Sudan University of Science and Technology

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Title: Antitrypanosomal Activity of Quinapyramine Sulphate and Diminazene Diaceturate against *T. evansi* in Experimentally Infected Wistar Albino Rats

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الاية

قال الله تعالى :

(اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ{1} خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ{2} اقْرَأْ وَرَبُّكَ الْأَكْرَمُ{3} الَّذِي

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سورة العلق

صدق الله العظيم

Dedication

We dedicate this thesis to our dear

Mothers

The strong and gentle spirit that taught us to trust in God, believe in hard work, and that much can be done with little

Fathers

Who have earned a sincere living for us, supported us and always encouraged us to believe in ourselves

&

Teachers and colleagues of the study

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Abstracts

Trypansoma evansi causes an important disease of camel called Surra which is transmitted mechanically by various tabanids and other flies. Surra causes economic losses from decreased productivity in working animals, reduced weight gain, decreased milk yield, reproductive losses and the cost of treatment.

This study aimed to evaluate the efficacy of quinapyramine sulphate compared with diminazene diaceturate in the treatment of *T. evansi* isolated from camel in Wistar albino rats.

Twenty rats were divided into four groups (I, II, III, & IV), group I was kept as normal control, group II as a positive control, group III as a standard drug control, rats were treated with diminazene diaceturate at a dose of 3.5 mg/kg bw subcutaneously at day 4 of parasitaemia, while group IV was treated with the test drug quinapyramine sulphate at a dose of 3g/600-1000 kg bw subcutaneously at day 4 of parasitaemia. Parasitaemia was investigated daily using wet smear method. The level of parasitaemia in treated groups were compared with uninfected untreated rats.

The results indicated that quinapyramine sulphate has low efficacy against *T. evansi* compared to diminazene diaceturate used as a standard drug. However the parasitaemia of quinapyramine sulphate group increased significantly ($p < 0.05$) than the parasitaemia of infected untreated group. Diminazene diaceturate produced significant efficacy which indicated by the sharp clearance of parasite from the blood from day one of treatment till the end of experiment.

Infected untreated control rats produced significant decrease ($p < 0.05$) in Packed Cell Volume (PCV) compared to normal control and other groups.

It is concluded that *T. evansi* showed high level of resistance against antitrypanosomal drug; quinapyramine sulphate compared to diminazene diaceturate in rats. Clinical trials are recommended to evaluate the efficacy of quinapyramine sulphate in camel, horses and other livestock.

Arabic abstract

طفيل التربانوسوما ايفانساي يسبب مرضا هاما في الإبل يسمى "السرة" التي تنتقل ميكانيكيا من قبل العديد من عائلة ذباب الخيل والذباب الأخر. تسبب السرة خسائر اقتصادية بسبب انخفاض الإنتاجية في الحيوانات العاملة، انخفاض الوزن، انخفاض إنتاج اللبن، خسائر الإنجاب وتكلفة العلاج.

هدفت هذه الدراسة إلى تقييم فعالية عقار الكينابايرامين بالمقارنة مع فعالية عقار الدايمنازين داي اسيتيوريت في علاج طفيل التربانوسوما ايفانساي (المعزول من الجمال) فى الجرذان.

تم تقسيم 4 مجموعات من جرذان ويستار ألبينو ، في اليوم الرابع من حقن الطفيل في الجرذان تم علاج اثنين من المجموعات باستخدام عقار الكينابايرامين كعقار اختبار و عقار الدايمنازين كعقار قياسي.

أشارت النتائج إلى أن عقار الكينابايرامين لا يملك فعالية ضد طفيل التربانوسوما ايفانساي مقارنة مع عقار الدايمنازين داي اسيتيوريت المستخدم كدواء قياسي ، إلا أن طفيليات مجموعة عقار الكينابايرامين زادت بشكل اكبر من طفيليات المجموعة غير المصابة. كما ان عقار الدايمنازين داي اسيتيوريت أدى الى إزالة كاملة للطفيل

الفئران المصابة غير المُعالجة اظهرت انخفاض كبير في حجم خلايا الدم الحمراء المكدسة مقارنةً بالمجموعة الغير مصابة والمجموعات الأخرى.

وخلاصة ذلك ان طفيل التربانوسوما ايفانساي أظهر مستوى عالي من المقاومة ضد عقار الكينابايرامين. يوصى باجراء تجارب معملية لفحص فعالية عقار الكينابايرامين في المواشي.

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Introduction

The most widely used curative trypanocide against surra is diminazene diaceturate. However, other drugs can be used, such as isometamidium chloride (both curative and preventive), cymelarsan (only recommended for camels), suramin, and quinapyramine (Desquesnes et al., 2013). It is better to use the existing drugs by rational combination chemotherapy, because the discovery of a new chemotherapeutic agents is very expensive and time consuming.

Although quinapyramine sulphate is a drug of choice in the treatment of *T. evansi* in Sudan, (El Rayah et al., 1999) reported that all *T. evansi* Sudanese isolates were found to be resistant to quinapyramine compound in experimentally infected mice, so the present study was aimed to

1- Evaluate the efficacy of quinapyramine sulphate in experimentally infected Wistar albino rats against *T. evansi* isolated from camels.

2- Compare the efficacy of quinapyramine sulphate with diminazene diaceturate in the treatment of *T. evansi* in wistar albino rats.

CHAPTER ONE

1. Literature review

1.1 Origin of *T. evansi*

Trypanosoma evansi (*T. evansi*) was the first trypanosome shown to be pathogenic for mammals (Muieed et al., 2011). Its principal host is originally the camel but it is present in dromedaries, horses, and other Equidae as well as in a large range of other hosts. It is derived from *T. brucei brucei* (cyclically transmitted by tsetse flies), but it is no longer able to undergo its cycle in *Glossina* due to the loss of the maxicircles of kinetoplastic mitochondrial DNA (ONAH).

