

بسم الله الرحمن الرحيم

Comparison of the pathogenicity of Quinapyramine Sulphate drug-resistant and drug-susceptible *Trypanosoma evansi* in experimentally infected mice.

مقارنة إمرضية طفيليات التريبانوسوما إيفانساى المقاومة لعقار سلفات

الكوينابايرامين مع تلك الغير مقاومة للعقار تجريبياً في الفئران.

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Dedication

To my wife and children for their understanding, encouragement and tireless support ,To my mother and father For their love and patience.

To my sisters and brother with love and gratitude.

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Abstract

The pathogenicity of two stabilates of *Trypanosoma evansi* isolated from camels in El-Gedarif and North Kordofan States, to study and evaluate the biochemical and microscopical pathology of camel strains of *T. evansi* in vivo in experimentally infected Albino mice. Total of 36 adult male outbred Albino mice, weighing between 133-137g were used in this study. The mice were divided into 6 groups of 6 mice per group (A, B, C, D, E and F). Group A and B were infected, intraperitoneally, with *T. evansi*, drug-resistant, while group C and D were infected with *T. evansi*, drug-susceptible, with 1×10^4 trypanosome for the inoculum. Group B and D were given quinapyramine sulphate (20 mg/Kg bwt) after parasitaemia was evident. Group E and F were left as healthy uninfected controls for the two stabilates. (When parasite counts were one or more parasites per field, counting in haemocytometer was used for exact number of parasite per cubic millimeter using the method of Paris *et al.* 1982). Parasites from tail blood were first fixed, stained and diluted in trypanosome diluting reagent. The parasites were diluted to the level that can easily be counted in WBC counting chamber in the haemocytometer and the total number was expressed as log₁₀ number of parasites per ml of blood. The presence and degree of parasitaemia were determined daily for each rat by examining tail blood. There was significant reduction in serum glucose, potassium and phosphorus as well as significant increase in Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT), creatinine, albumin, calcium, total protein, urea, and cholesterol in groups A, B and C. The different tissue samples were collected in 10% neutral buffered formal saline and were used to study the histopathological changes. Microscopically, brain revealed acute congestion of meningeal capillaries,

perivascular oedema, neuroncrosis (vaculation), gliosis and trypanomastigotes in dilated capillaries. The lung revealed oedema, congestion, multifocal alveolar emphysema, hyperplasia of the peribronchiolar lymphoid tissues and haemorrhages. The spleen showed extensive haemorrhages, haemosiderosis and aggregation of histiocytes resulting in multinuclear giant cell formation. The kidneys showed acute congestion of the glomerular tuft. All tissues obtained showed exactly the same histopathological changes. No significant histopathological alterations were observed in the liver and heart. The most consistent histopathological changes were seen in the brain, lungs, spleen and kidneys. These changes were consistent with trypanosome infection and were confirmed by presence of trypanosomes in most of the tissue sections examined.

Key words: Parasitemia, Histopathological, biochemical, El-Gedarif, Kordofan States, Sudan.

ملخص الاطروحة

تم استخدام ستة وثلاثين من ذكور فئران التجارب البيضاء التي يتراوح وزنها بين 133-137 جرام في هذه الدراسة وقسمت إلى ستة مجموعات (A, B, C, D, E and F) كل مجموعة تحتوي علي ستة فئران. أصيبت المجموعة A و B بالترانسوما إفانساي المعزولة من منطقة الشواك بولاية القضارف شرق السودان، بينما أصيبت المجموعة C و D بالترانسوما إفانساي المعزولة من منطقة أمبادر بولاية شمال كردفان بغرب السودان. تم حقن الفئران في البريتون بجرعة قدرها 104×1 من الترانسوما تقريباً بينما تم علاج المجموعه B و D عند ظهور الطفيل بالدم بعقار الكوينابيرامين سلفيت بجرعة 20 ملجم/كج من وزن الحيوان وتركت المجموعة E و F من دون إصابة ولا علاج كضابط طبيعي . تم استخدام طريقة باريس وآخرون (Paris et al. (1982 للكشف عن طفيل الترانسوما بالدم وذلك بأخذ نقطة دم من زيل الفأر ووضعها في شريحة وتغطيتها بغطاء شريحة وفحصها تحت المجهر وملاحظة وجود الطفيل. أيضا تم عد طفيل الترانسوما إفانساي وذلك بأخذ نقطة من الدم من الذيل وتنبيتها ثم صبغها وتخفيفها بواسطة محلول تخفيف الترانسوما ليسهل عدها في شريحة عد كريات الدم البيضاء ومن ثم وضع نتائج العد في شكل لوغريثمات.

كان هناك انخفاض كبير في مستوى السكر والفسفور والبوتاسيوم في الدم بينما هنالك زيادة كبيرة في الغلوتامات أوكسالوأسيتات ناقلة (GOT)، الغلوتامات بيروفات ناقلة (GPT) والبروتين الكلي والكرياتين والألبومين والكالسيوم واليوريا والكولسترول في المجموعات C , B , A و تم جمع عينات الأنسجة المختلفة وحفظها في محلول الفورمول سلاين 10٪ لدراسة التغيرات التشريحية المرضية التي قد تحدث في هذه الأنسجة بعد قراءتها مجهريا. تبين وجود إزدحام شديد في الشعيرات الدموية السحائية بالدماغ ووذمة حول الأوعية الدموية وتتكس في العصبونات ووجود أطوار الترانسوماستيقوت *trypomastigotes* في الشعيرات الدموية المتوسعة. كشفت الدراسة وجود وذمة واحتقان وانتفاخ في الأسناخ الرئوية ووجود البؤر المتعددة وحدوث تضخم في الأنسجة اللمفاوية بالقصبيات وحدوث أنزفة. أظهر الطحال نزف في نطاق واسع وتلون دموي ووجود الخلايا العملاقة بسبب تجمع الأنسجة متعددة الأنوية ووجود احتقان حاد في الكبيبات الكلوية. أظهرت جميع الأنسجة نفس التغيرات المرضية في المجموعتين. أما بالنسبة للكبد والقلب فلم تظهر فيهما أي تغيرات نسيجية مرضية واضحة. أما جميع التغيرات المرضية الأكثر وضوحا فكانت في الدماغ والرئتين والطحال والكلي وقد إتفقت هذه التغيرات مع عدوى المتقيبات في الدراسات السابقة. وقد تم تصميم هذه الدراسة لتقييم أمراض الكيمياء الحيوية والتغيرات النسيجية

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Chapter One

Introduction

1.1 Definition of *Trypanosoma evansi*

Trypanosoma evansi is a widely-distributed haemoflagellate of veterinary importance that infects a variety of large-mammals including horses, mules, camels, buffalo, cattle and deer. It causes epidemics of a disease called *surra*, which is of great economic importance in Africa, Asia and South America, where thousands of animals die from *T. evansi* infection each year. The infection is mechanically transmitted by blood-sucking insects of the genera *Tabanus*, *Stomoxys*, *Atylotus* and *Lyperosia* (Hoare, 1972; Stephen, 1986).

1.2 Morphology and ultrastructure

T. evansi cannot be distinguished from the slender forms of *T. brucei brucei* or the human pathogenic sub species *T. brucei rhodesiense* and *T. brucei gambiense*. Although pleomorphism may appear to some extent, generating stumpy and intermediate forms in some strains, *T. evansi* is generally monomorphic, assuming a long slender form. Strains from different geographical areas and various host sources are morphologically indistinguishable (Hoare, 1972; Stephen, 1986). The trypanosomes measure 14-33 μm in length with a width of 1.5-2.2 μm . They possess a free flagellum and a small sub-terminal kinetoplast, Dyskinetoplastic forms, in which the circular kinetoplast DNA (kDNA) is absent, are found in wild strains as a result of mutation, or after treatment with trypanocides such as diminazene, prothidium (Hajduk, 1978). Some dyes such as ethidium bromide can also cause the appearance of a high percentage of dyskinetoplastic forms. Dyskinetoplastic forms of *T. evansi* were also reported to appear after long term in vitro cultivation (Zweygarth *et al.*, 1990).

1.3 Susceptibility of mammalian hosts

T. evansi has a wide range of hosts and is pathogenic for most domestic, wild and laboratory mammals. However, equines, camels, dogs, deer and Asian elephants are more frequently found infected by this trypanosome species than buffalo and cattle (Hoare, 1972; Lun *et al.*, 1993). Infection in pigs can occur, but is either asymptomatic or associated with only light clinical signs. Goats experimentally infected with *T. evansi* showed chronic infection even with CNS involvement (Zweygarth, 1992).

1.4 Route of transmission

Many blood-sucking insects, especially horseflies (*Tabanus spp.*) and stableflies (*Stomoxys spp.*) can mechanically transmit *T. evansi* from one infected host to another, however, their role as vectors may vary under different conditions. For example, horseflies and stableflies are the most efficient vectors for transmission of this parasite in China and in Indonesia (Luckins, 1988; Lun *et al.*, 1993). 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 co-exist. In general, the shorter the 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 (Hoare, 1972; Luckins, 1988). 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 (Hoare, 1965). Besides mechanical transmission by insects and vampire bats, *T. evansi* can be directly transmitted through milk or during coitus (Wang, 1988). In both Eastern and Western Hemispheres, canids (wolves and foxes) are believed to become infected by eating freshly-killed infected animals (Woo, 1977). Developmental stages were

not observed in any of the vectors mentioned above. A procyclic or insect stage does not exist in *T. evansi*, a finding which has been explained by the lack of maxicircles in the kinetoplast DNA of this parasite (Borst *et al.*, 1987).

1.5 Pathogenicity

T. evansi is pathogenic to most domestic and many wild mammals, but its effect on the host varies according to the virulence of the strain of trypanosome, the species of the host, non-specific factors affecting the animal such as other concurrent infections, general stress and the local epizootiological conditions (Hoare, 1972).

In camels, the disease is manifested by elevation of body temperature which is directly associated with parasitaemia. Milder cases develop recurrent episodes of fever. Anaemia appears to be a major component of the pathology of surra. It is mainly macrocytic and hypochromic type of anaemia (Jatkar and Purohit, 1971). Infected animals show progressive anaemia, marked depression, dullness, loss of condition, and often rapid death. Other clinical manifestations include progressive emaciation, oedema in the dependent parts of the body, corneal opacity, weight loss and reduced draught power (Luckins, 1998). Nervous signs such as incoordination, trembling and sudden collapse in severely stressed and or worked animals (Manuel, 1998) appear to be the results of brain tissue disturbance or damage by the parasites. Evidence of *Trypanosoma evansi* being found in the cerebrospinal fluid has been presented by Rottcher *et al.* (1987).

T. evansi, in most cases produces a chronic wasting disease with anaemia as the major outcome. Dysfunctions of many vital organs were reported (Anosa, 1988) and disturbances of blood chemistry and enzymes were reported by many authors (Boid, 1980). Fifteen stocks of *T. evansi* isolated in Sudan were compared using isoenzyme banding patterns. Differences

between the stocks were found but, in overall, they formed a homogeneous group (Boid, 1988).

1.6 Diagnosis and treatment

Diagnosis of *T. evansi* is based on demonstrating either the parasite or parasite antigens, or antibodies directed against the trypanosomes. For the direct demonstration of the parasite, the techniques used for the diagnosis of sleeping sickness or *Nagana* can be employed: (1) haematocrit centrifugation, (2) mini anion-exchange chromatography with DEAE-cellulose, (3) inoculation of laboratory mice. 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* (Diall *et al.*, 1994). A simple latex agglutination test for the detection of *T. evansi* antigens has been reported (Nantulya, 1994) as well as antigen ELISAs to detect infections in horses in Argentina (Monzon *et al.*, 1995) or infections in buffalo and horses or camels, respectively, in India (Singh *et al.*, 1995; Pathak *et al.*, 1993). A highly sensitive method to detect single parasites is the PCR-based amplification of trypanosomal DNA (Wuyts *et al.*, 1994). 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 (Raynaud *et al.*, 1989) and its efficacy has been demonstrated in camels, cattle, goats, pigs and water buffaloes (Lun *et al.*, 1991; Zweygarth, 1992; Musa *et al.*, 1994; Payne *et al.*, 1994). 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 *T. evansi* strains have been reported from various places, such as the Sudan (Boid *et al.*, 1989). Resistance of *T. evansi* to suramin and also

to diminazene and cymelarsan could be induced in mice in the laboratory, indicating that in-appropriate use of drugs may lead to a resistance in the field (Zhang *et al.*, 1993). Since in-vitro assays to determine drug sensitivity of *T. evansi* isolates are available (Kaminsky and Zweygarth, 1989; Zhang *et al.*, 1992; Brun and Lun, 1994), drug resistance monitoring can be done for areas where it 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 (Zhang *et al.*, 1992; Brun and Lun, 1994).

1.7 Biochemical and molecular characteristics

Isoenzyme analysis has widely been used to group morphologically similar or identical forms in a variety of parasitic protozoa, especially among the trypanosomes (Gibson *et al.*, 1980; Boid, 1988; Lun *et al.*, 1992a; Stevens *et al.*, 1992). Analysis of small or large subunit ribosomal RNA sequences (Johnson and Baverstock, 1989; Qu *et al.*, 1990; Hide and Tait, 1991), restriction fragment length polymorphisms (RFLPs) in DNA (Borst *et al.*, 1981; Gibson *et al.*, 1985; Paindavoine *et al.*, 1986; Dietrich *et al.*, 1990; Lun *et al.*, 1992b; Zhang and Baltz, 1994), ribosomal DNA restriction (Camargo *et al.*, 1992), the polymorphisms of PCR amplification products (Artama *et al.*, 1992; Dirie *et al.*, 1993) and molecular karyotypes (Lun *et al.*, 1992a; Waitumbi *et al.*, 1994; Waitumbi and Young, 1994) have been successfully used in distinguishing morphologically similar protozoa. Results from isoenzyme analysis of *T. evansi* strains isolated from Asia, West Africa and South America indicated that the majority of *T. evansi* strains worldwide have a single origin (Gibson *et al.*, 1980; Boid, 1988; Stevens *et al.*, 1989).

1.8 The overall objective

To evaluate the differences in pathogenicity (if any) induced by drug-resistant (Eastern Sudan) as opposed to drug-susceptible (North Kordofan) isolates of *Trypanosoma evansi* in experimentally infected rats.

1.8.1 Specific objectives

- 1) To compare the levels of parasitaemia and the number of serodemes expressed and the effect of treatment on levels of parasitaemia of the two isolates in infected mice.
- 2) To compare the pathogenicity of drug-resistant and drug-susceptible *T. evansi* isolated from Showak (Gedarif State) and Umbadir (North Kordofan State) in mice.
- 3) To evaluate the changes in blood chemistry and electrolytes resulted from infections induced by drug-resistant and drug-susceptible *T. evansi* isolates.
- 4) To compare the degree of tissue destruction between the two stocks as expressed by the levels of kidney and liver enzymes released in the serum.
- 5) To evaluate the gross lesions and histopathological changes produced by the two isolates of *T. evansi* in mice.
- 6) To determine the phylogenetic relatedness of the two isolates of *T. evansi* and relate it to the known phylogenies of Asia, Africa and South America using standard molecular phylogenetic assays.

The present study showed in chapter one (introduction), chapter two (literature review), chapter three (materials and method), chapter four (results), chapter five (discussion), conclusion, recommendations, references and appendixes.

Chapter Two

Literature Review

Trypanosoma evansi is a widely distributed flagellate protozoa that causes a disease called *surra* in domestic animals and is transmitted mechanically by biting flies such as *Tabanus* and *Stomoxys spp.* (Lun and Desser 1995; Reid 2002; Sumba *et al.*, 1998; Otte and Abuabara, 1991). The disease causes significant morbidity and mortality in camels in the Sudan, which has a population of over 3 million camels (Omer *et al.*, 2004). Other species of trypanosomes, e.g. *Trypanosoma congolense*, *Trypanosoma brucei* and *Trypanosoma vivax* have also been isolated from camels in Sudan, but their role in camel trypanosomosis is insignificant (Mahmoud and Gray, 1980; Elamin *et al.*, 1999). Natural and experimental camel trypanosomosis have been described in different parts of the world (Dia *et al.*, 1997; Al-Rawashdeh *et al.*, 2000; Pacholek *et al.*, 2001; Njiru *et al.*, 2002). Severe outbreaks had occurred in different parts of the world where several thousands of animals died in the 1970s. In 1994 and 1995, for instance, in Pantamal, Brazil, severe incidences had been well documented (Luckins, 1998). These epidemics posed a major constraint to camel productivity, given their importance as a source of meat and milk, wool, hair, skin and hides in addition to their use in transportation and as a draught power. Further, they are important foreign currency source through their exportation, live or as by-products (Elamin *et al.*, 1999). *Trypanosoma evansi* is thought to be 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 (Borst *et al.*, 1987; Lai *et al.*, 2008). When this phenomenon occurred is not known, and some authors even recently suggested that it might have occurred in several instances (Lai *et al.*, 2008).

Camel Trypanosomosis has been reported to be transmitted mechanically by a number of species of haematophagous biting flies including the genera *Tabanus*, *Stomoxys*, *Lyprosia* and *Haematobia* (Diptera) (Rutter, 1967; Scott, 1973). In the Sudan tabanid flies play an important role in the mechanical transmission of animal Trypanosomosis (Karib, 1961). They were also considered as major cause of the seasonal migration of cattle from the South to North during the rainy season (Kheir *et al.*, 1995). More than 20 different species of *Tabanus* have been shown experimentally to transmit *Trypanosoma evansi* (Luckins, 1998). Furthermore, surveys of *Tabanus* in the various tropical areas have shown a definite correlation between the seasonal outbreaks of *Trypanosoma evansi* infections and the increase in number of *Tabanus* during the rains (Mahmoud and Gray, 1980; Njiru *et al.*, 2002). Thus, rainfall, suitable moisture-retaining clay, soil and surface water pools also support, in addition to the *tabanus* multiplication, the development of suitable camel browsing conditions, where *Acacia senegal* shrubs grow in abundance. The prevalence of some *Tabanus spp.* all year round ensures that transmission of the parasite occurs wherever reservoir hosts, vectors and susceptible hosts co-exist. This finding may explain the sporadic occurrence of the disease during the dry season and outbreaks domination during the rainy season (Njiru *et al.*, 2002). However, the efficiency of the different flies to transmit *Trypanosoma evansi* appears to vary in different geographic conditions and is also dependent on the interval between two successive feeds and intensity of the fly challenge (Luckins, 1998). The transmission of *T. evansi* can only occur if the donor host exhibits high parasitaemia. This is because *T. evansi* is mechanically transmitted by biting insects (due to the very small amount of blood transferred from one host to another) (Dia and Desquesnes, 2007).

In Africa and the Middle East, *T. evansi* is responsible for an acute or chronic disease principally found in camels, horses and dogs in the north of the tsetse belt (Dia, 1997). In camels kept close to the tsetse-fly belt, some cases of *T. brucei brucei* have been recorded. *T. congolense* infections are fatal to camels. Therefore, camels should not be allowed to enter the tsetse belt unless they are permanently protected; e.g. with the use of chemicals. Consequently, *T. evansi* is not found in the tsetse belt. Thus, the epidemiology of surra in Africa is mainly governed by camel infections. The latter are seasonal because the vectors activity is seasonal and the disease is expressed seasonally, at times when animals are exposed to stress from over work, food shortages, and/or insufficient or poor quality water (Dia, *et al.*, 1997). For example, in Mauritania, by using CATT and IFAT blood smears, it was shown that *T. evansi* infection was widely spread in the country, with an overall prevalence of 1.3% by parasitological detection. This level reached 18.4% to 31% with serological tests (Dia *et al.*, 2000). Other host species, such as goats, may be infected occasionally and could act as a reservoir. However, their impact was never demonstrated (Jacquiet *et al.*, 1993). In cattle, especially transhumant herds that spend part of the year within the tsetse belt and the rest of the year in the northern region, it is difficult to distinguish between infections due to *T. brucei* and *T. evansi*. The latter is probably rare because, even under experimental conditions, the infection of African cattle by *T. evansi* proved to be difficult as a consequence of their low susceptibility (Dia and Desquesnes, 2007). In Africa, only camels and horses may be a source for this type of transmission. Hosts that have a low susceptibility, such as cattle and goats, are likely to constitute dead ends, even if they may occasionally be infected when come close to infected camels or horses (Dia and Desquesnes, 2007). Finally, given that camels and horse can not enter the tsetse belt without being at risk from Nagana, which is fatal to both

hosts, and because other hosts that could be infected by *T. evansi* do not exhibit sufficient parasitaemia to play an important role in surra's epidemiology, there is a reciprocal exclusion of Nagana in the southern territory (among tsetse flies, livestock and wild animals) and surra, which is restricted to the northern region (among mechanical vectors and camels) (Dia and Desquesnes, 2007).

