: تم تقسيم الجرذان إلي ٣ مجموعات، كل مجموعة مكونة من ٨ جرذان أعطيت المجموعة الأولي محلول جلوكوز ٢٥٪ عن طريق الفم في حين حقن كل جرذ من المجموعة الثانية عن طريق الغشاء البريتوني بجرعة يومية عبارة عن ٢,٥ مللي جلوكوز ٢٥٪ لمدة ٣ أيام متتالية، وتركت جرذ المجموعة الثالثة بدون علاج كضوابط للتجربة. فحصت شرائح دموية مصبوغة بصبغة جيمسا بعد يوم واحد من العلاج ثم أسبوعياً.

بالنسبة للتجربتين تم دراسة وجود الطفيل وكذلك التغيرات الوصفية له. لوحظ أن بعض التغيرات الوصفية لطفيل التريبانوسوما إيفانزاي في الجرذان بعد إعطائها محلول الجلوكوز فقط (عن طريق الفم أو الحقن) كانت تقريباً مشابهة لما تم ملاحظته علي الطفيل في الجرذان المعالجة بالتراي كوين.

من هذه الدراسات يتبين كيفية تأثير التراي كوين علي التريبانوسوما إيفانزاي في الجمال. هذا وفي حالة الإصابات الحادة وتحت الحادة فإنه ينصح بإعطاء محلول السكريات مع العلاج.