1.2. Distribution of T. evansi

Trypanosomosis caused by *Trypanosoma evansi* (Surra) is widely distributed in Africa, South America and the Middle East. In Sudan, this parasite has been reported in different regions since 1908, affecting camels and to lesser extent horses (El Rayah and El Malik, 2006; Salim et al., 2011). In Africa it is found in Kenya, Morocco, Algeria, Tunisia, Libya, Egypt, Sudan, Eritrea, and Ethiopia, the northern parts of Mali, Burkina Faso, Niger, Nigeria, Chad, Somalia, and Kenya. Nowadays, its geographical distribution is continuous from the northern part of Africa through the Middle East to South-East Asia. Although it is not possible to date the initial spread of *T. evansi* eastwards, the analysis of historical data suggests that surra was already present in India since time immemorial (Desquesnes et al., 2013).

1.3 Epidemiology

T. evansi has a more complex epidemiology due to the diversity of its hosts and vectors. *T. evansi* causes epidemics of a disease called surra, a Hindi word meaning rotten as described by Soulsby (Soulsby, 1982), which is of great economic importance in Africa, Asia and South America,

where thousands of animals die from *T. evansi* infection each year (Brun et al., 1998; Desquesnes et al., 2013).

The disease affects camels, horses, donkeys, cattle, buffalos, pigs, dogs and certain species of wild animals (Cheah et al., 1999). The epidemiology of a disease depends on the characteristics of a pathogen, its hosts, reservoir, and vectors and their environment and interrelations. Consequently, in the peculiar case of surra, which is a multispecies disease, it can exhibit highly variable characteristics because of its highly complex epidemiology.

The study of the epidemiology of surra requires various specific diagnostic tools. Detailed procedures are available from the World Animal Health Organization (WHO/OIE) website, terrestrial manual (Desquesnes et al., 2013).

1.4 Transmission

T. evansi is transmitted mechanically, non-cyclically, by many blood-sucking insects, especially horseflies (Tabanus) and stable flies (Stomoxys) which are endemic in Africa, Asia and South America; although in America the vampire bat also acts as a vector as well as reservoir hosts (Brun et al., 1998; Eyob and Matios, 2013; Muieed et al., 2011). In Africa the tsetse fly (*Glossina spp*), like other blood-sucking flies, can act as a mechanical vector in the areas where both *T. evansi* and these flies occur. In general the shorter interval between the two feedings, the greater the chances of successful transmission, since trypanosomes have a restricted survival time in the mouth parts of the vector. In South and Central America, *T. evansi* can be transmitted by vampire bats (*Desmodus rotundus*) which serve as both vectors and reservoir hosts. Transmission by the bats is not cyclical because the trypanosomes multiply in them only as blood forms, and are not undergoing a developmental cycle including non infective forms. Besides mechanical transmission by insects and vampire bats, *T. evansi* can be directly transmitted through milk or during coitus. In both Eastern and Western Hemispheres, canids (wolves and foxes) are believed to become infected by eating freshly-killed infected animals. Developmental stages were not observed in any of the vectors mentioned above (Desquesnes et al., 2013).

1.5 Clinical Sings in animals

Surra is a disease that causes various symptoms in a given host; from subclinical evolution to abortion or death, with or without vascular, nervous, or genital signs. Also produce various symptoms from one host to another (mostly lethal in horses, acute or chronic in camels, variable in bovines and buffaloes, acute in dogs, and generally mild but sometimes acute in pigs, sheep, and goats, etc.); as well as various aspects in different places (surra in buffaloes and cattle is virtually absent in Latin American, although it is a major constraint in South East Asia) (Desquesnes et al., 2013).

The pathogenic effects of *T.evansi* are including fever, anaemia, loss of appetite, loss of condition and productivity, nervous signs, abortion, cachexia, and death, with or without more peculiar signs related to the host species (Abdel-Rady, 2008).

1.5.1 Camels and Horses

Surra in camels may be acute with high fever, weakness, and death; it is also frequently fatal sometimes within a few months; however it is more often chronic than in horses and can frequently last 2-3 years (also called Tibersa) Signs of illness appear with intermittent fever, the animals appear dull and restless and become progressively weaker with staring hair, loss of appetite and weight, abortion, odema (ventral parts, udder or scrotum, and sheath), anemia with pale mucous membrane, and petechial or ecchymotic haemorrhages. All the age groups can be infected but surra generally starts occurring shortly after weaning. Nervous signs are sometimes observed, such as periodic convulsions .A specific odour of the urine is detected by camel owners, which is efficient for diagnosing the disease(Desquesnes et al., 2013).

1.5.2 Cattle and Buffalo

It is a mild, chronic, or asymptomatic disease especially in Africa and Latin America, where it is sometimes even difficult to infect animals experimentally similarly, in Venezuela, although some clinical signs have been recorded, the economic impact is not demonstrated(Desquesnes et al., 2013).

1.5.3 Sheep and Goat

Natural infection is generally considered as mild or asymptomatic in sheep. In some cases experimental infections can even fail, but in others they can lead to clinical signs, mainly fever, lack of appetite, and anemia; during hyperthermia modification of behavior such as exhaustion or sudden aggressiveness has been observed; anemia can recede after 2 months; parasitaemia is generally low (105 parasites/mL) and decreases until undetectable for several months; however, under certain circumstances such as food restriction or transport stress, parasites can relapse into the blood and clinical signs reappear(Desquesnes et al., 2013).

1.5.4 Pigs

Infection is very mild or symptomless; however, symptoms such as fever, anorexia, emaciation, and abortion were reported in an outbreak in pigs in Malaysia, and there were reports of low fertility in Thailand.

Even under experimental conditions, clinical expression is mild or delayed for several months .The immunosuppressive effects of the parasite have been considered to be responsible for interference with the efficacy of the vaccine against Classical Swine Fever (Desquesnes et al., 2013).