In the Middle East and towards Asia, the geographical distribution of *T. evansi* is closely related to that of camels and dromedaries (Hoare, 1972). However, no difference was observed in terms of the pathogenic effects of the parasite in this host species, which, like horses, are highly sensitive to the infection. In overall, surra affects mainly camels with acute and chronic infections that cause death. Infection is contracted during the rainy season when there is a peak level of biting insects. Camels constitute the main reservoir of *T. evansi* in this region (Hoare, 1972).

In Latin America, the disease is called *Mal de Caderas* (Brazil), *Murrina* (Central America), or *Der-rengadera* (Venezuela) (Wells, 1984). *T. evansi* is principally pathogenic in horses and induces outbreaks with very high morbidity and mortality. It also affects buffaloes (*Bubalus bubalis*). In Venezuela, although infection in buffaloes by *T. evansi* showed significant signs such as spleen, liver and glandular enlargement, together with lymphoproliferation, the economic impact of infections has not been assessed (García *et al.*, 2006).

Trypanosoma evansi regularly affects dogs (especially hunting dogs) and even cats. In both cases, the disease is usually fatal. In Latin American cattle, sheep, goats, and pigs, *T. evansi* is generally considered as a low pathogenic agent (Desquesnes, 2004). It is regularly found in a wide range of wild reservoirs, including capybaras (*Hydrochoerus hydrochaeris*), which is the most well known, together with white tail deer (*Odocoileus virginianus chiriquensis*), brocket deer (*Mazama satorii*), coati (*Nasua*

nasua), vampire bats (*Desmodus rotundus*), wild pigs (*Tayassu tajacu*), Guinea pig (*Cavia porcellus*), wild dog (*Canis azarae*) and ocelot (*Felis pardalis*). Llamas are also receptive to the disease and infected animals have been found, although little is known about its impact (Desquesnes, 2004). There is no obvious link between wild and domestic fauna. In some places, the prevalence of infection may be very high in capybara and coati, while it remains low in horses (Herrera *et al.*, 2004).

In French Guyana, the parasite has never been found in livestock, including horses, but it was first described in 1995 in a hunting dog, which was probably infected by wild fauna when hunting in the forest (Desquesnes, 2004). Surra remains a major disease in Latin America, especially, because horses (sensitive host) are used for herding cattle (reservoir) in extensive conditions in Venezuela and Brazil, for example. Overall, in Latin America, surra is predominantly a disease that affects horses. However, a large range of wild and domestic mammals can act as a reservoir. In most cases, farmers use chemoprophylactic drugs regularly to protect horses against *T. evansi* (isometamidium or quinapyramine). This treatment ensures that they stay alive and efficient for work. As a result, two groups of livestock are kept in close contact: a low susceptible reservoir made up of bovines and a highly susceptible host made up of horses under chemoprophylaxis (Desquesnes, 2004).

In Asia, the geographical distribution of *T. evansi* is spreading steadily. It is present in large areas in India, China, and Russia (Lun *et al.*, 1993; Singh *et al.*, 2004). It is sometimes difficult to distinguish it from *T. equiperdum* (Zablotskij *et al.*, 2003) . It is present in *Camelus bactrianus* and horses in Mongolia, with low prevalence. It is more frequent in Uzbekistan and Kazakhstan. In South East Asia, it affects principally horses, dogs and buffaloes (*Bubalus bubalis*), as well as cattle, pigs and deer. It has been described in tigers in India (Bhaskararao *et al.*, 1995) as

well as in Thai elephants (Tuntasuvan *et al.*, 1997). In the water buffalo, *T. evansi* causes production losses, abortion and early calf mortality. It also has immuno-suppressing effects, which decrease the efficacy of some vaccines (especially the vaccine for hemorrhagic septicaemia). In bovines, its pathogenicity in Asia is superior to that of African and American strains. We do not know whether the difference is due to the presence of more sensitive dairy breeds in Asia, or if the local populations of *T. evansi* are more pathogenic to cattle, or both. In India, surra is present all over the country in various hosts, such as cattle, buffaloes, camels, donkeys, dogs, and horses (Ravindran *et al.*, 2008).

A recent survey carried out on horses showed a maximum seroprevalence (20%) for *T. evansi* infection in Uttar Pradesh. There was an overall seroprevalence of 11% in north and north-western regions of India, which confirmed that surra is endemic in equids in these areas (Kumar *et al.*, 2013). In Thailand, a study on seroprevalence carried out in dairy cattle demonstrated the presence of the parasite in most parts of the country. The mean seroprevalence was 8%, ranging from 0 to 100% at farm level and 25% of dairy cattle are exposed to the infection (Desquesnes *et al.*, 2009). Similar studies conducted on buffaloes and beef cattle showed seroprevalence of 10 – 12% . Molecular evidence of *T. evansi* was also obtained in various wild rodents (Jittapalapong *et al.*, 2008; Milocco *et al.*, 2013). However, their role in the epidemiology of the disease is not known. In horses, several outbreaks are recorded every year and are frequently fatal. Indeed, serological studies show very low evidence of positive animals; in other words, there are few survivors after the outbreaks (Cherdchutham *et al.*, 2012 ; Kongkaewa *et al.*, 2012). Elephants are affected by surra. Cases are reported rarely but regularly. The disease may be fatal or it develops into a chronic or subclinical evolution, depending on the case (Arjkumpa *et al.*, 2012). Surra outbreaks occur seasonally and are

generally linked to the activity of biting flies. In North east Thailand, seasonal occurrence is observed at the beginning of the rainy season (June-July) and in winter (October-November) (Kashemsant *et al.*, 1989). Bovines (cattle and buffaloes) exhibit moderate signs and impact. However, they constitute a permanent threat to themselves and horses, which may die or survive under permanent chemoprophylaxis. In the Philippines, over the past decade, the number and severity of surra outbreaks have increased dramatically. The highest mortality is in horses, carabao (Asian water buffalo) and cattle. As a result, the Philippine government regarded surra as the second most important livestock disease (Reid, 2002). Indeed, surra has emerged as the most important cause of livestock mortality in the Philippines, prompting the government to implement a national control strategy. In Indonesia, the disease appears in sporadic outbreaks, mainly in horses, buffaloes, cattle and dogs, although it is also present in sheep, goats, pigs, and wild animals (Reid, 2002; Payne *et al.*, 1991). The parasite was found throughout most of the archipelago. Its regular occurrence suggests the existence of enzootic stability, including an efficient reservoir (Payne *et al.*, 1992; Payne *et al.*, 1991). However, an update of information is required.

In Vietnam, Laos and Cambodia, the disease occurred especially in horses, buffaloes and cattle, although it was given little attention. Serological surveys demonstrated the presence of infection in all the areas investigated (Mohamad *et al.*, 2004). Limited means are available for carrying out studies on surra since priority is given to other diseases in these countries. Hence, the situation is not well documented. Similarly, in Malaysia, although the disease has been known for years, a national survey has not yet been organized to evaluate its impact. Surra is regularly detected in horses, deer, pigs, buffaloes, cattle and, rarely, in dogs. It was also reported in Sumatran rhinoceroses (Mohamad *et al.*, 2004).

In Malaysia, diagnosis is routinely carried out using a mouse inoculation test and buffy coat examination. Thin blood smears are also conducted in regional laboratories. Prophylactic treatment is administered to livestock in high-risk areas where cattle and buffaloes live in close proximity to pigs or horses (Cheah *et al.*, 1999; Arunasalam *et al.*, 1995). In domesticated deer, the infection is observed regularly. Fulminating parasitaemia is detected when the animals become weak and recumbent. Nervous symptoms are not clearly evident; however, fatality is most often observed during the outbreaks. *Trypanosoma evansi* is not present in Australia, but it may spread eastward from Indonesia to Papua New Guinea and then Australia (Reid and Copeman, 2000).

Overall in Asia, surra is mainly a disease of horses and buffaloes. It benefits from a large reservoir made up of buffaloes, cattle, deer and possibly, wild animals such as rodents. On cattle farms, little attention is given to the disease, even though it may cause serious economic losses, via abortion, weight loss and immunosuppressive effects. An evaluation of the economic impact is needed to determine whether it would be profitable to eliminate the infection. Horse breeders generally avoid close contact between buffaloes and horses to avoid the risk of infection, which is generally fatal and uncontrollable because of the limited efficacy of trypanocides (Tuntasuvan *et al.*, 2003a). When horses are bred in the same area as cattle or buffaloes, farmers regularly use chemoprophylactic drugs to protect horses against *T. evansi* (isometamidium or quinapyramine). Nonetheless, *T. evansi* remains a permanent threat to livestock throughout South-East Asia, with a decreasing gradient of impact for horses, buffaloes, dogs, cattle, deer, pigs, sheep and goats (Tuntasuvan *et al.*, 2003b).

Trypanosoma evansi can infect a variety of hosts and causes a species-specific pathology. The following descriptions are taken from the accounts of Mahmoud and Gray (1980) and Luckins (1998). In camels, the disease is

manifested by elevation of body temperature which is directly associated with parasitaemia. Infected animals show progressive anaemia, marked depression, dullness, loss of condition and often, rapid death. Anaemia was observed to be a major clinical finding in camel trypanosomosis in Morocco (Rami *et al.*, 2003). Milder cases develop recurrent episodes of fever. Some camels develop oedema in their dependent parts of the body, urticaria, plaques and petechial haemorrhages in serous membranes. Death finally ensues if untreated. However, some may harbour trypanosomes for 2-3 years; thus constituting reservoirs of infection to susceptible camels and other hosts. Other well documented field reports are death (Tuntasuvan *et al.*, 1997), abortion (Lohr *et al.*, 1986), weight loss, reduced draught power (Luckins, 1998) and nervous signs such as circling movement and trembling, unusual aggressiveness, running aimlessly and sudden collapse in severely stressed and over-worked animals (Manuel, 1998). At post mortem, necrotic foci in the liver and spleen as well as generalized lymphoid tissue hyperplasia are common in camels suffering from surra (Rottcher *et al.*, 1987).

The pathogenicity of *T. evansi* varies significantly among strains and in different animal species (De Menezes *et al.*, 2004; Queiroz *et al.*, 2000). *T. evansi* infection is usually regarded as a serious disease of horses and camel, causing high mortality, where as the disease is typically mild in other domestic animals (Luckins, 1988; Silva *et al.*, 1995).

Trypanosomiasis in camels occurs both in chronic and acute forms (Gutierrez *et al.*, 2000). The chronic form of the disease is most common and is likely to be associated with secondary infections due to immunosuppression (Njiru *et al.*, 2004). In regard to the disease pathology and pathogenesis, generally, anaemia is a major component of the pathology of surra and of African trypanosomosis. Anaemia in *Trypanosoma evansi* infections of camels is reportedly macrocytic and

hypochromic (Jatkar and Purohit, 1971). In the early phases of infection the anaemia is haemolytic and haemophagocytic. The mechanism(s) responsible for this increased erythrophagocytic activity are not fully understood. Several have been proposed, viz, immune complexes, expanded mononuclear phagocytic system per se, haemolytic factor produced by the trypanosome, fever and disseminated intravascular coagulation (FAO, 1979). In the late stages, anaemia continues to be a major factor, with probably additional causes. However, irrespective of the cause of anaemia the primary abnormality of function are the anoxic conditions created by the persistent anaemia. Following this are signs of dysfunction which appear in the various organs. An increase in cardiac output due to increases in stroke volume and heart rate; and a decrease in circulation time are obvious manifestations (Rottcher *et al.*, 1987). The central nervous system is reported to be most susceptible to anoxia with consequent development of cerebral anoxia. The marked depression observed in camel trypanosomosis is a mental state and is a manifestation of depression of cerebral cortical function in various degrees. Other nervous signs reported, such as circling movement, incoordination and dullness, appear to be the results of brain tissue disturbance or damage by the parasites. Evidence of *Trypanosoma evansi* being found in the cerebrospinal fluid has been presented (Rottcher *et al.*, 1987).

Humans were considered to be refractory to *T. evansi* infection (Vanhamme *et al.*, 2003); however, a case of human infection due to a lack of apolipoprotein (Apo) that lyses the trypanosoma was reported for the first time in 2005 in an Indian farmer (Joshi *et al.*, 2005). Apo1 is absorbed by the parasite by endocytosis and triggers the formation of anion selective pores in the lysosomal membrane, which induces uncontrolled osmotic swelling of this compartment and subsequently cell death (Perez-Morga *et al.*, 2005; Pays *et al.*, 2006; Vanhollebeke *et al.*, 2006).

In Sudan, *Trypanosoma evansi* infection is the most important disease of dromedary camels. Some work was carried out to evaluate a simple PCR-based technique for field diagnosis of *T. evansi* infection in camels from Eastern and Western regions of the Sudan (Nahla *et al.*, 2011). A representative number of 600 camels (*Camelus dromedarius*) from different areas of Gedarrif State (Eastern) and North Kordofan State (Western) were examined from May 2005 to July 2007 for *Trypanosoma evansi* infection. The tests used were parasitological (Wet Smear Film, WSF; Thin Smear Film, TSF and Buffy Coat, BC), serological (Card Agglutination Test/*T. evansi*, CATT), and DNA amplification by polymerase chain reaction (PCR) (Nahla *et al.*, 2011). The prevalence of *T. evansi* infection in camels was detected in 36 (out of 40), 100 (out of 210), 36, 22, 10 (Out of 600); by PCR, CATT, TSF, BC and WSF with sensitivity of 90%, 47.6%, 6%, 3.7% and 1.7%, respectively. PCR revealed a specific 200 bp band in positive samples. The intensity of PCR bands was variable in different test samples depending upon the level of infection in the test samples (Nahla *et al.*, 2011). The history of intermittent fever, emaciation, oedema, poor body condition significantly correlated with positive serological status in CATT as well as trypanosome DNA detection by PCR. As there were no previous studies in the Sudan on the molecular characterization of the parasite, this research was considered useful in formulating strategic control programmes (Nahla *et al.*, 2011).

In a study aimed to diagnose the natural infection of captive and free-living procyonids with *Trypanosoma evansi* in the states of Amapá and Pará, Brazil, from February 2012 to August 2013, whole blood samples and blood smears were obtained from 45 free-living procyonids and from nine procyonids kept in captivity in wildlife refuges and zoo-botanical parks in the states of Amapá and Pará. Whole blood samples were collected and kept at -20°C for the detection of *T. evansi* DNA by PCR

using the RoTat 1.2 forward and RoTat 1.2 reverse primers (Paulo *et al.*, 2016). In addition, the blood smears were processed and examined for the presence of trypomastigote forms of *T. evansi*. *Trypanosoma evansi* DNA was detected in 18.52% (10/54) of the procyonids, namely, in captive crab-eating raccoons and captive and free-living coatis in Pará State. No trypomastigote forms were observed in the blood smears. DNA from *T. evansi* was detected in *Procyon cancrivorus* and *Nasua nasua* in Pará State (Paulo *et al.*, 2016).

The most common tools are the Card Agglutination Test for *T. evansi* (CATT/*T. evansi*) (Bajyana and Hamers, 1988) and the ELISA *T. evansi* (Desquesnes *et al.*, 2009; Desquesnes *et al.*, 2008). CATT can detect IgM and, therefore, early infections, whereas ELISA is generally used to detect IgG, that is, established infections. Consequently, these tests are complementary and work well together. ELISA for *T. evansi* detection is quite robust, regardless of the host species. It provides the same range of sensitivity and specificity (90–95%) in the various host species investigated; for example, camels, cattle, buffalo and horses. The sensitivity of CATT *T. evansi* varies from one host to another. CATT seems to be highly sensitive in camels and horses, although it has a very low sensitivity in cattle (12%), even under experimental conditions (Desquesnes *et al.*, 2011). In Sudan, a few molecular studies have been carried out on *T. evansi* using isoenzyme characterizations (Boid, 1988) or on drug resistance of *T. evansi* (El Rayah *et al.*, 1999; El Rayah and El Malik, 2006). Parasite prevalence and infection pattern were also performed with varying estimates of prevalence: 5.4% by using parasitological examination and 31.3% with ELISA (Elamin *et al.*, 1999). The overall prevalence estimated by, using molecular epidemiological tools, ranged between 33.9 to 42.1% (Salim *et al.*, 2011).

Trypanosoma evansi is generally considered a mild pathogen in bovines. However, in Asia, acute and chronic signs have been observed in cattle, with high levels of parasitaemia, abortion and death. Investigations in Asian cattle are needed to better understand this epidemiological situation (Desquesnes *et al.*, 2009). To generate comparable data at a regional level, development and standardization of an antibody-enzyme linked immunosorbent assay for *T. evansi* (ELISA/*T. evansi*) was initiated and applied in an epidemiological survey carried out in dairy cattle in Thailand. A batch of 1979 samples was collected from dairy farms located throughout the country's four regions. Soluble *T. evansi* antigens initially produced in France were also produced in Thailand for comparison and technology transfer (Desquesnes *et al.*, 2009). Screening of 500 samples allowed to identify reference samples and to determine the cut-off value of the ELISA. Some of the seropositive animals was confirmed by PCR and were found in the four regions; in 12 out of 13 provinces, in 22 out of 31 districts, in 56 farms out of 222 (25%, 95%CI \pm 6%) and in 163 animals out of 1979 (8.2, 95%CI \pm 1.2%) (Desquesnes *et al.*, 2009). Estimated seroprevalence in 35 farms ranged between 1% and 30%, and in 21 farms it was over 30%. Approximately, 25% of the surveyed cattle were exposed to the infection, in various situations. A sub-sample of 160 sera was tested on both antigens. Wilcoxon's ($Z = 1.24$; $p = 0.22$) and McNemars's tests ($\text{CHI}^2 = 3.55$; $p = 0.09$) did not show any significant differences, showing that the locally produced antigen is suitable for further evaluation in the surrounding countries. Use of this standardized serological method will broaden knowledge of the prevalence and impact of the disease at the regional level in South-East Asia. Further validation of this ELISA will be necessary in other host species such as buffalo, horse and pig (Desquesnes *et al.*, 2009).

In a study aimed to evaluate the ameliorative effects of Isometamidium chloride (standard trypanocide) and Buparvaquone (anti-theilerial drug) treatments on the clinical signs of *T. evansi* infection in donkeys as part of study on the efficacies of the drugs against *T. evansi* infection, twenty four apparently healthy donkeys were used for the experiment (Garba *et al.*, 2015). The animals were housed, fed on hay and concentrate feed and water were provided *adlibitum*. Animals were identified with neck-tags and grouped into 4 groups namely; A1, A2, A3 (*T. evansi*-infected groups) and B (control) of 6 animals each (3 males and 3 females) at random. Two milliliters of buffered, parasitaemic Wister rat blood containing 2×10^6 of *T. evansi* (Sokoto isolate) were used to infect each of all donkeys in the (A) category through jugular vein. On day 28 post-infection, groups A2 and A3 animals were treated with Isometamidium chloride and Buparvaquone, respectively. Groups A1 and B remained as infected-untreated and uninfected-untreated, respectively (Garba *et al.*, 2015). Animals were monitored and evaluated post-infection and post-treatment for clinical signs including vital parameters and body weight changes. The effects of treatments on the observed-parameters were evaluated. Result showed that *T. evansi* infection in donkeys is predominantly a chronic disease, with an incubation period of 3-7 days. Isometamidium chloride treated group showed greater reduction in prevalence of signs than buparvaquone treated group which did not differ much from the un-treated group. It was concluded that *Trypanosoma evansi* infection in donkeys is a chronic disease and treatment with Isometamidium chloride, ameliorates the clinical signs while buparvaquone does not (Garba *et al.*, 2015).

In a study using a PCR approach based on the sequence of maxicircle kinetoplast DNA (kDNA) of *Trypanosoma brucei* to distinguish *T. brucei*/*T. equiperdum* from *T. evansi* and to evaluate its diagnostic use for their detection in blood samples (Li *et al.*, 2006), primers derived from the

sequence of the maxicircle kDNA of *T. brucei*, encoding the NADH dehydrogenase subunit 5 (*nad5*) gene, were used to test the PCR–amplification from *T. brucei* (including *T. b. brucei* and *T. b. rhodesiense*), *T. equiperdium*, *T. evansi*, *T. vivax* and *T. congolense* (Li *et al.*, 2006) . A primer pair to a nuclear DNA region incorporated into a separate PCR was employed to control for the presence of amplifiable genomic DNA (representing the subgenus Trypanozoon) in each sample subjected to PCR (Li *et al.*, 2006). Products of 395bp were amplified from all *T. brucei* and *T. equiperdium* samples tested using the *nad5*-PCR, but not from *T. evansi* DNA samples or any of the control samples representing *T. vivax*, *T. congolense*, or host. The current PCR approach allows the rapid differentiation of *T. brucei*/*T. equiperdium* from *T. evansi* and can detect the equivalent of 20-25 cells of *T. brucei* or *T. equiperdium* in purified genomic DNA or infected blood samples (Li *et al.*, 2006).

A wide range of DNA-based techniques have been described for trypanosome detection, including hybridization using repetitive DNA sequences, random amplification of polymorphic DNA (RAPD), polymerase chain reaction (PCR) and kinetoplast DNA (kDNA) minicircle analysis, as reviewed by (Boid *et al.*, 1996). However, there are differences in the sensitivity claimed for these assays in the literature. Nonetheless, the polymerase chain reaction (PCR) has been widely used as a highly sensitive and specific assay for detection of trypanosomes (Moser *et al.*, 1980; Masiga and Nyang’ao, 2001). Comparative studies have led to the recommendation of TBR primers as the most sensitive primers for detecting *T. evansi* (Masiga *et al.*, 1992; Pruvot *et al.*, 2010) and the Phenol-Chloroform method as the most sensitive DNA preparation method (Sambrook and Russell, 2001). A combination of these methods provided a sensitivity of around 5–10 trypanosomes/ml of blood (or other fluid). In addition to the use of parasitological or molecular tools for detecting *T.*

evansi infection, serological tests are quite useful as they can be applied to investigations at herd or population level (prevalence or incidence studies), follow-up (seasonal or inter-annual variations), or control method assessment (trypanocide treatment or vector control) (Pruvot *et al.*, 2013). Many molecular markers are used to detect, differentiate and study trypanosome species. The rRNA internal transcribed spacer one (ITS1) and internal transcribed spacer two (ITS2), which are separated by the 5.8S gene and flanked by the small subunit and large subunit rRNA genes in most eukaryotes are subject to higher evolutionary rates leading to greater variability in both nucleotide sequence and its length (Hillis and Dixon, 1991). The ITS1 and ITS2 spacers have been found to be reliably valuable in more discrete phylogenetic separation of closely related species including piroplasms and their subspecies (Fazaeli *et al.*, 2000; Collins and Allsopp, 1999) and trypanosomes. Generally, the ITS region has been used extensively in characterization of *T. evansi* (Beltrame *et al.*, 2005; Amer *et al.*, 2011). The ITS1 region has been successfully used as target for PCR-based detection of trypanosomes by Njiru *et al.* (2005) and Cox *et al.* (2005) who documented specific PCR product length corresponding to each Trypanosoma species, which was the base of differentiation among Trypanosoma species. For example, *T. congolense* savannah, lead to an ITS1 PCR product size of 700 bp, 400 bp for *T. simiae* and 250 bp for *T. vivax*. The product for *T. evansi* and *T. brucei* subspecies was the same size, 480 bp. The variable surface glycoprotein of trypanosomes RoTat 1.2 VSG is a predominant variant antigen type thought to be expressed in all *T. evansi* stocks examined so far (Verloo *et al.*, 2001). It has been used in routine diagnostic antigen tests such as card agglutination test CATT/*T. evansi*, but negative CATT/*T. evansi* reactions in *T. evansi* parasitaemic animals have been reported (Elsaid, 1998; Davison *et al.*, 1999). Thereafter, it has been shown that a number of *T. evansi* in Kenya were not

detected by tests based on RoTat 1.2 VSG gene and four of the isolates were lacking the RoTat 1.2 VSG gene (Ngaira *et al.*, 2004).

In a study where Bandicoot rat (*Bandicota bengalensi*) received intraperitoneal inoculation of *Trypanosoma evansi* flagellates, the experimental animals showed acute disease, leading to death during the 2nd peak of parasitaemia [14th day post infection (pi)]. Damage in brain and choroid plexus of the infected bandicoot is studied on the 5th, 8th, 12th and 14th day post inoculation (Biswas *et al.*, 2010). Signs of histopathological changes in the brain and choroid plexus of the bandicoot are detected after 1st peak of parasitaemia. Infiltration of lymphocyte and plasma cells, congestion, perivascular cuffing, gliosis and brain lesions were observed during the 12th–14th day post infection. Multiple sclerosis, neuronophagia, focal haemorrhage, cerebral hyperplasia, oligodendrocytoma, astrocytoma and fatty degeneration of brain tissue were also found (Biswas *et al.*, 2010). Alteration in the ependymal cells of choroid plexus was noticed. Extensive oedema, infiltration of inflammatory cells and rupture of ventricular ependymal layer were found. Parasites were found both in the brain tissue and choroid plexus. No intracellular stage of the parasite was observed. The nature of damage in the brain tissue and choroid plexus showed similarity with the cases of African trypanosomiasis. This salivarian species causes more deleterious effect in the brain much earlier in the disease course than its African relatives (Biswas *et al.*, 2010).

The pathology of *Trypanosoma evansi* infection was studied in Swiss albino mice using cattle isolate of the parasite. Sixteen Swiss albino mice were used in the experiment and were divided into two groups; viz. infected group (I) and uninfected healthy control group (II) comprising 12 and four mice, respectively (Bal *et al.*, 2012). Twelve mice from group I were infected with 1×10^5 purified trypanosomes. Systematic necropsy

examination specifically of the infected mice (group I) as well as of healthy control (group II) was performed and pathological changes were recorded. The different tissue samples were collected in 10% neutral buffered formal saline and were used to study the histopathological changes (Bal *et al.*, 2012). Gross post-mortem examination revealed enlargement of the spleen, petechial haemorrhages in the liver in the terminal stages of disease. Tissue sections revealed presence of numerous trypanosomes in the blood vessels of the liver, spleen, brain and kidneys. Microscopically, the liver revealed lesions varying from vacuolar degeneration, coagulative necrosis along with congestion and haemorrhages (Bal *et al.*, 2012). Spleen showed extensive haemorrhages in red pulp area, haemosiderosis and aggregation of histiocytes resulting in multinuclear giant cell formation. Lungs revealed oedema, congestion and mild inflammatory changes (Bal *et al.*, 2012). Brain revealed mild degenerative changes along with congestion of meningeal blood vessels. Kidneys showed tubular degeneration, congestion and cellular infiltration. Heart revealed mild degenerative changes along with interstitial oedema. All changes were consistent with trypanosome infection and were confirmed by presence of trypanosomes in most of the tissue sections examined (Bal *et al.*, 2012).

Thirty Wistar albino rats were challenged with the local strain of *Trypanosoma evansi*. Each animal was infected with 5×10^5 trypanosomes intraperitoneally. The animals were examined daily for the development of clinical signs and infection status by wet blood films made from the tail veins (Sivajothi *et al.*, 2015b). The infected rats were dull and depressed from 3 DPI on wards. Postmortem examination from 5 to 8 DPI (the maximum period of observation) revealed splenomegaly, hepatomegaly, marked congestion of lungs and presence of fluid in the peritoneal cavity. Histopathologically, heart muscles showed hyaline degenerative changes and haemorrhages. Liver parenchyma revealed congestion of central vein

and sinusoids, binucleated hepatocytes and fatty degeneration (lipid accumulation) of hepatic cells. Thickening of interstitial space with mononuclear infiltration, areas of collapse, areas of emphysema, edema and dilated and congested blood vessels were the histopathological changes noticed in the lungs of the infected rats (Sivajothi *et al.*, 2015b). In the spleen, giant cells aggregation, hyperplasia, thickening of capsule and trabecule were the observed changes which indicate irreversible degeneration. The affected kidney showed inter-tubular hemorrhages in the cortex, medullary hemorrhages, congested glomerulus, atrophied glomerulus, desquamated tubular epithelium and disruption of renal tubules at some places (Sivajothi *et al.*, 2015b) .

Based on the previous reports on the existence of variations in the infectivity and pathogenicity of *Trypanosoma evansi* in the field, sixteen West African Dwarf (WAD) goats were experimentally infected (IV) with (2.0×10^6) Sokoto isolate of *Trypanosoma evansi*. Clinical signs, temperature, hematological, gross and histopathological changes in response to the experimental infection were determined in the goats (Ogbaje *et al.*, 2011). All the infected goats developed parasitaemia 3 days post infection (dpi) with slight increase in temperature in the infected goats, decreased mean PCV % in the infected groups to as low as 18 ± 1.47 from 22.50 ± 0.87 . There were leucopenia and lymphocytosis in both infected and uninfected groups (Ogbaje *et al.*, 2011). Serous atrophy of fat around the heart and kidneys was observed in goats from two groups (A and B) and a goat from group C revealed pale liver. Microscopically, there were no pathological lesions in the organs of the sacrificed goats from both infected and uninfected groups (Ogbaje *et al.*, 2011). The ability of the parasite to be infective and pathogenic to mice and rat after serial passaging through WAD goats was also tested and proven to be infective and pathogenic (Ogbaje *et al.*, 2011). It was discovered that the parasite

disappeared from both peripheral circulation and body tissues/organs after 14 days. It was observed that infection of WAD goats with *Trypanosoma evansi* (Sokoto isolate) did not produce noticeable clinical signs, gross and histopathological lesions and it is self-limiting (Ogbaje *et al.*, 2011) .

Five cats were experimentally inoculated with *Trypanosoma evansi* in order to evaluate the pathological changes induced by this protozoan infection. Clinical signs observed included vomiting, diarrhoea, hyperthermia, weight loss, facial oedema, corneal opacity, lymphadenopathy and hind limb instability. Reduction in hematocrit was observed from 7 days post-infection (dpi). One cat died at 40 dpi and the other four cats were humanely destroyed (Da Silva *et al.*, 2010). Necropsy examination was performed in two cats at 56 dpi and two cats at 120 dpi. Gross findings in all cats included generalized muscle atrophy, pale mucosae, icterus of the subcutaneous and serosal tissue and the intima of arteries, lymphadenopathy and splenomegaly. Other findings included corneal opacity, subcutaneous oedema (mainly of the head) and hydropericardium (Da Silva *et al.*, 2010). Trypomastigotes of *T. evansi* were observed in impression smears prepared from the aqueous humor. Microscopically, there was lymphoid hyperplasia of the spleen and lymph nodes. The animals with corneal opacity had mild corneal oedema and accumulation of fibrin and inflammatory cells (neutrophils and plasma cells) in the anterior chamber. Similar inflammatory cells infiltrated the iris, ciliary body, corneoscleral limbus and conjunctiva (Da Silva *et al.*, 2010).

The biochemical changes associating *T. evansi* infection in pregnant and non-pregnant camels were investigated. Based on pregnancy diagnosis and serological findings, camels were classified into four groups as non-pregnant healthy camels, non-pregnant camels infected with *T. evansi*, pregnant healthy camels and pregnant camels infected with *Trypanosoma evansi* (Megahed *et al.*, 2012). The results revealed significant decreases

in serum total proteins, albumin and globulins levels, and significant increases in serum total cholesterol and blood urea nitrogen (BUN) levels in pregnant camels infected with *T. evansi* compared with healthy pregnant camels (Megahed *et al.*, 2012). On the other hand, there were hyperproteinemia and hyperglobulinemia in healthy pregnant camels compared with non-pregnant camels. It was concluded that the biochemical changes associating *T. evansi* infection in pregnant camels were hypoproteinemia, hypoalbuminemia, hypoglobulinemia and increased serum total cholesterol and blood urea nitrogen (BUN) levels (Megahed *et al.*, 2012).

In another study which allowed establishing values of certain biochemical parameters in 48 donkeys selected in seven villages of the agro-pastoral zone of Sideradougou, Burkina Faso (Sow *et al.*, 2014) values found in donkeys of this agro-pastoral zone were within the ranges of those found in donkeys elsewhere in the world, except that values of creatinine and aspartate aminotransferase (AST) were lower in Burkinabese donkeys. The values of these biochemical parameters varied with trypanosomosis infection in donkeys. Donkeys suffering from trypanosomosis got higher values of alanine transaminase than the noninfected donkeys (Sow *et al.*, 2014). The values of AST in the healthy donkeys were significant. The values of total proteins were significantly higher in the infected donkeys, while the albumin values did not vary significantly; the variation in proteins concentration was due to globulins levels. The gamma globulin level was increased in the trypanosomosis-infected donkeys (Sow *et al.*, 2014).

In a study aimed to assess the antitrypanosomal activity of leaf ethanolic extracts against *Trypanosoma evansi* experimental infection, seven medicinal plants; *Camellia sinensis*, *Thymus vulgaris*, *Mentha longifolia*, *Azadirachta indica*, *Olea europaea*, *Rosmarinus officinalis* and *Salvia*

officinalis, were selected based on information from traditional healers on their curative effect against parasites (Barghash, 2016). They were investigated for phytochemical screening and antitrypanosomal properties. Results revealed the presence of saponins, tannins, flavonoids, alkaloids, and phenols (Barghash, 2016). The in-vitro antitrypanosomal activity in microtiter plates showed that all of them had pronounced trypanocidal effects and inhibited the growth of *T. evansi* that was observed either disappeared, dissected or non-motile within 10-60 min. of incubation except for *O. europoea* and *R. officinalis* which could not clear the parasites completely. Therefore, only five plants were evaluated for their in vitro extracts administration and in vivo against *T. evansi* experimental infection. Haematological analysis of the infected and treated rats revealed a marked decline in hemoglobin, haematocrit, total red blood corpuscles and anaemia persisted until the end of the experimental period (Barghash, 2016). Whereas the biochemical assay showed increased serum levels of urea, creatinine, aspartate aminotransferase and alanine aminotransferase, total bilirubin, and decreased albumin, globulin, total protein, cholesterol, triglycerol and glucose in some treated groups. It was concluded that *S. officinalis* followed by *C. sinensis* possess high antitrypanosomal activity compared to diminazene aceturate, and could be useful in the management of trypanosomiasis.

Animals affected with *Trypanosoma evansi* show rare serum hormonal disturbances. One of the important hormones for livestock is thyroxin, and the level of thyroxin may be reduced during *T. evansi* infection. In a study aimed to investigate thyroxin level during experimentally induced *T. evansi* infection in Wister albino rats (Sivajothi *et al.*, 2015a), the white rats were challenged with the local strain of *T. evansi* (at 5x10⁵ trypanosomes/animal subcutaneously). At the high parasitemia, blood was collected from the rats and serum was separated and then subjected to

biochemical evaluation. Decreased total serum thyroxin ($2.91 \pm 0.04 \mu\text{g/dl}$) and free thyroxin ($1.30 \pm 0.05 \text{ ng/dl}$) levels were recorded in *T. evansi* infected rats as compared to the control group of rats (Sivajothi *et al.*, 2015a). Along with lowered thyroxin levels, decreased levels of total erythrocyte count, packed cell volume, hemoglobin, total leucocyte count, total serum proteins, albumin and glucose levels were recorded. On the other hand, significant increase in cholesterol, blood urea nitrogen, serum alkaline phosphatase, serum aspartate aminotransferase, and serum alanine aminotransferase levels were observed. Thus, it was concluded that trypanosomiasis induces stress on rat, which has direct effect on thyroid hormone (Sivajothi *et al.*, 2015a).

Effect of intramuscular administration of Trypanocides on body temperature and serum chemistry in Wistar rats infected with *Trypanosoma brucei brucei* was investigated in 25 Wistar rats with body weights range of $200\text{-}240 \pm 20\text{g}$. The rats were randomly divided into five groups (Ajakaiye *et al.*, 2014). Group A had received 0.5 ml of normal saline only, while group B was given 0.1×10^6 of *T. brucei brucei* only. Group C, D and E were inoculated by the same dose of the parasite as in group B and they were intramuscularly administered with 3.5 mg/kg b.w. of Trypadim, 1 mg/kg b.w. of Trypamidium, and 1 mg/kg b.w. of Novidium, respectively (Ajakaiye *et al.*, 2014). Body temperature increased consistently in all groups except group A. However, there was a significant difference in all treated groups compared to group B on 28 day post-infection (DPI). All serum indicators increased significantly in group B when compared with all other groups (Ajakaiye *et al.*, 2014). Values observed for creatinine in all treated groups were only significantly higher in group E when compared to group A. In conclusion, administered trypanocidal drugs possess anti- pyrexia activity and could off-set the negative effect of *T.*

brucei brucei on the measured serum chemistry in Wistar rats (Ajakaiye *et al.*, 2014).

Haemoparasitic diseases, such as trypanosomiasis, have an adverse impact on health, productivity and working capacity of camels in many camel-rearing regions in the world. In a study, a total of 70 camels were examined for the presence of *Trypanosoma evansi* parasite where the antioxidant status, coagulation disorders and cytological changes associated with *T. evansi* infection as well as the hematological and biochemical changes were studied in both naturally infected camels and experimentally infected rats (Abeer and Shaymaa, 2011). The results revealed significant increases in the activities of antioxidant enzymes and prolonged increases in the values of prothrombin time (PT) and activated partial thromboplastin time (APTT) which were seen in both camels and rats (Abeer and Shaymaa 2011). Anemia and significant leucocytosis were observed in both camels and rats. Serum biochemical parameters showed significant increases in the activities of hepatic enzymes in both of them. In contrast to camel results, there was an increase of blood urea nitrogen (BUN) and serum creatinine concentrations in experimentally infected rats. Different cytological changes associated with *T. evansi* infection were observed in both camels and rats (Abeer and Shaymaa, 2011).

Chapter Three

Materials and Methods

3.1 Parasites selected

Two stocks of *T. evansi* were selected according to sensitivity of Quinapyramine sulphate. They were named as Showak stock and Umbadir stock; following the locations where they were isolated. The pathogenicity of these two *T. evansi* stocks was compared in experimentally infected rats.

3.1.1 Showak Stabilate

This parasite was isolated from a camel at village within the vicinity of the Showak area, Gedarif State, North eastern Sudan. About 0.2 ml of EDTA camel blood was inoculated intraperitoneally into a rat. The rat was transported to the College of Veterinary Medicine, Sudan University of Science and Technology (CVM- SUST). At the peak of parasitaemia the rat was scarified and heparinized blood was cryopreserved and stabilized as “Showak Stabilate”. Preliminary studies showed this stabilate is resistant to higher doses of Quinapyramine sulphate (40mg/kg bwt). On basis of this result, it is selected to represent drug resistant stocks of *T. evansi*.