1.5.5 Carnivores

Dogs are highly susceptible to *T. evansi*, and they often exhibit strong clinical signs leading to death sometimes within a week and most often within a month in acute cases especially in stray dogs which are not treated and also sometimes even despite treatments clinical signs are intermittent fever, odema of the head, including larynx (to be differentiated from rabies), odema of the abdominal wall and legs, anemia, weakness, lack of appetite leading to emaciation and sometime paresis of the hindquarters; myocarditis has been described and can be fatal, as described in the first record of *T. evansi* in French Guiana sexual excitement has also been mentioned. Ocular signs are most often observed in dogs, with conjunctivitis, lacrimation, keratitis, corneal opacity and heamorrhagic signs, which can lead to fibrin deposits in the anterior chamber of the eye' parasites have sometimes been observed in ocular aqueous fluid (Desquesnes et al., 2013).

1.6 Diagnosis

The diagnosis of T. evansi infection is still difficult because the clinical signs are varied and non-specific and, in enzootic areas, the natural hosts frequently present mild chronic forms of the disease. The routine of T. evansi diagnosis is mainly based on finding the flagellates in wet films, smears or by the michrohematocrit test. These methods are specific but less sensitive, principally in detecting parasites during low levels of parasitemia (Herrera et al., 2004; Muieed et al., 2011; Silva et al., 1999). The demonstration of specific antibodies for the diagnosis of T. evansi infections in camels has been used by employing a modified card agglutination test (CATT) initially developed for T. brucei gambiense. A simple latex agglutination test for the detection of T. evansi antigens has been reported as well as antigen ELISAs to detect infections in horses. Sensitive method to detect single parasites is the PCR-based amplification of trypanosomal DNA (Herrera et al., 2004; Muieed et al., 2011).

1.7 Treatmeant

Treatment of surra is based on the drugs suramin, diminazene, quinapyramine and cymelarsan. While the first three have been used for 40 years or more, cymelarsan was developed less than 10 years ago and its efficacy has been demonstrated in camels, cattle, goats, pigs and water buffalo . Choice of drug, dosage and route of application depend on the animal species and the management in a given area as well as on the chemosensitivity of the trypanosome strains. The appearance of resistance to these drugs is a severe threat which will restrict their use. Suramin resistant toT. evansi strains have been reported from various places, such as the Sudan and also to diminazene and cymelarsan and it could be induced in mice in the laboratory, indicating that inappropriate use of drugs may lead to a resistance in the field . Since in vitro assays to determine drug sensitivity of T. evansi isolates are available, drug resistance monitoring can be done for areas where drug resistance might be encountered. These studies also provided evidence from in vitro and in vivo experiments that the majority of T. evansi isolates are innate resistant (tolerant) or non-responding to isometamidium. For the treatment of T. equiperdum infection the same drugs which are used for T. evansi are available. Evidence from in vitro drug sensitivity determination of T. equiperdum indicates that suramin, diminazene, quinapyramine and cymelarsan are effective against this trypanosome species, although no reports on clinical efficacy have been published (Brun et al., 1998).

1.8 Drug resistance

Suramin was used as an effective trypanocide until 1975. However, due to the occurrence of suramin-resistant isolates, the drug was withdrawn. Today, the most commonly used drug in Sudan is quinapyramine (Trypacide1). However, there is no information available on the present quinapyramine sensitivity of T. evansi isolates. Frequent reports by camel owners on treatment failure of quinapyramine would indicate reduced sensitivities of *T. evansi* in various areas in Sudan(Gillingwater et al., 2007)

1.8.1 Diminazene aceturate

There are now numerous reports of resistance to the diminazene aceturate in various Trypanosoma species and from many different part of Africa though on the whole, this still seems to be limited to highly endemic areas where use of the drug is highest.

Yet, the high local levels of resistance are creating genuine problems, especially where cross-resistance with the only viable alternative treatment Isometamidium . DA is relatively cheap, very widely available and active against trypanosomiasis as well as various tick-borne diseases including babesiosis . These clear advantages often make it the first-line treatment for sick cattle, and this inexpert use may well have contributed to the development of resistance. A major reason that DA resistance is indeed not far more widespread than it is, after decades of intensive use, is believed to be the rapid clearance of the drug from the body, reducing the chance of new infections being exposed to sub-curative residual levels of DA. Given the highly charged nature of diamidine compounds, specific carriers are required to translocate the drugs across biomembranes. Conversely, the absence or loss of these transport activities would render cells impervious to this class of drugs, explaining both their selective toxicity and the probable resistance mechanism. The TeDR40 protein, which has low similarity only to variant surface glycoproteins of T. evansi and T. brucei, could only be detected in the adapted trypanosomes and over expression of the gene in a sensitive line induced significant diminazene resistance.

DA has never been licensed for human treatment, yet it has been used to treat thousands of patients with early stage rhodesiense and gambiense sleeping sickness in experimental studies or when other drugs were not available. The administration is usually intra-muscular but it has also been given oraly and relapse rates and toxicity were generally low. Advantages of DA over pentamidine include shorter and less painful treatment and lower cost. Despite ongoing discussions pro and con, there do not seem to be any immediate plans to seek a licence for human use of DA. However, a much newer diamidine, DB-75 (furamidine), is in phase III clinical trials for early stage gambiense . It is administered orally as the N-methoxy prodrug DB289. Structurally, it is closer to diminazene than to pentamidine , with a very rigid furan linker between the benzamidine groups. Neither the active compound nor the prodrug have sufficient penetration into the central nervous system to act on late stage trypanosomiasis However, in an exciting new development, the prodrug of a related compound (DB-820, azafuramidine) was reported to completely cure a murine late-stage model. This prodrug, DB-844, could potentially become the first aromatic diamidine with activity against late stage sleeping sickness, and is currently in preclinical development (Delespaux and de Koning, 2007).