3.1.2 Umbadir Stabilate

This parasite was isolated from a camel at Umbadir, North Kordofan. About 0.2 ml of EDTA camel blood was inoculated intraperitoneally into a rat. The rat was transported to the College of Veterinary Medicine, Sudan University of Science and Technology (CVM- SUST). At the peak of parasitaemia, the rat was scarified and heparinized blood was cryopreserved in liquid Nitrogen as “Umbadir stabilate”. Preliminary studies showed that this stabilate is very sensitive to Quinapyramine sulphate (can be treated with 5mg/kgbwt). On basis of this, it is selected to represent drug sensitive stocks of *T. evansi*.

3.2 Preparation of the inocula (Donor rats)

One albino rat was infected intraperitoneally with blood that was cryopreserved in liquid nitrogen, containing 1×10^4 parasites/animal to obtain a large amount of the parasite for blood inoculation of experimental groups. Parasitaemia in the inoculated rat was regularly monitored by collecting a drop of fresh blood from the tip of the tail, placed on a clean slide, covered with 22x22 mm cover slip and then examined microscopically for the presence of the parasite (in >30 fields) using X10 eye piece and X40 objective lenses. Blood samples showing actively motile organisms with characteristic flagellar movement was considered as positive for the presence of *T. evansi*. At the peak of parasitemia, the rat was anesthetized with chloroform inhalation; and with the help of a disposable syringe, blood was collected aseptically in tubes containing EDTA anticoagulant by cardiac puncture. Using Neubauer's counter the trypanosome titre was determined in order to be diluted to 1×10^4 trypanosoma for the inoculum.

3.3 Experimental animals

Thirty six (36) adult male outbred Albino mice, weighing between 133 to 137 g were used in this study. The rats were kept in a group of 6 rats per cage with controlled temperature (25 – 30 °C) and humidity around 60-70% RH. The food prepared was according to that stated by Ismail (1988).

3.4 Trypanosome infection

The distribution of the experimental mice into 6 groups of 6 mice each is presented in Table 1. Group 1 (A group), the control group, was infected with *T. evansi* (Showak stabilate) and left without treatment. Group 2 (B group) was infected with *T. evansi* (Showak stabilate) and was treated with the Quinapyramine sulphate (20mg/kg bwt), after the parasite was seen (at the patency). Group 3 (C group) was a control group as was infected with *T. evansi* (Umbadir stabilate) and left without treatment. Group 4 (D

group) was infected with *T. evansi* (Umbadir stabilate) and then treated with Quinapyramine sulphate (20mg/kg bwt), after the parasite is seen (at the patency). Group 5 (E group) was uninfected healthy control for Showak Stock (6 rats). Group 6 (F group) was uninfected healthy control for Umbadir Stock.

3.4.1 Trypanosome sub-inoculation

Sub-inoculation of the experiment first group (A group), second group (B group), third group (C group) and fourth group (D group) was carried out intraperitoneally with the help of a sterile insulin syringe. Rat blood containing 1×10^4 trypanosomes in 0.2 ml volume was inoculated in each mice individually at day one. The number of inoculated flagellates was estimated by Neubauer chamber and the dilutions to obtain the titre of the inoculum were made in sterile phosphate buffer saline with glucose (PSG).

Table 1. The experimental design of the two stabilates (Showak stabilate and Umbadir stabilate) and protocol of treatment with Quinapyramine sulphate.

Group	Stabilate	Parasite	Treatment protocol
A	Showak	<i>T.evansi</i>	Infected not treated
B	Showak	<i>T.evansi</i>	Infected and Treated with Q.S. (20mg/kgbwt)
C	Umbadir	<i>T.evansi</i>	Infected not treated
D	Umbadir	<i>T.evansi</i>	Infected and Treated with Q.S. (20mg/kgbwt)
E	Uninfected Healthy Control Showak Stock		
F	Uninfected Healthy Control Umbadir Stock		

3.5 Parasitological Studies

This research was conducted to study parasite characteristics and to generate data needed to evaluate the differences in the pathogenicity of the two types of *Trypanosoma evansi* stocks.

3.5.1 Parasites detection

All infected rats were bled daily as preferred by Eisler *et al.* (2001), from the tip of the tail for trypanosomes detection using the following parasitological diagnostic methods:

3.5.1.1 Wet blood film count

A drop of fresh blood from the tip of the tail of the infected mice was placed on a clean slide, then covered with 22x22 mm cover slip and examined microscopically for the presence of the parasite using X10 eye piece and X40 objective lenses. The number of parasites were recorded as number of parasites in the whole preparation = No. of parasites per preparation or number of parasites per field. These parasites were expressed as log₁₀ number of parasites per ml of blood following the enumeration of Ismail (1988).

3.5.1.2 Haemocytometer count

When parasite counts are one or more parasites per field, counting in haemocytometer were used for exact number of parasite per mm³ using the method of Paris *et al.* (1982). The presence and degree of parasitaemia were determined daily for each mice by examining tail blood. A drop (5 µl) of blood was collected from the tail, mixed and diluted with trypanosome counting reagent (45 µl). The stained parasites were diluted to the stage that the count on Haemocytometer could be done easily. The parasites were counted in the WBC counting chamber and the total number was expressed as log₁₀ a number of parasites per ml of blood. The parasites were counted using X40 magnification during the 60 days of experiment.

3.6 Sera collection

Blood for sera was collected in plain containers from the retro-orbital plexus. Serum samples were collected at four days intervals and were kept at -20°C until needed for biochemical studies.

3.7 Drug dosages

Dosage was calculated according to the WHO drug testing protocol (2003). Quinapyramine sulphate was used at a dose rate of 20mg/kg bwt and dissolved in sterile water such that the required dose was contained in 0.2 ml of water for each mice and then inoculated intra-peritoneally.

3.8 Biochemical methods

3.8.1 Total Serum Proteins

Total protein was measured using commercial kits (Spinreact S.A./S.A.U. Ctra. Santa Coloma, Spain). The proteins are macromolecular organic compounds, widely distributed in the organism. They act as structural and transport elements. The proteins of the serum are divided in two fractions, albumin and globulins. The determination of total proteins is useful in the detection of high protein levels caused by hemoconcentration like in the dehydrations or increase in the concentration of specific proteins; and secondly, low protein level caused by hemodilution by an impaired synthesis or loss (as by hemorrhage) or excessive protein catabolism (Burtis and Tietz 1999; Tietz 1995).

3.8.1.1 Principle of the method

Proteins give an intensive violet-blue complex with copper salts in an alkaline medium. The change in colour was measured at 540nm using spectrophotometer (Jenway 6305 U.V./vis. Spectrophotometer, U. K). Iodide is included as an antioxidant. The intensity of the color formed is proportional to the total protein concentration in the sample (Burtis and Tietz, 1999; Koller and Kaplan, 1984).

3.8.1.2 Procedure

1 ml of Biuret (sodium potassium tartrate 15 mmol/l, sodium iodide 100 mmol/l, potassium iodide 5 mmol/l and copper (II) sulphate 19 mmol/l) reagent (R) was placed in three test tubes, then 25 µl of standard of total

protein calibrator (bovine albumine primary standard 7 g/dl) was added in tube of standard, also 25 µl of serum sample was added in tube of sample, mixed well and incubated for 10 minutes at room temperature. The absorbance of the samples and standard was against the blank (Table 2).

Table 2. Procedure of Total Serum Protein .

	Blank	Standard	Sample
R (ml) (Blank)	1	1	1
Standard (µl)	-	25	-
Sample (µl)	-	-	25

3.8.1.3 Calculations

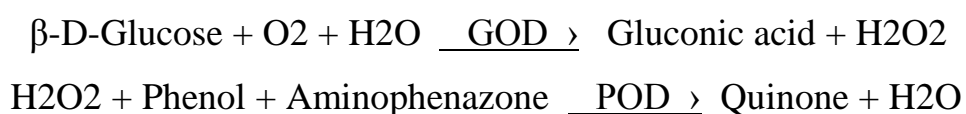
$$\frac{\text{Sample}}{\text{Standard}} \times 7 = \text{g/dl of protein in the sample}$$

3.8.2 Serum Glucose

Glucose was measured using commercial kits (Spinreact S.A./S.A.U. Ctra., Santa Coloma, Spain).

3.8.2.1 Principle of the method

Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H₂O₂) is detected by a chromogenic oxygen acceptor, phenol, 4 – aminophenazone (4-AP) in the presence of peroxidase (POD):



The intensity of the color formed is proportional to the glucose concentration in the sample by Kaplan (1984a) and Trinder (1969). The change in colour was measured at 520 µm using spectrophotometer (Jenway 6305 U.V./vis. Spectrophotometer, U. K).

3.8.2.2 Procedure

Preparation of working reagent (WR): The enzymes (glucose oxidase 15000 U/l , peroxidase 1000 U/l and 4- Aminophenazone 2.6 mmol/l) were

dissolved in buffer (TRIS (pH 7.4) 92 mmol/l and phenol 0.3 mmol/l). One ml of working reagent (WR) was placed in three test tubes, then 10 µl of the standard of glucose calibrator (glucose aqueous primary standard 100 mg/dl) was added in the tubes of the standard, also 10 µl of the serum sample was added in the tube of sample, mixed well and incubated for 10 minutes at room temperature. The absorbance of the samples and Standard was read against the Blank (Table 3).

Table 3. Procedure of Serum Glucose

	Blank	Standard	Sample
WR (ml)	1	1	1
Standard (µl)	-	10	-
Sample (µl)	-	-	10

3.8.2.3 Calculation

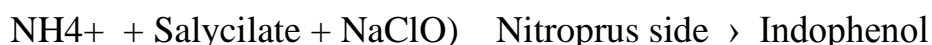
$$\frac{\text{Sample}}{\text{Standard}} \times 100 = \text{mg/dl of glucose in the sample}$$

3.8.3 Serum Urea

Urea was measured using commercial kits (Spinreact S.A./S.A.U. Ctra., Santa Coloma, Spain).

3.8.3.1 Principle of the method

Urea in the sample is hydrolyzed enzymatically into ammonia (NH₄⁺) and carbon dioxide (CO₂). Ammonia ions formed react with salicylate and hypochlorite (NaClO), in presence of the catalyst nitroprusside, to form a green indophenol:



The change in colour was measured at 580 nm using spectrophotometer (Jenway 6305 U.V./vis. Spectrophotometer, U. K). The intensity of the color formed is proportional to the urea concentration in the sample by Kaplan (1984b), Tabacco, et al 1979) and Fawcett (1960).

3.8.3.2 Procedure

Preparation of working reagent (WR): One tablet R3 enzymes (urease 30000 U/l) was dissolved in one bottle of R1 buffer phosphate (pH 6.7) 50 mmol/l, EDTA 2 mmol/l, sodium salicylate 400 mmol/l and sodium nitroprusside 10 mmol/l). 1 ml of working reagent (WR) was placed in three test tubes, then 10 µl of the standard of urea calibrator (Urea aqueous primary standard 50 mg/dl) was added in the tubes of the standard, also 10 µl of the serum sample was added in tube of sample, mixed well and incubated for 10 minutes at room temperature, then 1ml of reagent 2 (R2) (sodium hypochlorite (NaClO) 140 mmol/l and sodium hydroxide 150 mmol/l) was added in each test tube, mixed well and incubated for 10 minutes at room temperature. The absorbance of the samples and Standard was read against the Blank (Table 4).

Table 4. Procedure of Serum Urea

	Blank	Standard	Sample
WR (ml)	1	1	1
Standard (µl)	-	10	-
Sample (µl)	-	-	10
R2 (ml)	1	1	1

3.8.3.3 Calculation

$$\frac{\text{Sample}}{\text{Standard}} \times 50 = \text{mg/dl of urea in the sample}$$

3.8.4 Serum Albumin

Albumin was measured using commercial kits (Spinreact S.A./S.A.U. Ctra. Santa Coloma, Spain).

3.8.4.1 Principle of the method

Albumin in the presence of bromocresol green at a slightly acid pH, produces a colour change of the indicator from yellow-green to green-blue.

The intensity of the color formed is proportional to the albumin concentration in the sample (Gendler and Kaplan, 1984; Rodkey, 1965; Webster, 1974 and Doumas *et al.*, 1971). The change in colour was measured at 630µm using the spectrophotometer (Jenway 6305 U.V./vis. Spectrophotometer, U. K).

3.8.4.2 Procedure

1 ml of reagent R (bromcresol pH (4.2) 0.12 mmol/l) was placed in three test tubes, then 5 µl of the standard of albumin calibrator (Albumin aqueous primary standard 5g/dl) were added in tube of standard, also 5 µl of serum sample were added in tube of sample, mixed well and incubated for 10 minutes at room temperature. The absorbance of the samples and Standard was read against the Blank (Table 5).

Table 5. Procedure of Serum Albumin

	Blank	Standard	Sample
R (ml)	1	1	1
Standard (µl)	-	5	-
Sample (µl)	-	-	5

3.8.4.3 Calculation

$$\frac{\text{Sample}}{\text{Standard}} \times 5 = \text{g/dl of Albumin in the sample}$$

3.8.5 Serum Calcium

Calcium was measured using commercial kits (Spinreact S.A./S.A.U. Ctra. Santa Coloma, Spain).

3.8.5.1 Principle of the method

The measurement of calcium in the sample is based on formation of color complex between calcium and O-cresolphtalein in alkaline medium:



The intensity of the colour formed is proportional to the calcium concentration in the sample (Farell and Kaplan, 1984a; Kessler, 1964;

Connerty, 1996). The change in colour was measured at 570 µm using spectrophotometer (Jenway 6305 U.V./vis. Spectrophotometer, U. K).

3.8.5.2 Procedure

2 ml of reagent 1 (R1) (Ethanalamine 500 mmol/l) was placed in three test tubes and 1 drop of Reagent 2 (R2) (chromogen) (0-Cresolphtalein 0.62 mmol/l and 8-hydroxyquinolein 69 mmol/l) was added in each test tube, then 20 µl of standard of calcium calibrator (Calcium aqueous primary standard 10 mg/dl) was added in tube of standard, also 20µl of serum sample was added in tube of sample, mixed well and incubated for 5 minutes at room temperature. The absorbance of the samples and Standard was read against the Blank (Table 6).

3.8.5.3 Calculation

$$\frac{\text{Sample}}{\text{Standard}} \times 10 = \text{mg/dl of Calcium in the sample}$$

Table 6. Procedure of Serum Calcium

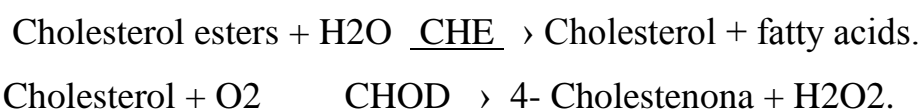
	Blank	Standard	Sample
R1(ml)	2	2	2
R2 (drop)	1	1	1
Standard (µl)	-	20	-
Sample (µl)	-	-	20

3.8.6 Serum Cholesterol

Cholesterol was measured using commercial kits (Spinreact S.A./S.A.U. Ctra., Santa Coloma, Spain).

3.8.6.1 Principle of the method

The cholesterol present in the sample originates a coloured complex, according to the following reactions:



2 H₂O₂ + Phenol + 4-Aminophenazone POD > Quinonimine + 4H₂O

The intensity of the color formed is proportional to the cholesterol concentration in the sample (Naito and Kaplan, 1984; Meiattini, 1978). The change in colour was measured at 520 µm using spectrophotometer (Jenway 6305 U.V./vis. Spectrophotometer, U. K).

3.8.6.2 Procedure

Preparation of working reagent (WR) Dissolve the enzymes (R2) (Cholesterol esterase 300 U/l, Cholesterol oxidase 300 U/l, Peroxidase 1250 U/l and 4-Aminophenazone 0.4 mmol/l) in buffer (R1) (PIPES - pH 6.9 - 90 mmol/l and phenol 26 mmol/l). One ml of working reagent (WR) was placed in three test tubes, then 10 µl of the standard of cholesterol calibrator (Cholesterol aqueous primary standard 200 mg/dl) was added in the tubes of the standard, also 10 µl of the serum sample was added in the tube of sample, mixed well and incubated for 10 minutes at room temperature. The absorbance of the samples and Standard was read against the Blank (Table 7).

Table 7. Procedure of Serum Cholesterol

	Blank	Standard	Sample
WR(ml)	1	1	1
Standard (µl)	-	10	-
Sample (µl)	-	-	10

3.8.6.3 Calculation

$$\frac{\text{Sample}}{\text{Standard}} \times 200 = \text{mg/dl of Cholesterol in the sample}$$

3.8.7 Serum Creatinine

Creatinine was measured using commercial kits (Spinreact S.A./S.A.U. Ctra. Santa Coloma, Spain).

3.8.7.1 Principle of the method

The assay is based on the reaction of creatinine with sodium picrate as described by Jaffé (1886). Creatinine reacts with alkaline picrate forming a red complex. The time interval chosen for measurements avoids interferences from other serum constituents. The intensity of the color formed is proportional to the creatinine concentration in the sample (Murray and Kaplan, 1984a).

The change in colour was measured at 490 μm using spectrophotometer (Jenway 6305 U.V./vis. Spectrophotometer, U. K).

3.8.7.2 Procedure

Preparation of working reagent (WR) mix equal volumes of picric reagent (R1) (Picric acid 17.5 mmol/l) with alkaline reagent (R2) (sodium hydroxide 0.29 mol/l). One ml of working reagent (WR) was placed in three test tubes, then 100 μl of the standard of creatinine calibrator (Creatinine aqueous primary standard 2 mg/dl) were added in tube of the standard, also 100 μl of the serum sample were added in tube of sample, mixed and started stopwatch. The absorbance (A1) of the samples was read after 30 seconds and after 90 seconds (A2) of the sample addition then the standard was read against the Blank (Table 8) and calculated (Calculated $\Delta A = A_2 - A_1$).

Table 8. Procedure of Serum Creatinine

	Blank	Standard	Sample
WR(ml)	1	1	1
Standard (μl)	-	100	-
Sample (μl)	-	-	100

3.8.7.3 Calculation

$$\frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times 2 = \text{mg/dl of creatinine in the sample}$$

3.8.8 Serum Phosphorus

Phosphorus was measured using commercial kits (Spinreact S.A./S.A.U. Ctra., Santa Coloma, Spain).

3.8.8.1 Principle of the method

Inorganic phosphorus reacts with molybdic acid forming a phosphomolybdic complex. Its subsequent reduction in alkaline medium originates a blue molybdenum colour. The intensity of the color formed is proportional to the inorganic phosphorus concentration in the sample (Farrell and Kaplan, 1984b; Daly and Gerhard, 1972). The change in colour was measured at 710 nm using spectrophotometer (Jenway 6305 U.V./vis. Spectrophotometer, U. K.).

3.8.8.2 Procedure

Preparation of working reagent (WR) mix equal volumes of molybdic reagent (R1) (Molybdate-Borate 1.21 mmol/l and Sulphuric acid 100 mmol/l) with catalyzer reagent (R2)(1,2 Phenylenediamine 2.59 mol/l). 1.5 ml of working reagent (WR) were placed in three test tubes, then 50 µl of the standard of phosphorus calibrator (Phosphorus aqueous primary standard 5 mg/dl) were added in tube of the standard, also 50 µl of the serum sample were added in tube of sample, mixed well and incubated for 30 minutes at room temperature. The absorbance of the samples and Standard was read against the Blank (Table 9).

Table 9. Procedure of Serum Phosphorus

	Blank	Standard	Sample
WR (ml)	1.5	1.5	1.5
Standard (µl)	-	50	-
Sample (µl)	-	-	50

3.8.8.3 Calculation

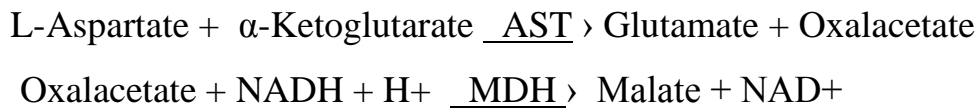
$$\frac{\text{Sample}}{\text{Standard}} \times 5 = \text{mg/dl of phosphorus in the sample}$$

3.8.9 Aspartate aminotransferase (AST) or Glutamate oxaloacetate transaminase (GOT)

Aspartate aminotransferase (AST) was measured using commercial kits (Spinreact S.A./S.A.U. Ctra., Santa Coloma, Spain).

3.8.9.1 Principle of the method

Aspartate aminotransferase (AST) formerly called glutamate oxaloacetate (GOT) catalyses the reversible transfer of an amino group from aspartate to α -ketoglutarate forming glutamate and oxalacetate. The oxalacetate produced is reduced to malate by malate dehydrogenase (MDH) and NADH:



The rate of decrease in concentration of NADH, was measured at 340 μm using spectrophotometer (Jenway 6305 U.V./vis. Spectrophotometer, U. K). It is proportional to the catalytic concentration of AST present in the sample by Murray and Kaplan (1984b).

3.8.9.2 Procedure

Preparation of working reagent (WR) mix four volumes of buffer reagent (R1)(TRIS pH (7.8) 80 mmol/l, Lactate dehydrogenase 800 U/l, Malate dehydrogenase 600 U/l and L-Aspartate 200 mmol/l) with one volume from substrate (R2)(NADH 0.18 mmol/l and α -Ketoglutarate 12 mmol/l). One ml from working reagent (WR) was placed in a test tube then 100 μl of serum sample was added, mixed well and the solution was incubated for 1 minute at room temperature and the initial absorbance (A) of the sample was read, the stopwatch was started and the absorbance was read at 1 minute interval for 3 minutes. The difference between absorbance and the average absorbance was calculated as differences per minute ($\Delta A/\text{min}$) (Table 10).

Table 10. Procedure of Aspartate aminotransferase (AST)

WR (ml)	1
Sample (μ l)	100

3.8.9.3 Calculation

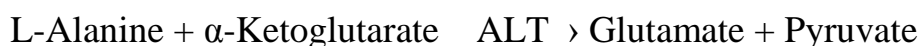
$$\Delta A/\text{min} \times 1750 = \text{U/L of the AST in the sample}$$

3.8.10 Alanine aminotranferase (ALT) or Glutamate pyruvate transaminase (GPT)

Alanine aminotranferase (ALT) was measured using commercial kits (Spinreact S.A./S.A.U. Ctra., Santa Coloma, Spain).

3.8.10.1 Principle of the method

Alanine aminotranferase (ALT) or Glutamate pyruvate transaminase (GPT) catalyses the reversible transfer of an amino group from alanine to α -ketoglutarate forming glutamate and pyruvate. The piruvate produced is reduced to lactate by lactate dehydrogenase (LDH) and NADH:



The rate of decrease in concentration of NADH, was measured at 340 μ m using spectrophotometer (Jenway 6305 U.V./vis. Spectrophotometer, U. K.), is proportional to the catalytic concentration of ALT present in the sample (Murray and Kaplan, 1984c).

3.8.10.2 Procedure

Preparation of working reagent (WR): Four volumes of buffer reagent (R1)(TRIS pH (7.8) 100 mmol/l, Lactate dehydrogenase 1200 U/l and L-Alanine 500 mmol/l) were mixed with one volume from substrate (R2)(NADH 0.18 mmol/l and α -Ketoglutrare 15 mmol/l). One ml from working reagent (WR) was placed in a test tube then 100 μ l of serum sample were added, mixed well and the solution was incubated for 1 minute at room temperature and the initial absorbance (A) of the sample

was read, the stopwatch was started and the absorbance was read at 1 minute interval for 3 minutes. The difference between absorbance and the average absorbance was calculated as differences per minute ($\Delta A/\text{min}$) (Table 11).

Table 11. Procedure of Alanine aminotranferase (ALT)

WR (ml)	1
Sample (μl)	100

3.8.10.3 Calculation

$$\Delta A/\text{min} \times 1750 = \text{U/L of the ALT in the sample}$$

3.8.11 Serum Sodium

Sodium was measured using commercial kits (BioMed schiffgraben 41, 30175 Hannover, Germany).

3.8.11.1 Principle of the method

Sodium is precipitated with Mg-uranyl acetate; the uranyl ions remaining in suspension form as a yellow-brown complex with thioglycolic acid. The difference between reagent blank (without precipitation of sodium) and analysis is proportional to the sodium concentration (Trinder, 1951; Henry, 1974). The absorbance was measured at 630 μm using spectrophotometer (Jenway 6305 U.V./vis. Spectrophotometer, U. K).

3.8.11.2 Procedure

1 ml of color reagent (R2) was placed in three test tubes, 10 μl of distilled water were added in tube of blank, then 10 μl of standard of sodium calibrator (Sodium aqueous primary standard 150 mEq/l) were added in tube of standard, also 10 μl of serum sample were added in tube of sample, then mixed well and incubated for 5 minutes at room temperature. The absorbance of the samples and Standard was read against the blank (Table 12).

Table 12. Procedure of Serum Sodium

	Blank	Standard	Sample
Reagent2 (R2) (ml)	1	1	1
Distilled Water (µl)	10	-	-
Standard (R1)(µl)	-	10	-
Sample (µl)	-	-	10

3.8.11.3 Calculation

$$\frac{\text{Sample}}{\text{Standard}} \times 150 = \text{mEq/l of Sodium in the sample}$$

3.8.12 Serum Potassium

Potassium was measured using commercial kits (BioMed schiffgraben 41, 30175 Hannover, Germany).

3.8.12.1 Principle of the method

Potassium ions in a protein-free alkaline medium react with sodium tetraphenylboron to produce a finely dispersed turbid suspension of potassium tetraphenyl boron. The turbidity produced is proportional to the potassium concentration and read photometrically at 630 µm using spectrophotometer (Jenway 6305 U.V./vis. Spectrophotometer, U. K) (Young, 1995; Young , 2001).

3.8.12.2 Procedure

1 ml of color reagent (R2)(Sodium tetraphenylboron 0.2 mmol/l) was placed in three test tubes, 20 µl of distilled water were added in tube of blank, then 20 µl of standard of potassium calibrator (Potassium aqueous primary standard 5 mEq/l) were added in tube of standard, also 20 µl of serum sample were added in tube of sample, then mixed well and incubated for 5 minutes at room temperature. The absorbance of the samples and Standard were read against the blank (Table 13).

Table 13. Procedure of Serum Potassium

	Blank	Standard	Sample
Reagent2 (R2) (ml)	1	1	1
Distilled Water (µl)	20	-	-
Standard (R1) (µl)	-	20	-
Sample (µl)	-	-	20

3.8.12.3 Calculation

$$\frac{\text{Sample}}{\text{Standard}} \times 5 = \text{mEq/l of Potassium in the sample}$$

3.9 Histopathology

Vital organs were preserved in 10% neutral buffered formalin immediately after removal from the animal.

3.9.1 Tissue processing

The tissues were placed in 10% formalin (diluted to 10% with normal saline) for one hour to rectify shrinkage due to high concentration of formalin. The tissues were dehydrated by ascending grades of ethyl alcohol by immersing in 50%, 70%, 90%, and 100% ethyl alcohol for 2 hour. The dehydrated tissues were cleared in two changes of xylene, 2 hour each. The wax impregnated tissues were embedded in paraffin blocks using the same grade wax. The paraffin blocks were cut with rotary microtome (Leica) at 5 micron thickness. The sections were floated on a tissue floatation bath at 40°C and taken on glass slides and smeared with adhesive media by gelatin. The sections were then melted in an incubator at 60°C and after 15 minutes the sections were allowed to cool.

3.9.2 Tissue staining

The sections were deparaffinized by immersing in xylene for 6 min in vertical staining jar. The deparaffinized sections were washed in 100% absolute alcohol, 90% alcohol, 70% alcohol, distilled water (DW) and

stained in Harris's hematoxyline for 10 minutes in vertical staining jar. After staining in hematoxylin, the sections were washed in tap water and dipped in acid alcohol to remove excess stain (8.3% HCl in 70% alcohol). The sections were then placed in running tap water for 10 minutes for bluing (slow alkalization). The sections were counter stained in 1% aqueous eosin (one gm in 100 ml tap water) for one min and the excess stain was washed in tap water and the sections were allowed to dry. Complete dehydration of stained sections was censured by placing the sections in the incubator at 60°C for 5 minutes. When the sections were cooled, they were mounted in DPX mount having the optical index of glass (the sections were wetted in xylene and inverted on to the mount and placed on the cover slip).

3.10 Molecular Studies

3.10.1 Polymerase Chain Reaction (PCR)

3.10.1.1 DNA Extraction

For DNA extraction from blood using protein precipitation method, 5 ml of blood were collected in EDTA tube, 300 µl of blood were placed in a 1.5 Eppendorf tube, then 1000 µl of red blood cells lyses (RBCsL) were added and mixed by inversion 32 and centrifuged at 9000 rpm for 5 minutes. The supernatant was discarded and the pellet (white blood cells) was washed with 1000µl of RBCsL, then 300 µl of white blood cells lyses (WBCsL) were added followed by one µl of proteinase k and the solution was incubated at 37°C overnight. 100 µl of protein precipitation solution (6M NaCl) were added next day and mixed by vortexing gently and 200 µl of cold chloroform were added and centrifuged at full speed for 6 minutes. Then the aqueous phase was transferred into a clean Eppendorf tube. Double volume of cold Ethanol was then added to precipitate DNA, centrifuged at 14000 rpm for 5 minutes the supernatant was poured off without disturbing the precipitate, washed with 70% ethanol (600 µl), air

dried at room temperature, re-suspended in 100 µl of TE buffer or ddH₂O and left to be dissolved and then the DNA was stored in -20°C.

3.10.1.2 Amplification

Extracted genomic DNA was subjected to a PCR that amplifies the internal transcribed spacer 1 (ITS1) region of the rDNA gene of *T. evansi* by using TeRoTat 920 F 5'-CTGAAGAGGTTGGAAATGGAGAAG-3' and TeRoTat 1070 R, 5'-GTTTCGGTGGTTCTGTTGTTGTTA-3' primers set. To obtain the expected 151 bp PCR product, (maxime PCR premix kit) Master Mix, 2X in a 20 µl total volume was deployed. Each reaction includes, 1 µl of 10 mM of each primer, 4 µl extracted DNA and 14µl of ddH₂O. Thermocycling profile that starts with an initial hold for 2 minutes at 95°C, followed by 35 cycles of 95°C for 30 seconds, 64°C for 30 seconds and 72°C for one minute and final extension step of 5 minutes at 72°C, was adopted. PCR products were subjected to electrophoresis in 2% agarose (Vivantis technologies, Malaysia) in Tris-borate EDTA buffer and was visualized under UV light. The ITS1-PCR detection method constitutes a powerful molecular 33 diagnostic tool for *T. evansi* detection as well as discrimination from other trypanosomes in one PCR.

3.11 Data Analysis

The statistical analysis was performed using independent t-test and Statistical Package for the Social Science (SPSS) software. P. values less than 0.05 were considered statistically significant.

Chapter Four

Results

4.1. The response of Showak stabilate to Quinapyramine Sulphate

4.1.1 The Overall Mean of Parasitaemia

Generally, the overall mean of parasitaemia in the mice infected-not-treated was 5.4 ± 2.8 and in the mice infected-treated was 4.8 ± 2.9 (Table 14).

4.1.2. Mice infected with *T. evansi* (Showak Stabilate) not-treated control (A) group

Mice inoculated by 1×10^4 of Showak stabilate of *Trpanosoma evansi* but were not treated with Quinapyramine sulphate (A group) inflicted high mortalities during the experiment period where one died at day 16 post infection (pi), one at day 21, one at day 25, two at day 26 and one at day 28 with a mean survival period of 23.2 ± 4.8 .

4.1.3. Mice infected with *T. evansi* (Showak Stabilate) treated with Quinapyramine Sulphate after the patency at a dose of 20 mg/kgbw (B) group

Only two mice died at days 52 and day 53 pi. with a mean survival period of 52.5 ± 0.72 (Table 15).

Treatment of mice in group (B) which were infected and treated with Quinapyramine sulphate was commenced at day 6 when the parasitaemia level was $\log_{10} 4.2$. By day 8, the parasite was cleared from all mice in the group and remained so until day 10 during which period no protozoan can be detected in wet blood smears. Up to day 6 there was no significant difference between parasitaemia levels in both treated and control groups. By day 26, the treated group recorded a mean parasitaemia of $\log_{10} 6$ while that of the control was $\log_{10} 8.3$ which was significantly higher than the treatment group ($p < 0.05$). In the control mice, by day 17 the parasitaemia fluctuated between $\log_{10} 7.8$ to $\log_{10} 8.0$ until the end of study period. The

drug has effect on parasitaemia till day 26 (Fig 4.1). The effect of drug (Quinapyramine sulphate) % =

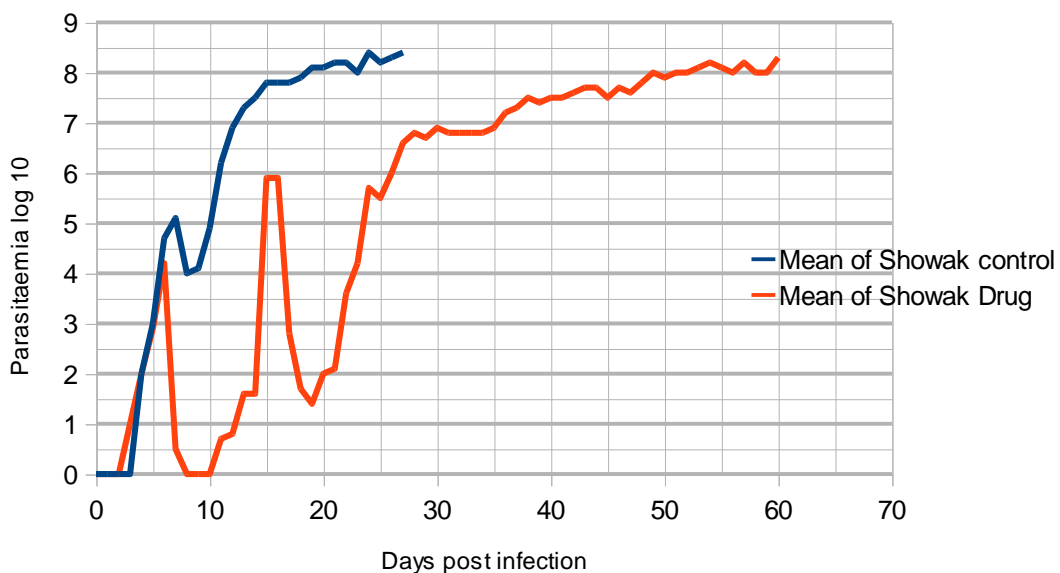
$$\frac{\text{Infected untreated} - \text{Inecfected Treated}}{\text{infected untreated}} \times 100 =$$

$$8.3 - 6.6 \div 8.3 \times 100 = 20.5\%$$

Table 14. Overall means and Std. Deviation of parasitaemia levels in mice infected-not-treated (A group) Showak stabilates and mice infected-treated (B group).

Treatment	Strains	Mean	Std. Deviation	N
Not treated (group A)	Showak	5.4	2.8	28
Treated (group B)	Showak	4.8	2.9	61

Fig. 1. Comparison of the means of parasitaemia levels (\log_{10}), between mice infected with *T. evansi* (Showak Stabilate) treated by Quinapyramine



Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.

Table 15 Comparison between mice infected with *T. evansi* (Showak Stabilate) treated by Quinapyramine Sulphate at dose rate of 20mg/kgbw (after patency group B) and mice infected-not-treated control (group A) .

Time to death	Control of 6 Mice (group A)	Time to death	Infected Treated of 6 Mice (group B)
Day 16	1mice n= 5mice	Day 52	1mice n= 5mice
Day 21	1mice n=4mice	Day 53	1mice n= 4mice
Day 25	1mice n=3mice		
Day 26	2 mice n= 1mice		
Day 28	1mice n= 0		
X= 23.2 ± 4.8		X= 52.5 ±0.72	

4.2. The response of Umbadir stabilate to Quinapyramine Sulphate

4.2.1 The overall mean of parasitaemia

Generally, the overall mean of parasitaemia in the mice infected-not-treated (group C) was 5.9 ± 2.01 and in the mice infected-treated (group D) was 0.07 ± 0.36 (Table 16).

4.2.2. Mice infected with *T. evansi* (Umbadir Stabilate) not-treated control group (C)

Mice inoculated by 1×10^4 of Umbadir stabilate of *Trpanosoma evansi* but were not treated with Quinapyramine sulphate (group C) inflicted low mortalities during the experiment period where one died at day 30 post infection (pi), one at day 50, one at day 51 and one at day 54, with a mean survival period of 46.3 ± 11 (Table 17).

4.2.3 Mice infected with *T. evansi* (Umbadir Stabilate) and treated with Interquin (Quinapyramine sulphate) after patency at a dose of 20 mg/kgbw group (D)

All mice survived until the end of the study period. Treatment of mice in group (D) was commenced at day 4 when the parasitaemia level was $\log_{10} 2.2$. By day 5, all treatment group were negative. Treated mice remained

negative until the end of the study period. Up to day 4, there was no significant difference between parasitaemia levels in both treated and control groups. By day 18, the treated group recorded a mean parasitaemia of $\log_{10} 0$ while that of the control was $\log_{10} 6.8$ which was significantly higher than the treatment group ($p < 0.05$). In the control mice, by day 31, the parasitaemia fluctuated between $\log_{10} 7.3$ to $\log_{10} 8.0$ until the end of study period (Fig.17).

Table 16 Overall means and Std. Deviation of parasitaemia levels in mice infected-not-treated (C group) Umbadir stabilates and mice infected-treated (D group).

Treatment	Strains	Mean	Std. Deviation	N
Not treated (group C)	Umbadir	5.9	2.01	61
Treated (group D)	Umbadir	0.07	0.36	61

Fig. 2. Comparison of the means of parasitaemia levels (\log_{10}), between mice infected with *T. evansi* (Umbadir Stabilate) treated by

Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.

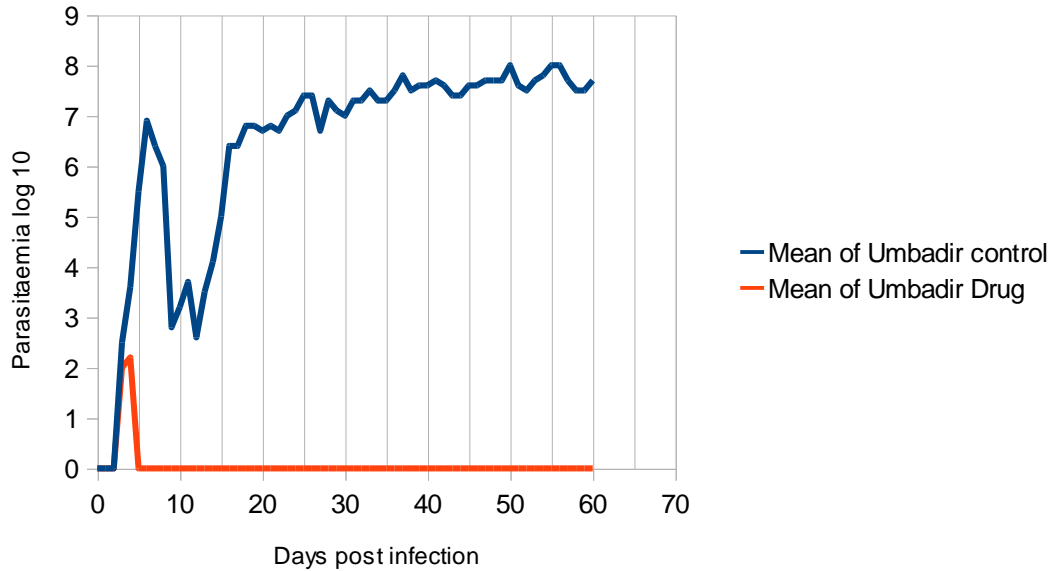


Table 17 Comparison between mice infected with *T. evansi* (Umbadir Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw (after patency group D) and mice infected-not-treated control (group C) .