1.8.2 Suramin

Suramin was announced in 1920 as a cure for sleeping sickness. Suramin treatment was recently shown to delay disease progression and improve quality of life in hormone-refractory prostate cancer, though the overall survival rate was not affected. At therapeutically obtainable concentrations (1.1 mM) of suramin, about 85% of the total amount of the drug was bound to proteins, approximately 15% of which was bound to LDL. The mode of action of suramin is related to the fact that it is a large polyanion exerting inhibitory activities on a wide spectrum of enzymes: dihydrofolate reductase, fumarase, glycerol-3-phosphate dehydrogenase, hexokinase, l-_-glycerophosphate oxidase, reverse transcriptase, receptor mediated uptake of low density lipoprotein, RNA polymerase and kinases, thymidine kinase, trypsin. Resistance of *Trypanosoma evansi* to suramin has been reported in isolated from Sudan to China and suramin is not effective against *T.vivax* and *T.congolense.* Even though T. b. rhodesiense isolate with reduced sensitivity to suramin, and treatment failures of up to 25–35% are not uncommon, suramin resistance is not considered a problem in the treatment of sleeping sickness today. The treatment failures are considered largely due to misdiagnosed late stage infections, for which suramin treatment is not effective due to poor penetration into the central nervous system. Yet, induction of suramin resistance in the laboratory, by long-term exposure to sub-curative concentrations, appears straight forward. It was also demonstrated that the resistant phenotype was stable after transmission through tsetse flies. Levels of suramin resistance up to 3000 fold have been reported in immunosuppressed mice, and were stable for at least ten passages in the absence of drug pressure. The stability of the resistant phenotype is confirmed by the observation that fairly recent Trypanosoma evansi isolates from Sudan were highly resistant to suramin even though this medication had not been used in the country for more than 20 years. No cross-resistance with arsenicals, diamidines, quinapyramine or isometamidium was observed in laboratory or field strains. Little is known, however, about the mechanism of suramin resistance. It has been argued that suramin resistance was unlikely to be coupled to a drastic reduction in drug uptake. This was primarily deduced from the fact that the molecule is too highly charged and too large to be taken up by a specific plasma membrane transporter yet, in the presence of serum proteins, taken up faster than could be explained by simple endocytosis.

It was subsequently shown that suramin enters trypanosomes through receptor-mediated endocytosis with LDL. Since LDL uptake is essential for proliferation in trypanosomes, which cannot synthesise their own fatty acids and cholesterol de novo , have presented strong evidence that at least in procyclics suramin uptake, while proceeding by receptormediated endocytosis, is not coupled to LDL uptake rates. An alternative mechanism of resistance would be increased drug efflux, as a result of expression of a P-glycoprotein-type efflux pump. Given the likelihood of a multifactorial mode of action for suramin, making resistance by mutation of a single target improbable, accumulation of the drug in resistant and sensitive bloodstream forms should be revisited and the role of the LDL receptor reassessed using molecular approaches (Delespaux and de Koning, 2007).

1.8.3 Pentamidine

Unlike DA, evidence for resistance to pentamidine from the field is at best anecdotal. Treatment failures with pentamidine are rare and believed to usually constitute misdiagnosed late stage disease. The lack of resistance is remarkable, as the drug has been in use since 1940 and has been the first line treatment for gambiense sleeping sickness for more than 60 years. Moreover, in the 1950s and 1960s population-scale prophylaxis programmes with pentamidine, consisting of a six-monthly intramuscular injection with 4mg/kg pentamidine isethionate (Pentacarinat®) or dimethanesulphonate (Lomidine®) saw millions of people treated with low doses over 2 decades. This programme, known as lomidinisation in French colonial Africa, was an apparent great success. By 1950, 2 million people in Congo were receiving Lomidine at regular 6-month intervals and by 1955, out of 6.6 million people examined, only 2117 were found infected with trypanosomes. Far from inducing mass-resistance the pentamidine prophylaxis programme, together with excellent surveillance, helped to reduce sleeping sickness to the point where it was almost eradicated in the late 1960s. Pentamidine resistance is inducible in the laboratory by stepwise exposure to sublethal pentamidine concentrations in vitro or in vivo, but the process is slow and incremental. Perhaps more significant is that the resistant strains were much less virulent in rodents than the un-adapted strains that had been cultured for the same length of time in the absence of drug. Both of these observations may help explain the absence of pentamidine resistance possibly a gene function important for infectivity is implicated in high-level pentamidine resistance.

high intracellular concentrations, it can be speculated that the mode of action is multi-factorial and that any resistance must therefore be linked to either decreased uptake or a significant and active efflux mechanism, especially since it was noted that pentamidine was not metabolised in either a resistant or the isogenic parental *T. b. brucei* strain, and that drug metabolism plays no role in either pentamidine action or resistance. transport of pentamidine was only inhibited to a maximum of about 50% by the main P2 substrates of adenosine and adenine , The adenosineinsensitive flux was attributed to two distinct, saturable transport activities of very high affinity (*K*m = 36 nM) and one of relatively low affinity for pentamidine (*K*m =56_M), designated HAPT and LAPT, respectively All three pentamidine transporters will contribute to uptake of the drug at pharmacologically relevant concentrations, explaining the difficulty in inducing pentamidine resistance(Delespaux and de Koning, 2007).