Time to death	Control of 6 Mice (group C)	Infected Treated of 6 Mice (group D)
Day 30	1mice n= 5mice	
Day 50	1mice n=4mice	All mice survived until the end of the study period
Day 51	1mice n=3mice	
Day 54	1mice n= 2mice	
X=46.3 ±11		

4.3 Confirmation of the identity of the test Trypanosoma by PCR:

The stabilates of Trypanosoms used in this study were confirmed to be *Trypanosoma evansi* by PCR using specific primers that specifically target the ITS1 region of the rDNA gene of *T. evansi*. Using this specific technique, DNA extract from whole blood of rat infected with the

trypanosome yielded an amplicon of the size 151 bp; a PCR product size expected for this species of the trypanosome (Fig. 3).

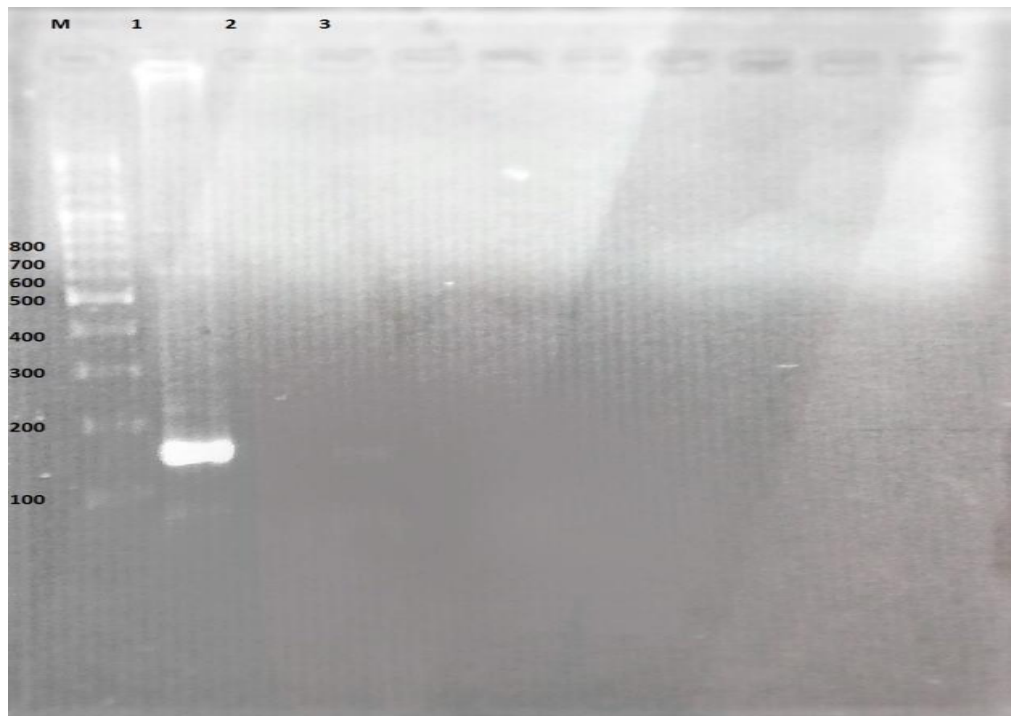


Fig. 3. The resultant amplicon of the ITS1 region of the rRNA gene of *Trypanosoma evansi* as (arrow) 151 bp detected in the DNA harvest of the

whole blood of mice infected by this species of trypanosome in samples 1 and 2.

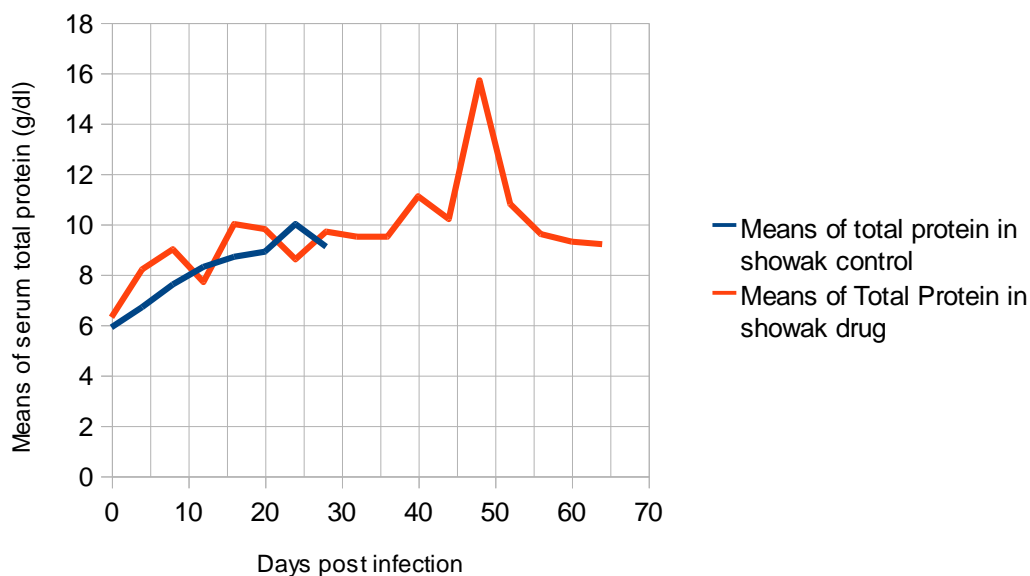
4.4 Serum biochemical changes

4.4.1 Serum total protein

4.4.1.1 Showak Stabilated

The mean serum values of total proteins in the mice infected-not-treated (A group) were increased progressively during the study. This increase started at day 12 post infection. The highest mean level recorded was 10 g/dl at day 24 post infection. The statistical analysis showed a mean of 8.2 ± 1.3 g/dl which was significant (P-value = 0.003). While the mean serum values of total proteins in the mice infected-treated (B group) were increased, starting at days 4 and 8 post infection with means of 8.2 and 9 g/dl, followed by normal levels at day 12 post infection with mean of 7.7 g/dl. Then the values increased till end of the study with the highest mean level recorded was 15.7 g/dl at day 48 post infection. The statistical analysis showed a mean of 8.7 ± 1.3 g/dl which was significant (P-value=0.003) (Table 18 and Fig. 4).

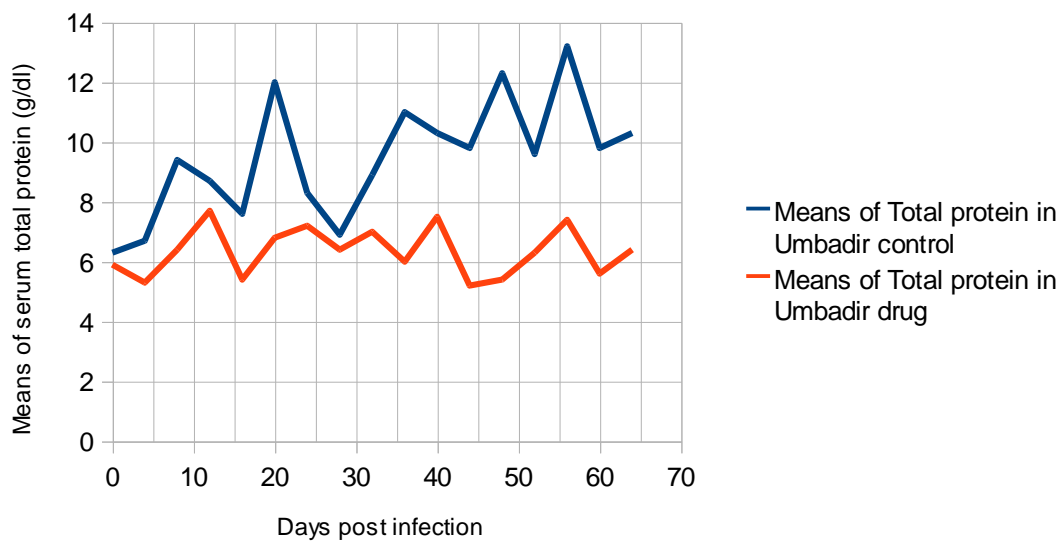
Fig. 4 Comparison of the means of serum total protein between mice infected with *T. evansi* (Showak Stabilate) treated by Quinapyramine Sulphate at dose rate of 20mg/kgbw and mice infected-not-treated control.



4.4.1.2. Umbadir Stabilated

The mean serum values of total proteins in the mice infected-not-treated (C group) were within normal levels at days 4, 16 and 28 post infection with means of 6.7, 7.6 and 6.9 g/dl. Followed by increase values in other days. The highest mean level recorded was 13.2 g/dl at day 56 post infection. The statistical analysis showed a means of 8.2 ± 1.9 g/dl which was significant (P-value=0.000). While the mean serum values of total protein in the mice infected-treated (D group) has showed no change at all days of the experiment. The statistical analysis showed a mean of 6.4 ± 0.84 g/dl which was not significant (P-value=0.300) (Table 18 and Fig. 5).

Fig. 5. Comparison of the means of serum total protein between mice infected with *T. evansi* (Umbadir Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.

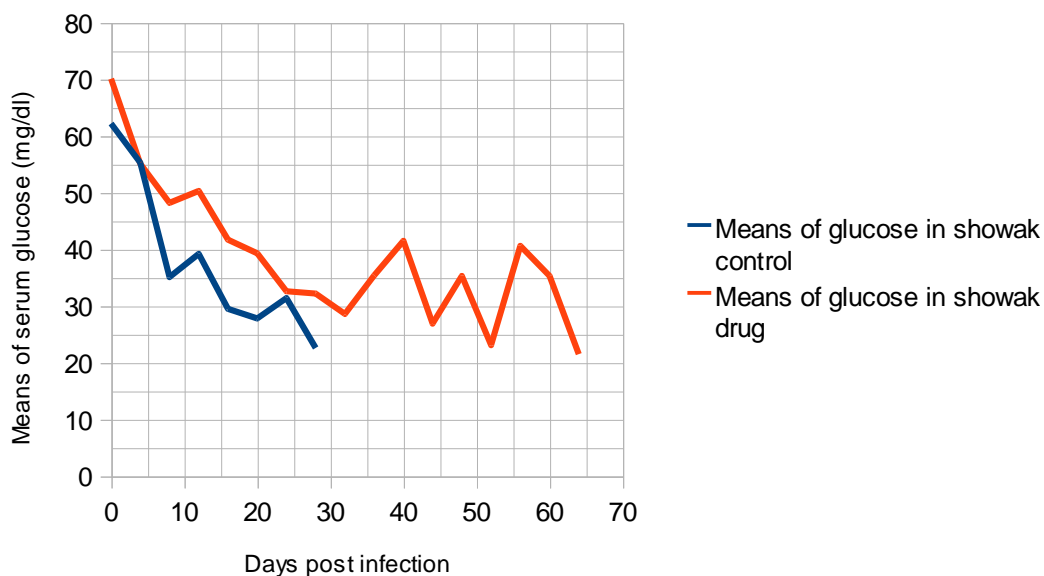


4.4.2. Serum glucose

4.4.2.1. Showak Stabilated

The mean serum values of glucose in the mice infected-not-treated (A group) were at normal levels at day 4 post infection with mean of 55.3 mg/dl, followed by decrease values till end of the study. This corresponded with the first parasitaemia build up, the lowest mean level recorded was 22.6 mg/dl at day 28 post infection. The statistical analysis showed a means of 37.9 ± 13.9 mg/dl which was significant (P-value = 0.001). While the mean serum values of glucose in the mice infected-treated (B group) were normal at days 4 and 12 post infection with means of 55.1 and 50.3 mg/dl, followed by decrease levels till end of the experiment. The lowest mean level recorded was 21.5mg/dl at day 64 post infection. The statistical analysis showed a means of 46.2 ± 12.6 mg/dl, which was significant (P-value = 0.002) (Table 18 and Fig. 6).

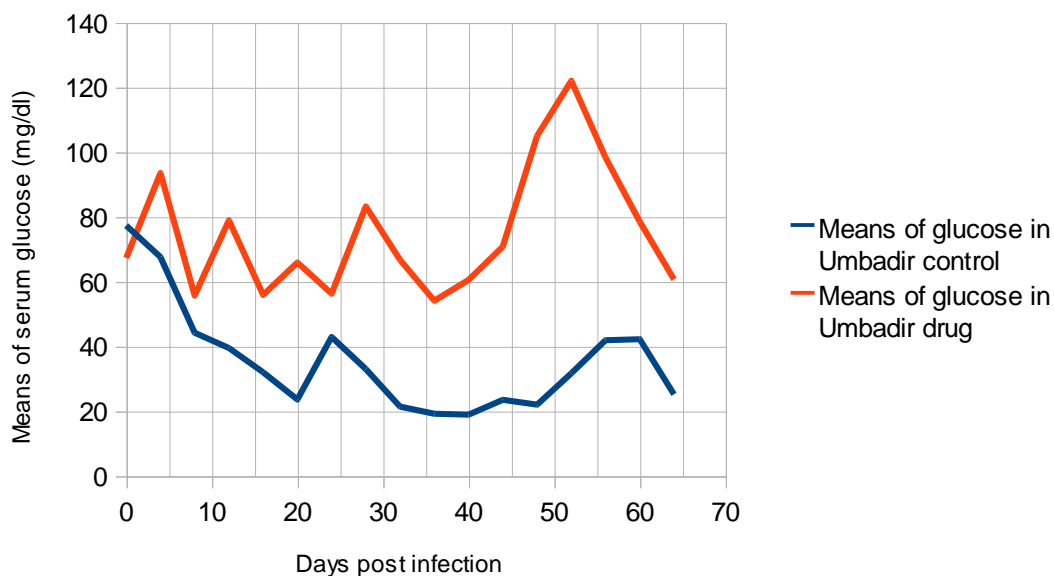
Fig. 6. Comparison of the means of serum glucose between mice infected with *T. evansi* (Showak Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.



4.4.2.2. Umbadir Stabilated

The mean serum values of glucose in the mice infected-not-treated (C group) were found normal at day 4 post infection with means of 67.6 mg/dl followed by decrease levels till end of the experiment. The statistical analysis showed a means of 45 ± 18.4 mg/dl was significant (P-value = 0.002), the lowest mean level recorded was 18.9 mg/dl at day 40 post infection. While the mean serum values of glucose in the mice infected treated (D group) were normal at all days of the experiment. The statistical analysis showed a means of 74.9 ± 19.8 mg/dl, which was not significant (P-value = 0.640) (Table 18 and Fig. 7).

Fig. 7. Comparison of the means of serum glucose between mice infected with *T. evansi* (Umbadir Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.

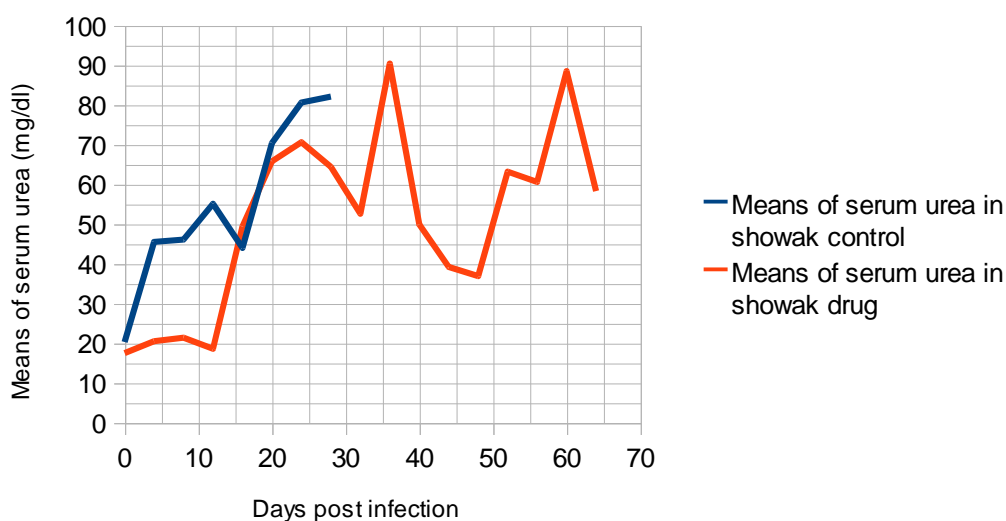


4.4.3. Serum Urea

4.4.3.1. Showak Stabilated

The mean serum values of urea in the mice infected-not-treated (A group) were increased not-progressively during the study. This increase started at day 4 post infection with mean 45.5mg/dl. The highest mean level recorded was 82.1 mg/dl at day 28 post infection. The statistical analysis showed a mean of 55.5 ± 21.1 mg/dl which was significant (P-value = 0.014). While the mean serum values of urea in the mice infected treated (B group) were increased during the study. This increase continued till end of the study. The highest mean level recorded was 90.4 mg/dl at day 36 post infection. The statistical analysis showed a mean of 41 ± 23.8 mg/dl, which was significant (P-value = 0.022) (Table 18 and Fig. 8).

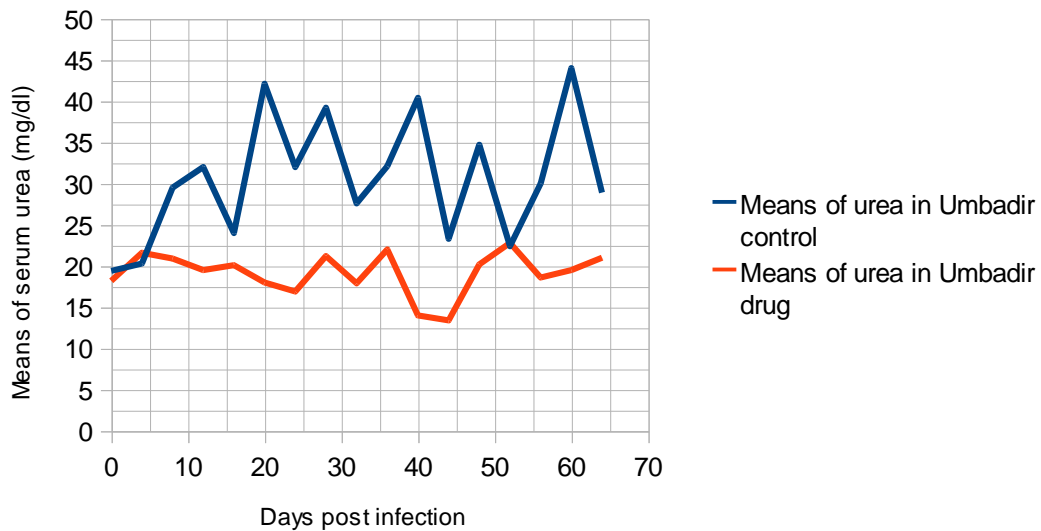
Fig. 8. Comparison of the means of serum urea between mice infected with *T. evansi* (Showak Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.



4.4.3.2. Umbadir stabilized

The mean serum values of urea in the mice infected-not-treated (C group) were within the normal levels at day 4 post infection with mean of 20.3 mg/dl. Serum urea levels had increased until end of the study. The highest mean level recorded was 44 mg/dl at day 60 post infection. The statistical analysis showed a mean of 29.8 ± 8.3 mg/dl which was significantly important (P-value = 0.011). While the mean serum values of urea in the mice infected-treated (D group) were normal at all days of the experiment. The statistical analysis showed a means of 19.6 ± 1.7 mg/dl which was not significant (P-value = 0.723) (Table 18 and Fig. 9).

Fig. 9. Comparison of the means of serum urea between mice infected with *T. evansi* (Umbadir Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.

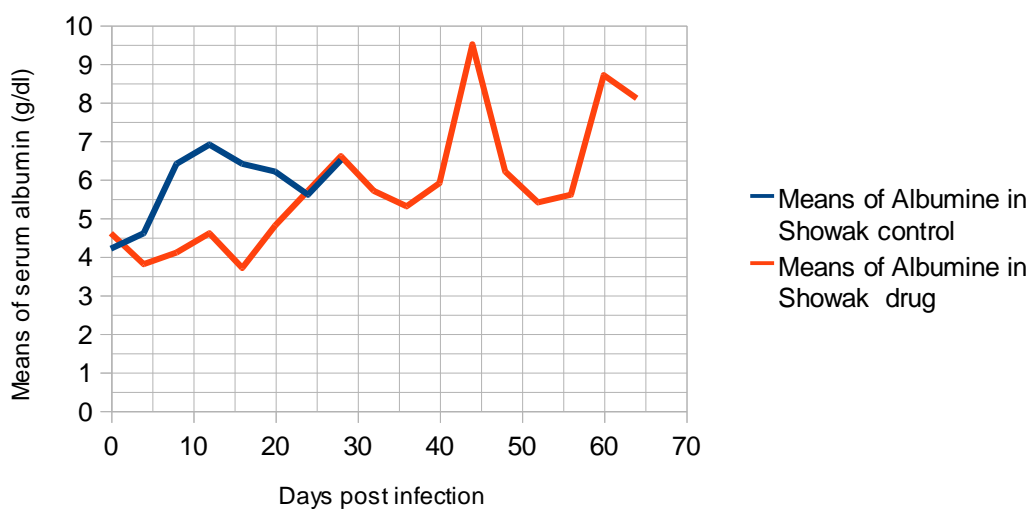


4.4.4. Serum Albumin

4.4.4.1. Showak Stabilated

The mean serum values of albumin in the mice infected-not-treated (A group) were increased, this increase was started at day 8 post infection with mean 6.4 g/dl. The highest mean level recorded was 6.9 g/dl at day 12 post infection. The statistical analysis showed a means of 5.9 ± 0.97 g/dl was significant (P-value = 0.039). While the mean serum values of albumin in the mice infected treated (B group) were increased, this increased was started at day 24 post infection, with mean 5.7 g/dl. The highest mean level recorded was 9.5 g/dl at day 44 post infection. The statistical analysis showed a means of 5.8 ± 1.7 g/dl which was significant (P-value=0.003) (Table 18 and Fig. 10).

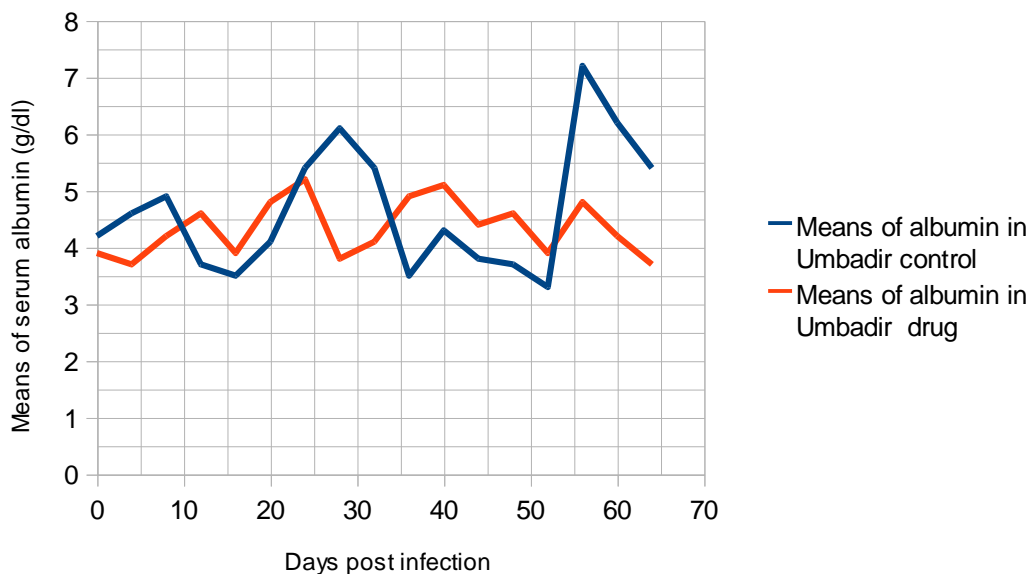
Fig. 10. Comparison of the means of serum albumin between mice infected with *T. evansi* (Showak Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.



4.4.4.2. Umbadir stablited

The mean serum values of albumin in the mice infected-not-treated (C group) had increased. This increase started at day 24 post infection with mean of 5.4 g/dl, the highest mean level recorded was 7.2 g/dl at day 56 post infection. The statistical analysis showed a mean of 4.7 ± 0.88 g/dl which was not significant (P-value = 0.961). While the mean serum values of albumin in the mice infected-treated (D group) were normal at all days of the experiment. The statistical analysis showed a means of 4.3 ± 0.54 g/dl which was not significant (P-value = 0.485) (Table 18 and Fig.11).

Fig. 11. Comparison of the means of serum albumin between mice infected with *T. evansi* (Umbadir Stablitate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.

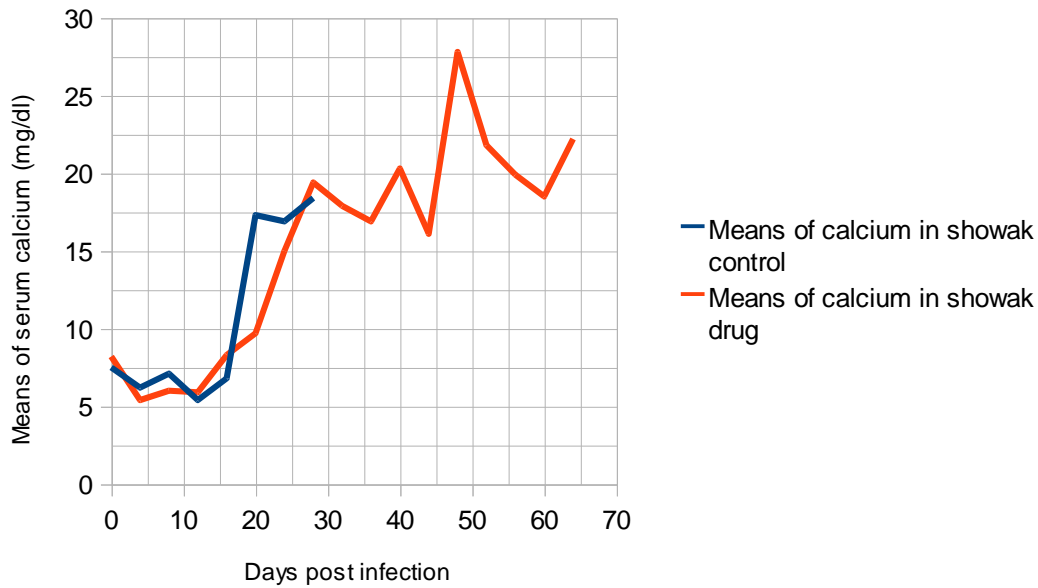


4.4.5. Serum calcium

4.4.5.1. Showak Stabilated

The mean serum values of calcium in the mice infected-not-treated (A group) were increased during the study. This increase started at day 20 post infection with mean of 17.3 mg/dl. The highest mean level recorded was 18.4 mg/dl at day 28 post infection. The statistical analysis showed a means of 10.7 ± 5.7 mg/dl which was not significant (P-value = 0.383). While the mean serum values of calcium in the mice infected-treated (B group) has increased, this increased started at day 24 post infection with mean of 15 mg/dl. The highest mean level recorded was 27.8 mg/dl at day 48 post infection. The statistical analysis showed a means of 15.3 ± 6.8 mg/dl was significant (P-value = 0.001) (Table 18 and Fig.12).

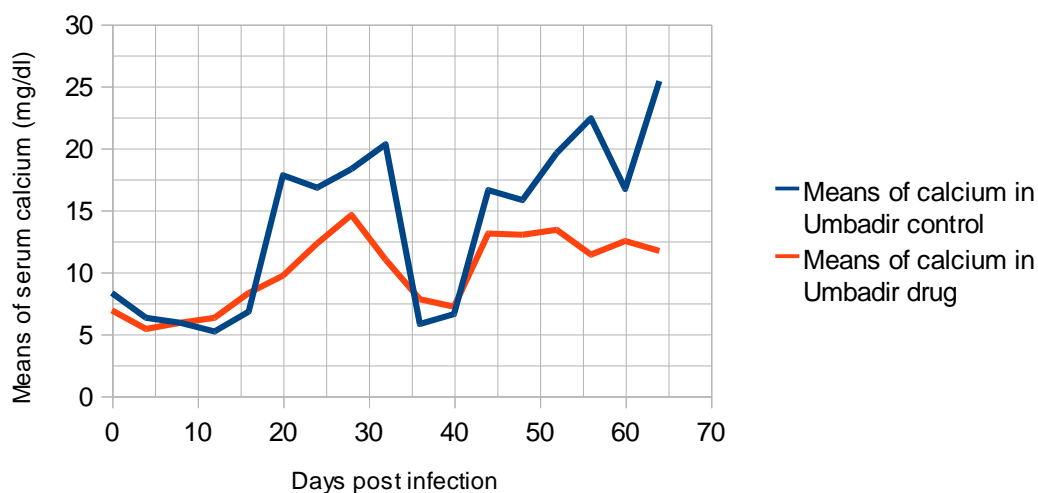
Fig. 12. Comparison of the means of serum calcium between mice infected with *T. evansi* (Showak Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.



4.4.5.2. Umbadir stabilized

The mean serum values of calcium in the mice infected-not-treated (C group) has increased. This increase started at day 20 post infection with mean 17.8 mg/dl. The highest mean level recorded was 25.4 mg/dl at day 64 post infection. The statistical analysis showed a means of 10.7 ± 5.8 mg/dl which was not significant (P-value = 0.907). While the mean serum values of calcium in the mice infected-treated (D group) were normal at all days of the experiment except day 28 post infection which showed some rise with mean of 14.6. The statistical analysis showed a means of 8.7 ± 3.3 mg/dl which was not significant (P-value= 0.105) (Table 18 and Fig.13).

Fig. 13. Comparison of the means of serum calcium between mice infected with *T. evansi* (Umbadir Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.

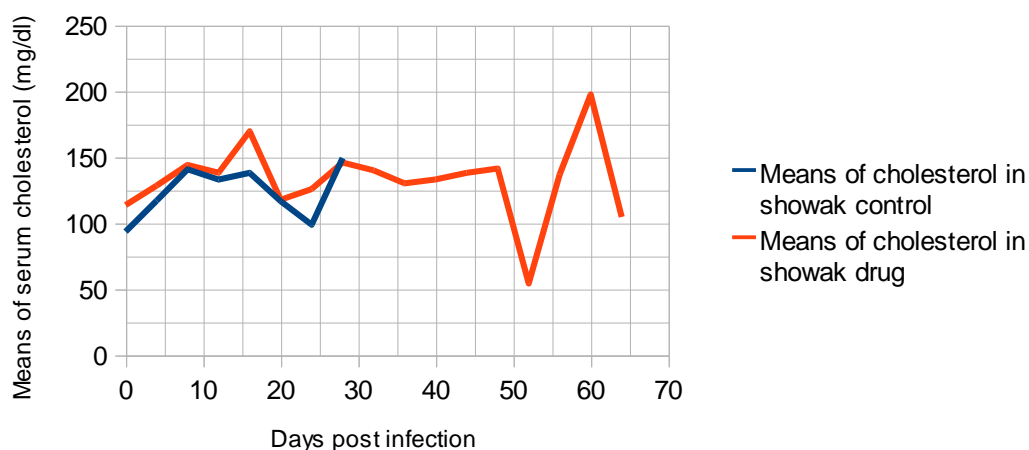


4.4.6. Serum Cholesterol

4.4.6.1 Showak Stabilated

The mean serum values of cholesterol in the mice infected -not- treated (A group) were increased this increased was started at day 8 post infection with mean 140.8 mg/dl. The highest mean level recorded was 149.3 mg/dl at day 28 post infection. The statistical analysis showed a means of 133.4 ± 19.5 mg/dl which was significantly important (P-value = 0.033). While the mean serum values of cholesterol in the mice infected -treated (B group), were elevated during the study this elevated started at day 8 post infection with a mean of 144.1mg/dl. The highest mean level recorded was 197.5 mg/dl at day 60 post infection. The statistical analysis showed a means of 135.4 ± 18.1 mg/dl which was significant (P-value = 0.022) (Table 18 and Fig.14).

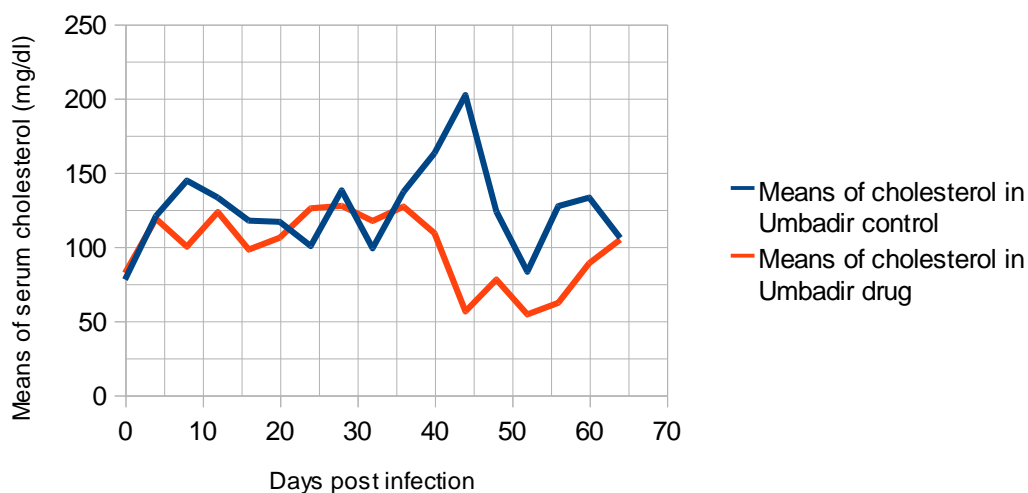
Fig. 14. Comparison of the means of serum cholesterol between mice infected with *T. evansi* (Showak Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.



4.4.6.2. Umbadir Stabilated

The mean serum values of cholesterol in the mice infected-not-treated (C group) were elevated during the study. This elevation started at day 8 post infection with a mean of 144.5 mg/dl. The highest mean level recorded was 202.1 mg/dl at day 44 post infection. The statistical analysis showed a means of 118.6 ± 21.6 mg/dl which was not significant (P-value = 0.195). While the mean serum values of cholesterol in the mice infected treated (D group) were normal at all days of the experiment. The statistical analysis showed a means of 98.8 ± 24.8 mg/dl which was not significant (P-value = 0.384) (Table 18 and Fig. 15).

Fig. 15. Comparison of the means of serum cholesterol between mice infected with *T. evansi* (Umbadir Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.

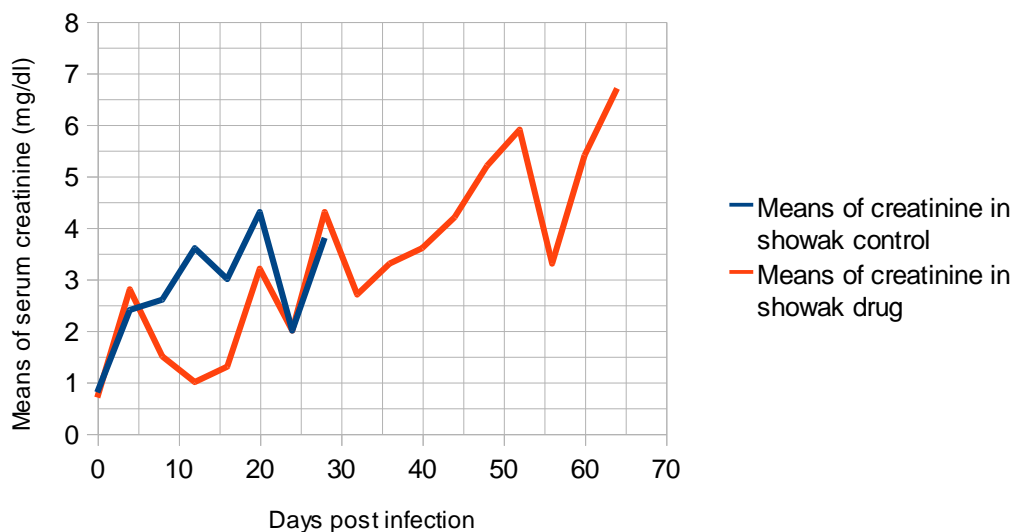


4.4.7 Serum creatinine

4.4.7.1 Showak Stabilated

The mean serum values of creatinine in the mice infected-not-treated (A group) were increased not progressively during the study. This increase was started at day 4 post infection with a mean of 1.6 mg/dl. The highest mean level recorded was 4.3 mg/dl at day 20 post infection. The statistical analysis showed a means of 2.8 ± 1.1 mg/dl which was significant (P-value = 0.016). While the mean serum values of creatinine in the mice infected-treated (B group) were increased till end of the study. The highest mean level recorded was 6.7 mg/dl at day 64 post infection. The statistical analysis showed a means of 2.1 ± 1.2 mg/dl which was significant (P-value=0.000) (Table 18 and Fig. 16).

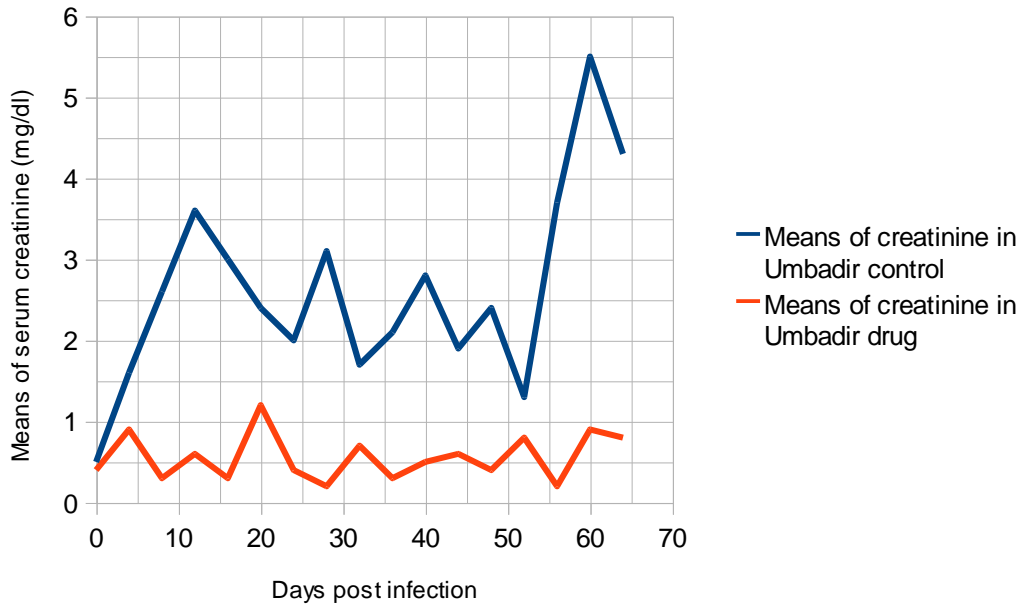
Fig. 16. Comparison of the means of serum creatinine between mice infected with *T. evansi* (Showak Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.



4.4.7.2. Umbadir Stabilated

The mean serum values of creatinine in the mice infected-not-treated (C group) has increased. This increase started at day 4 post infection with a mean of 1.6 mg/dl. The highest mean level recorded was 5.5 mg/dl at day 60 post infection. The statistical analysis showed a means of 2.4 ± 0.98 mg/dl which was not significant (P-value = 0.091). While the mean serum values of creatinine in the mice infected-treated (D group) were normal at all days of the experiment. The statistical analysis showed a means of 0.54 ± 0.35 mg/dl which was not significant (P-value = 0.397) (Table 18 and Fig. 17).

Fig. 17. Comparison of the means of serum creatinine between mice infected with *T. evansi* (Umbadir Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.