1.8.4 Isometamidium and homidium

The amphiphilic cationic phenanthridine, isometamidium chloride (ISM; Samorin, Trypamidium, Veridium) has been used in the field for several decades prophylactically or therapeutically for livestock suffering from trypanosomosis due to infection with *Trypanosoma congolense* and other *Trypanosoma* spp. It was first synthesized by coupling homidium (Ethidium) with *p*-aminophenyldiazonium chloride or in other words by coupling homidium with a part of the diminazene (Berenil) molecule. The first reports of ISM use date from 1963 and to the best of our knowledge, the first case of resistance to homidium and cross-resistance between homidium and ISM was reported in 1967 . Resistance to ISM is mostly associated with cross-resistance to homidium and it could be speculated that these structurally related compounds might share the same uptake mechanism albeit that their distributions within the trypanosome are slightly different. ISM is mainly concentrated in the kinetoplast, whereas homidium is spread much more diffuse throughout the trypanosome . The first study of ISM and diminazene uptake mechanisms concluded an energy dependent process for diminazene but not for ISM. Complete glucose privation induced a significant decrease of diminazene uptake and an increase in ISM uptake,

which is in contradiction with more recent studies concluding that the transport of ISM was energy dependent, as it was reduced in the presence of metabolic inhibitors such as SHAM/glycerol. The observed increase in ISM uptake in the absence of glucose was very likely due to cellular damage and loss of the integrity of the mitochondrial membrane, allowing the trypanocide direct access to the kinetoplast DNA, for which it shows ahigh affinity showed that the trypanosome kinetoplast was the primary site of ISM accumulation in *T. congolense*. This was later confirmed for the hemoflagellate *Cryptobia salmositica* (*Kinetoplastida, bodonina*) and for *T. brucei* using more sophisticated chromatographical and microscopical techniques. All seems to indicate that two compartments for the accumulation of the drug can be considered. The first one being freely diffusible, the second one consisting of retained ISM in the mitochondrion. When placed in ISMfree medium, no difference in ISM diffusion out of the cell was observed between sensitive and resistant strains. In the same conditions, a large proportion of the drug, sequestered within the mitochondrion of the sensitive strains, is retained The accumulation of ISM within the kinetoplast is impaired by agents affecting the mitochondrial function (oligomycin, 2,4-dinitrophenol) as measured by the accumulation of the lipophilic cations [^{3}H]methyltriphenylphosphonium or rhodamine 123. Those agents produce a dose dependent inhibition of the mitochondrial membrane potential strictly correlated with ISM accumulation showing that the uptake into the mitochondria is an active, transporter-mediated process. In *T. b. brucei*, the P2 adenosine transporter may be responsible for part of the ISM uptake as the drug inhibits P2-mediated adenosine uptake but the low level of cross resistance between diminazene and ISM suggests that this contribution is not an essential one. In *T.evansi*, RNAi of the TevAT1 gene conferred only low levels of ISM resistance. Resistance to ISM was increased 94-fold in a clone of *Trypanosoma congolense* by repeated subcurative treatment of infected mice for 11 months. Thiswas associated with 33-fold increases in resistance to homidium but only with 3.4-fold increases in resistance to diminazene ,while loss of TbAT1/P2 leads to almost 20-fold resistance to DA , Recent Amplified Fragment Length Polymorphism (AFLP) studies on two isogenic clones of *Trypanosoma congolense*, the parent clone being sensitive to ISM (CD50: 0.018 mg/kg in mice) and the derived one presenting a CD50 94-fold higher, showed at least 58 polymorphic

fragments only present in the derived resistant clone . Even though those fragments are unlikely to be all related to ISM resistance, these data suggest that high levels of ISM resistance may well be the result of changes in several genes. If so, this finding would parallel other drug resistance scenarios. For example, loss of the main melaminophenyl arsenical transporter, TbAT1, alone results only in low level resistance and though *pfcrt* is the main determinant of chloroquine resistance in Plasmodium, additional polymorphisms in pfmdr1 contribute to higher levels of resistance. Never the less, the AFLP study identified a putative protein that could be involved in the transport of ISM. This protein presents eight predicted transmembrane domains, a signal sequence, a putative ATP binding site and two conserved motifs of ABC transporters. All sensitive strains appeared to be heterozygous for the insertion, whereas most resistant strains were homozygous for the specified insertion – though a few clearly resistant isolates did not carry this marker. The fact that the sensitive isolates already appear to carry a recessive resistance allele would be consistent with the selection of an existing influx transporter with a decreased affinity for ISM through loss of heterozygosity. Alternatively, the resistance allele could encode a mitochondrial efflux pump with increased affinity for ISM. However, such an allele would be expected to be dominant, actively clearing the kinetoplast of the drug. More isolates should be screened to try to identify an ISM-sensitive phenotype for a strain homozygous for the insertion, whichwould challenge this model.Adeeper insight into the exact physiological role of this protein and its expression in the presence and absence of ISM would further increase our understanding of this particular resistance mechanism. The main mode of action of ISM chloride is thought to be the cleavage of kDNA-topoisomerase complexes, causing the desegregation of the minicircle network within the kinetoplast, though later showed that dyskinetoplastic trypanosomes are at least as sensitive to isometamidium as kinetoplastic lines. However, it was recently shown that amino acid substitutions in topoisomerase II genes conferred resistance to anticancer drugs in human and yeast cells and that two amino acid substitutions within the topoisomerase I gene conferred resistance to camptothecin, an antitumor compound, in *Leishmania donovani* . This led us to investigate a possible alteration of a *T. congolense* gene highly similar to the equivalent chromosome 11 gene of *T. brucei*.

The topoisomerase II genes of three strains, one sensitive and two resistant to ISM were sequenced from the open reading frame to the termination codon. Induction of resistance to ISM seems not to be an easy process as, in most cases, the drug is still effective in the field, even though it was first marketed nearly half a century ago. However, more and more cases of therapeutic failure are now being reported. The authenticity of the resistance phenotype in some of these field isolates of trypanosomes has been confirmed by in vivo testing of individual clones derived from the isolates. Resistance occurs where a large proportion of the trypanosome population is exposed frequently to the drug, for instance in commercial ranches, or after government policies based on large scale block treatments or where frequent under dosing of the trypanocide occurs . Eleven months of repeated sub-curative treatments in mice were necessary to induce resistance in one cloned isolate. Phenanthridine compounds are well-known mutagenic compounds and might induce mutations, the most resistant of which are selected under drug pressure. Taking into account the basic mutation rate in trypanosomes, which is estimated at 10−9 per base pair per cell generation in *T. brucei* the effects of this phenomenon should not be underestimated. It is of the greatest epidemiological importance that the development of resistance to ISM did not appear to affect infection rates or rates of cyclical development in *Glossina morsitans centralis* and seems to be a stable genetic trait. Field observations in Ethiopia, based on cloned populations, showed that the drug resistant phenotype of *T. congolense* had not altered over a period of four year (Delespaux and de Koning, 2007).

1.8.5 Quinapyramine Sulphate and Chloride

Quinapyramine sulphate (methyl sulphate) produce rapid and short duration of action while chloride compound is long acting, hence prophylactic. Quinapyramine chloride is unsuitable as general prophylactic in cattle and it is better to use the so called the (pro- salt) pro-salt is amixture of 2parts of Quinapyramine Chloride and 3parts of Quinapyramine Sulphate. The trypanocidal action is mainly due to inhibition of growth and cell division of the organism. The drug is administration IM and the local reaction may persist for few months. In cattle the drug is administered as a 10 percent solution and in horse as a 5 percent solution (Giordani et al., 2016).

CHAPTER TWO

2. Materials and Methods

2.1 Test parasite

T. evansi isolated from camels in Eastern Sudan in 2016. The parasite was confirmed as *T.evansi* using parasitological, molecular techniques and sequencing analysis (Mossaad *et al*., 2017). The parasite was kept in liquid nitrogen and maintained in rat. Infected rat was daily examined for parasitaemia using wet smear method.

2.2 Drugs

Diminazene diaceturate (Dophanil) drug, manufactured by DOPHARMA INTERNATIONAL B.V, Netherlands, was used as a standard antitrypanosomal drug

Quinapyramine sulphate drug, manufactured by ASHISH LIFE SCIENCE PVT. LTD., India, was used as a test drug to evaluate its antitrypanosomal activity compared with reference drug Diminazene diaceturate

2.3 Experimental animals

Wistar albino rats were obtained from Department of Parasitology, Faculty of Veterinary Medicine, University of Khartoum. Animals were housed in plastic cages in stander condition in laboratory animal house, Department of Clinical Study, Sudan University of Science and Technology. Rats were given a stander diet and free access water. They were given 3 days to adapt laboratory condition.

2.4 Harvest of parasite

T.evansi was harvested from donor infected rat at peak parasitaemia (8.1 antilog). The infected rat was anaesthetized using an overdose of chloroform and blood sample was collected from heart. About 2 ml infected blood was then diluted using 10 ml normal saline to be ready to infect experimental rats.

2.5 Inoculation of experimental rats (animals)

Experimental rats were infected with *T.evansi* by injecting of diluted infected blood, intraperitoneally at a dose of 0.3 ml. Blood diluted to $1x10^{75}$ ml *T.evansi* (antilog 8.1) as described by (Herbert and Lumsden, 1976)

2.6 Monitoring of parasitaemia

Parasitaemia was monitored daily according to the method of (Herbert and Lumsden, 1976). The blood was obtained from the tail vein by trimming the tip of tail. A drop of blood was put on a clean slide, covered with cover slip and examined under light microscope using x40 objective. The results were average of 5 rats.

2.7 Experimental design

Twenty rats were divided randomly into 4 groups of 5 rats each:

Group I kept as untreated uninfected control.

Group II served as infected untreated control (positive control).

Group III served as a standard drug control (rats were treated after infection with diminezine diaceturate at a dose of 3.5 mg/kg as a single dose (sc) at day 4 of parasitaemia.

Group IV rats were injected subcutaneously with quinapyramine sulphate at day 4 of parasitaemia.

2.8 Parameters

2.8.1 Body weight

Body weight was measured at day 0 and day 9 of parasitaemia.

2.8.2 Packed cell volume (PCV)

PCV was measured at the end of experiment (day 18), blood was collected from heart under anesthesia in EDTA tube

2.9 Statistical analysis

Data were analyzed using statistical package of social sciences (SPSS) version 20, ANOVA analysis of variant was measured to analyze the data of PCV. $P < 0.05$ was considered statistically significant (Gomez et al., 1984).

CHAPTER THREE

3. Results

3.1 Infected untreated group

The infected untreated rats maintained wavy wave (undulating wave) of parasitaemia from day 2 till the end of experiment (day 18).

3.2 Infected, treated rats with Diminazene Diaceturate:

The *T.evansi* infected, Diminazene Diaceturate treated rats showed sharp demise of all parasites from the blood (antilog <5.4) to zero (0.00± 0.00). A parasitaemia was observed from the 5^{th} day after treatment directly and continued to the end of experiment. The parastemia in this group significantly reduce in the 5^{th} day to 0% compared to infected untreated group.

3.3 Infected Quina pyramine sulphate treated group:

The infected Quina pyramine Sulphate treated rats, maintained wavy wave of parasitaemia from 5^{th} day till the end of experiment. Infected, Quina pyramine Sulphate treated group showed insignificantly ($P>0.05$) gradual decrease in parasitaemia on day 5 post treatment, compared to infected untreated group, but continued to rise again until reaching the highest level at day 13 post treatment (antilog 7.38).

The level of parasitaemia was found to be comparable to infected untreated group ($P>0.05$).The result was presented in Table (1), (2) and Fig.1.

Table 1 ***: Mean level of parasitaemia in rats infected with*** **T.evansi** ***(before treatment)***

Groups	**D0**	**D1**	**D2**	**D3**	**D4**
Un infected control	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	**0 ± 0.00**
Infected untreated control	0 ± 0.00	0 ± 0.00	5.96 ± 1.50	7.7 ± 0.25	**7.88 ± 0.30**
Diminazene diaceturate	0 ± 0.00	0 ± 0.00	7.58 ± 0.22	7.68 ± 0.12	**7.8 ± 0.00**
Quinapyramine sulphate	0 ± 0.00	0 ± 0.00	7.02 ± 0.21	7.34 ± 0.18	**7.7 ± 0.25**

Table 2 . Mean level of parastemia in rats infected with **T.evansi** *and treated with Diminzene diaceturate and Quina pyramine sulphate*

Groups	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18
Un infected control	**0 ± 0.00**	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Infected untreated control	**7.88 ± 0.30**	7.98 ± 0.15	5.64 ± 1.48	1.68 ± 1.68	3.66 ± 1.54	6.66 ± 0.40	7.08 ± 0.23	7.86 ± 0.06	7.86 ± 0.06	7.8 ± 0.09	8.04 ± 0.25	7.8 ± 0.25	4.62 ± 1.89	6.36 ± 1.59	6.42 ± 1.60
Diminazene diaceturate	**7.8 ± 0.00**	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Quinapyramine sulphate	**7.7 ± 0.25**	5.9 ± 1.48	4.32 ± 1.78	4.68 ± 1.22	3.6 ± 1.50	2.52 ± 1.56	3 ± 1.83	2.94 ± 1.81	4.56 ± 1.87	6.06 ± 1.52	5.32 ± 1.38	6.78± 0.50	7.14 ± 0.26	7.38 ± 0.07	6.98 ± 0.43

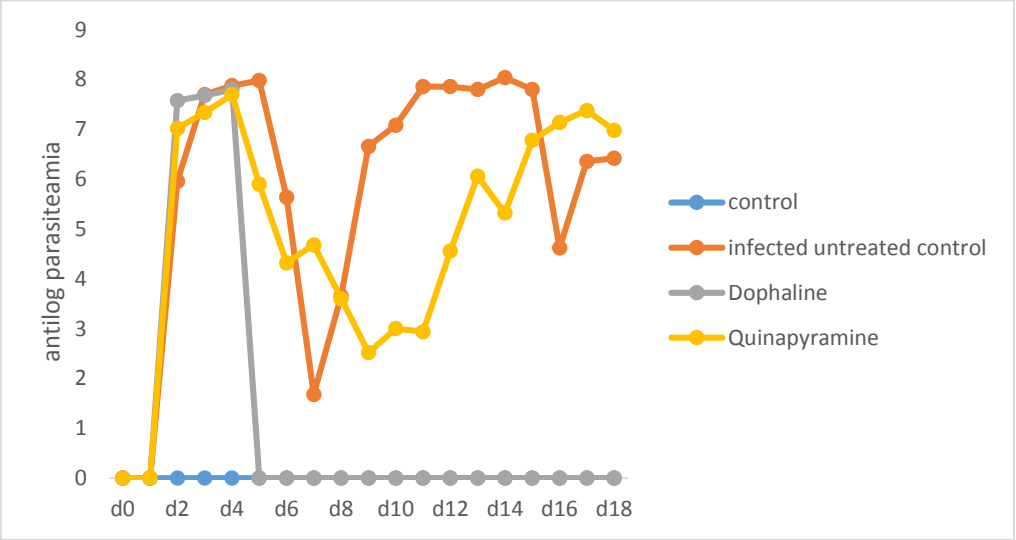

Figure 1 . ***Mean level of parasitaemia in rats infected with* T.evansi *and treated with Diminazene diaceturate and Quina pyramine sulphate***

Dophalline = Diminazene diaceturate

3.4 Package Cell Volume:

Severe anemia was significantly detected in rats infected with *T.evansi* (P>0.05) compared to normal control and other groups. There was no significant difference between the mean of Package Cell Volume in rats treated with Dophaline, Quina pyramine treated rats and normal control (P> 0.05).

Infected, Dophaline treated rats showed significant incrase in PCV compared with infected, Quina pyramine treated rats (P>0.05).

The Result was presented in Table (3), Fig (2).

Table 3 **.** ***PCV level of parasitaemia in rats infected with*** **T.evansi** ***and treated with Diminazene diaceturate and Quina pyramine sulphate***

Groups	**PCV % (Mean ± SE)**
Control	39.80 ± 1.16 ^{ab}
Infected untreated	27.40 ± 1.75 ^{c}
Diminezine diaceturate	40.00 ± 0.55^{a}
Quinapyramine sulphate	35.60 ± 1.94^{b}

a,b: means within the same column followed by different superscripts are significantly different ($p< 0.05$).

Chapter four

4. Discussion

The present study was achieved to investigate the efficacy of quinapyramine sulphate in experimentally infected rats.

The study showed remarkable level of parasitaemia on day two, this is in agreement with the result of (Mohamed, A. E., 2016)

Infected rats with Diminazene diaceturate showed significant decrease in all *T. evansi* parasites from the blood from the 1st day post treatment till the end of the experiments. The disappearance of parasitaemia continued to the end of experiment compared to infected untreated group. Infected quinapyramine sulphate treated rats showed wavy wave of parasitaemia from the 5th day post treatment. Quinapyramine sulphate group displayed insignificant gradual decrease in parasitaemia on day 5 post treatment compared to infected untreated group. However the level of parasitaemia in this group continued to increase until reaching the highest level at day 13 post treatment. The level of parasitaemia was found to be comparable with the infected untreated group. This is in the agreement with the result of El Rayah et al., (1999) who reported that all Sudanese isolates of *T. evansi* were found to be highly resistant to quinapyramine compound in experimentally infected mice. The study was also disagreed with (Delespaux and de Koning 2007) who stated that Diminzene diaceturate and isometamidium are the most trypanocides drugs have highly resistance against *T. evansi* parasite.

Infected untreated rats with *T.evansi* showed severe anemia compared to normal control and other groups. There was no significant difference between the mean of Package Cell Volume in rats treated with Dophaline, Quinapyramine treated rats and normal control. Infected, Dophaline treated rats showed significant increase in PCV compared with infected, Quinapyramine treated rats.

Conclusion and Recommendation

According to the given results of this study

- Diminazene diaceturate as antitrypanosomal drug exert high efficacy against *T. evansi*.
- Quinapyramine sulphate completely failed to treat *T. evansi* (isolated from camels) in rats.

It is recommended that using synergistic combinations between Quinapyramine sulphate and other antitrypanosomal agents. Investigate alternative antitrypanosomal agents of plant origin and use other drugs not established in Sudan.

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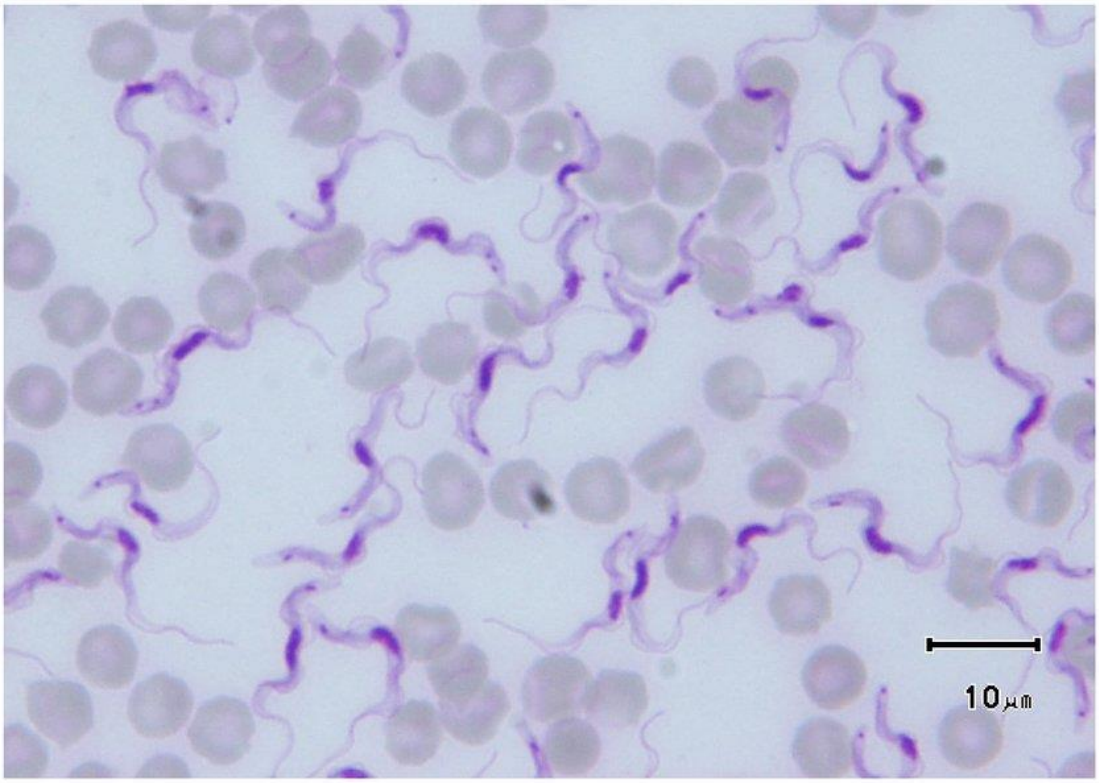
Appendix

Wistar albino rats used as an experimental animals

The **4 groups of rats** housed in laboratory animal house



T. evansi on a Giemsa-stained blood smear from an experimentally infected rat (credit, Marc Desquesnes). Size of the parasite (15–34 µm), small (diameter 0.6–0.7 µm) and subterminal kinetoplast, sharp posterior end, central nucleus, large undulating membrane, and free flagellum are the most striking features of the slender forms of the sub-genus Trypanozoon.

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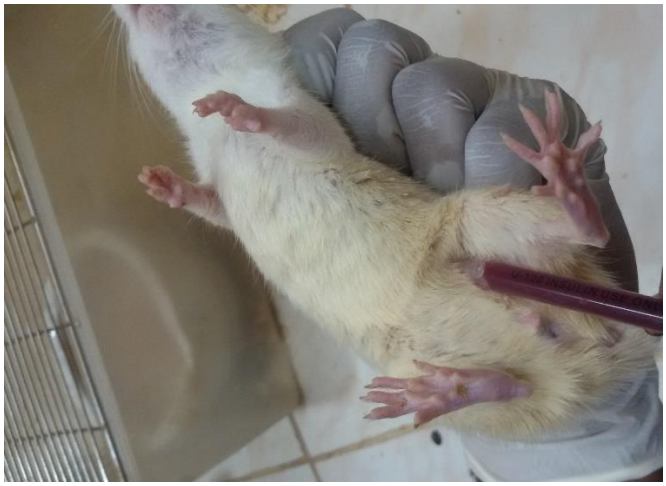
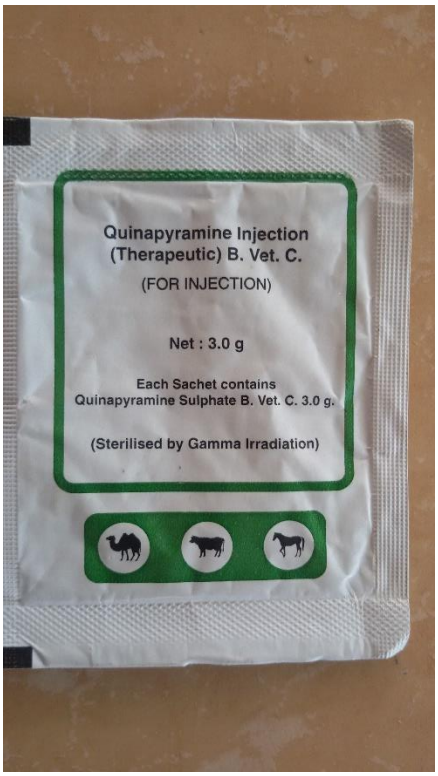


Intraperitoneal injection of **infected blood** with ***T. evansi***

The Standared drug Diminazine diacteurate



The test drug Quinapyramine sulphate