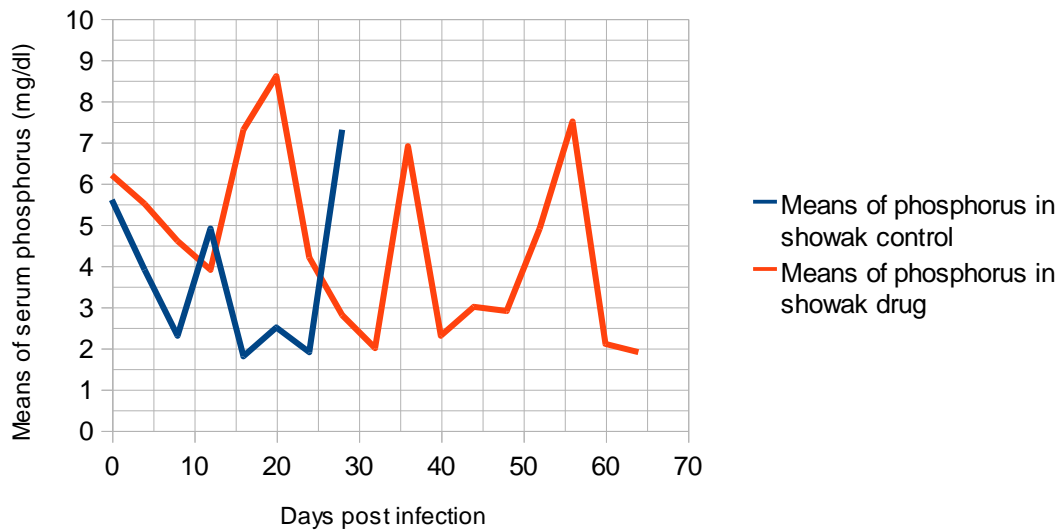


4.4.8. Serum phosphorus

4.4.8.1. Showak Stabilated

The mean serum values of phosphorus in the mice infected-not-treated (A group) were decreased. This decrease started at day 8 post infection with a mean of 2.3 mg/dl. The lowest mean level recorded was 1.8 mg/dl at day 16 post infection. The statistical analysis showed a means of 3.8 ± 2 mg/dl which was not significant (P-value = 0.194). While the mean serum values of phosphorus in the mice infected-treated (B group) were decreased during the study. This decrease started at day 28 post infection with a mean of 2.8 mg/dl. The lowest mean level recorded was 1.9 mg/dl at day 64 post infection. The statistical analysis showed a means of 5.4 ± 1.9 mg/dl which was not significant (P-value = 0.223) (Table 18 and Fig. 18).

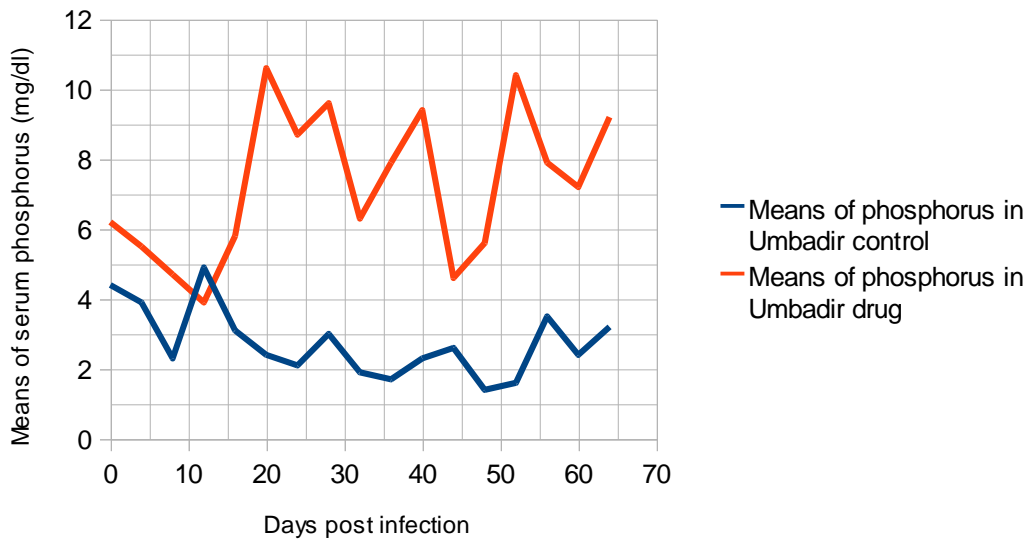
Fig. 18. Comparison of the means of serum phosphorus between mice infected with *T. evansi* (Showak Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.



4.4.8.2. Umbadir Stabilated

The mean serum values of phosphorus in the mice infected-not-treated (C group) were decreased during the study. This decrease started at day 8 post infection. The lowest mean level recorded was 1.4 mg/dl at day 48 post infection. The statistical analysis showed a means of 3.3 ± 1 mg/dl which was not significant (P-value = 0.152). While the mean serum values of phosphorus in the mice infected-treated (D group) were normal at all days of the experiment. The statistical analysis showed a means of 6.9 ± 2.4 mg/dl which was not significant (P-value = 0.890) (Table 18 and Fig. 19).

Fig. 19. Comparison of the means of serum phosphorus between mice infected with *T. evansi* (Umbadir Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.

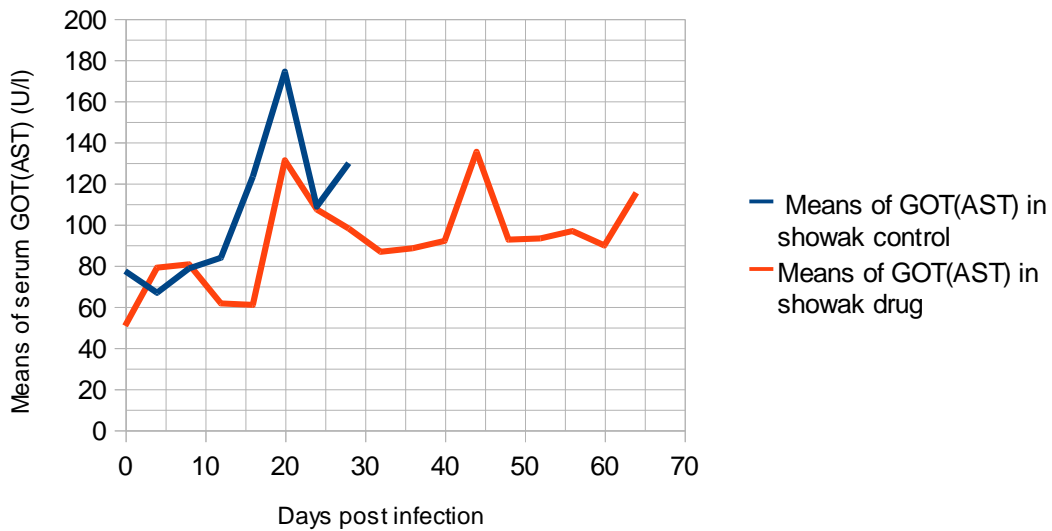


4.4.9. Serum glutamate oxaloacetate transaminase (GOT)

4.4.9.1 Showak Stabilated

The mean serum values of GOT in the mice infected-not-treated (A group) were not increased progressively during the study. This increase has started at day 12 post infection with mean 83.7 U/l. The highest mean level recorded was 174.2 U/l at day 20 post infection. The statistical analysis showed a means of 105.2 ± 36.1 U/l which was significant (P-value = 0.043). While the mean serum values of GOT in the mice infected-treated (B group) were also increased in this study. The highest mean level recorded was 135.2 U/l at day 44 post infection. The statistical analysis showed a means of 83.6 ± 0.35 U/l which was significant (P-value = 0.044) (Table 18 and Fig. 20).

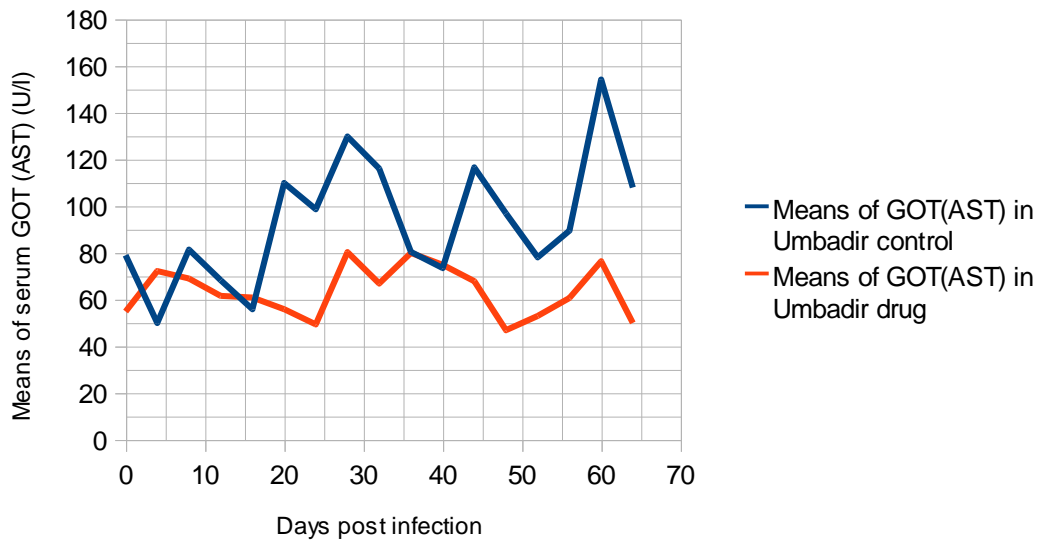
Fig. 20. Comparison of the means of serum GOT between mice infected with *T. evansi* (Showak Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.



4.4.9.2. Umbadir Stabilated

The mean serum values of GOT in the mice infected-not-treated (C group) were increased. This increase has started at day 20 post infection with mean of 109.8 U/L. The highest mean level recorded was 154.1 U/L at day 60 post infection. The statistical analysis showed a means of 84 ± 27.3 U/L which was not significant (P-value = 0.098). While the mean serum values of GOT in the mice infected-treated (D group) were normal at all days of the experiment. The statistical analysis showed a means of 62.9 ± 10.2 U/L which was not significant (P-value = 0.708) (Table 18 and Fig. 21).

Fig. 21. Comparison of the means of serum GOT between mice infected with *T. evansi* (Umbadir Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.

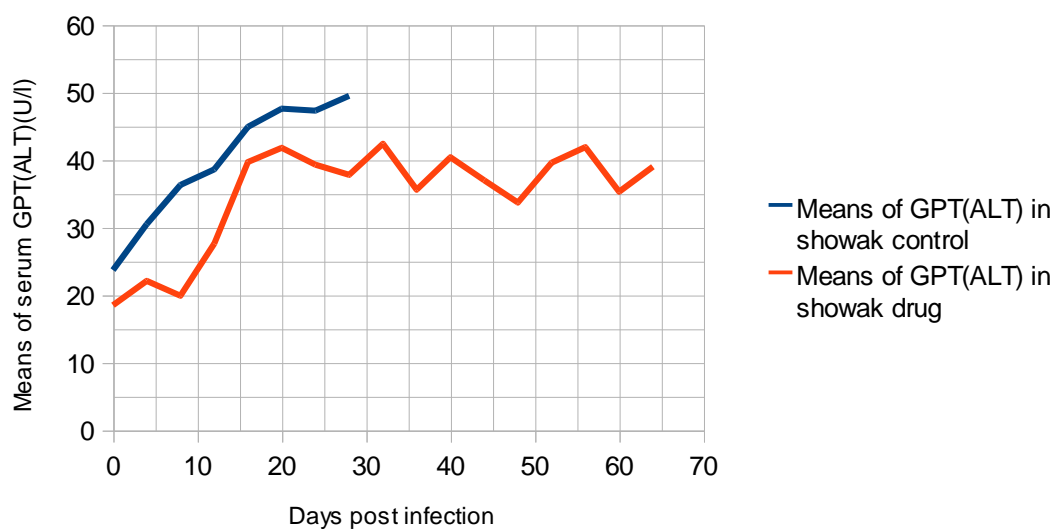


4.4.10. Serum glutamate pyruvate transaminase (GPT)

4.4.10.1 Showak Stabilated

The mean serum values of GPT in the mice infected-not-treated (A group) were increased progressively during the study. This increase has started at day 8 post infection with a mean of 36.3 U/l. The highest mean level recorded was 49.5 U/l at day 28 post infection. The statistical analysis showed a means of 39.8 ± 9.2 U/l which was significant (P-value = 0.001). While the mean serum values of GPT in the mice infected-treated (B group) were also increased in this study. This increase has started at day 16 post infection with a mean of 39.7 U/l. The highest mean level recorded was 42.4 U/l at day 32 post infection. The statistical analysis showed a means of 34.8 ± 7.9 U/l which was significant (P-value = 0.000) (Table 18 and Fig. 22).

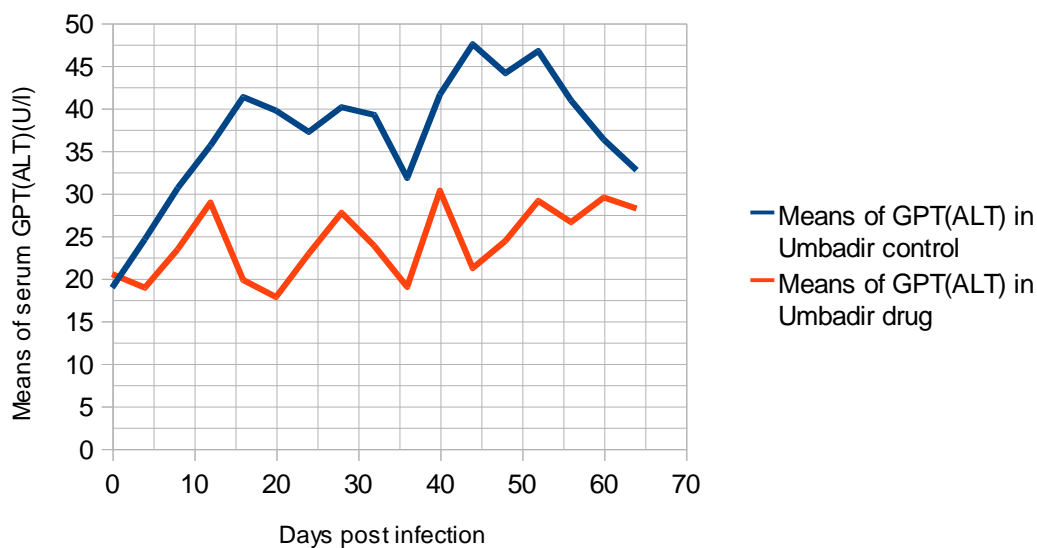
Fig. 22. Comparison of the means of serum GPT between mice infected with *T. evansi* (Showak Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.



4.4.10.2. Umbadir Stabilated

The mean serum values of GPT in the mice infected-not-treated (C group) were increased during the study. This increase was started at day 12 post infection with a mean of 35.6 U/L. The highest mean level recorded was 47.5 U/L at day 44 post infection. The statistical analysis showed a means of 33.5 ± 8.1 U/l which was significant (P-value = 0.000). While the mean serum values of GPT in the mice infected-treated (D group) were normal at all days of the experiment. The statistical analysis showed a means of 24.2 ± 4.2 U/L which was not significant (P-value = 0.368) (Table 18 and Fig. 23).

Fig. 23. Comparison of the means of serum GPT between mice infected with *T. evansi* (Umbadir Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.

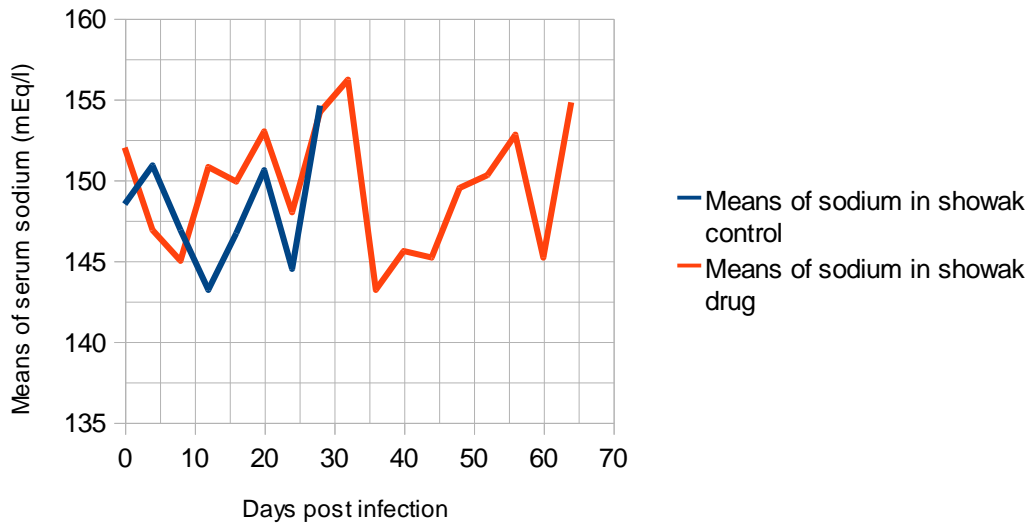


4.4.11. Serum sodium

4.4.11.1. Showak Stabilated

The mean serum values of sodium in the mice infected-not-treated (A group) were showed normal levels at all days of the experiment. The statistical analysis showed a means of 148.2 ± 3.7 mEq/l which was not significant (P-value = 0.272). While the mean serum values of sodium in the mice infected-treated (B group) were also normal at all days of the experiment. The statistical analysis showed a means of 150 ± 3.2 mEq/l which was not significant (P-value = 0.222) (Table 18 and Fig. 24).

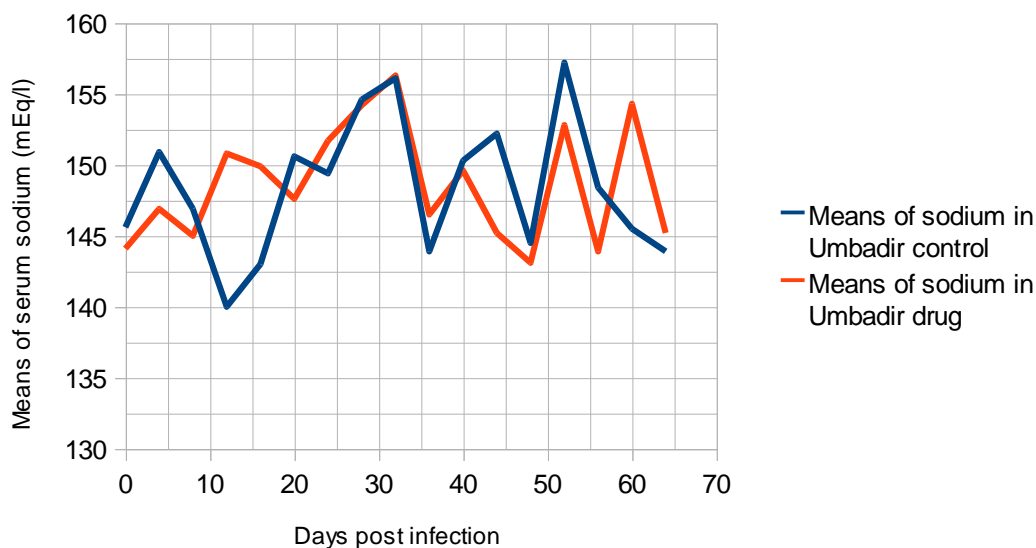
Fig. 24. Comparison of the means of serum sodium between mice infected with *T. evansi* (Showak Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.



4.4.11.2. Umbadir Stabilated

The mean serum values of sodium in the mice infected-not-treated (C group) were showed normal levels at all days of the experiment. The statistical analysis showed a means of 147.6 ± 4.7 mEq/l which was not significant (P-value = 0.550). While the mean serum values of sodium in the mice infected-treated (D group) were also normal at all days of the experiment. The statistical analysis showed a means of 148.8 ± 3.5 mEq/l which was not significant (P-value = 0.841) (Table 18 and Fig. 25).

Fig. 25. Comparison of the means of serum sodium between mice infected with *T. evansi* (Umbadir Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbwt and mice infected-not-treated control.

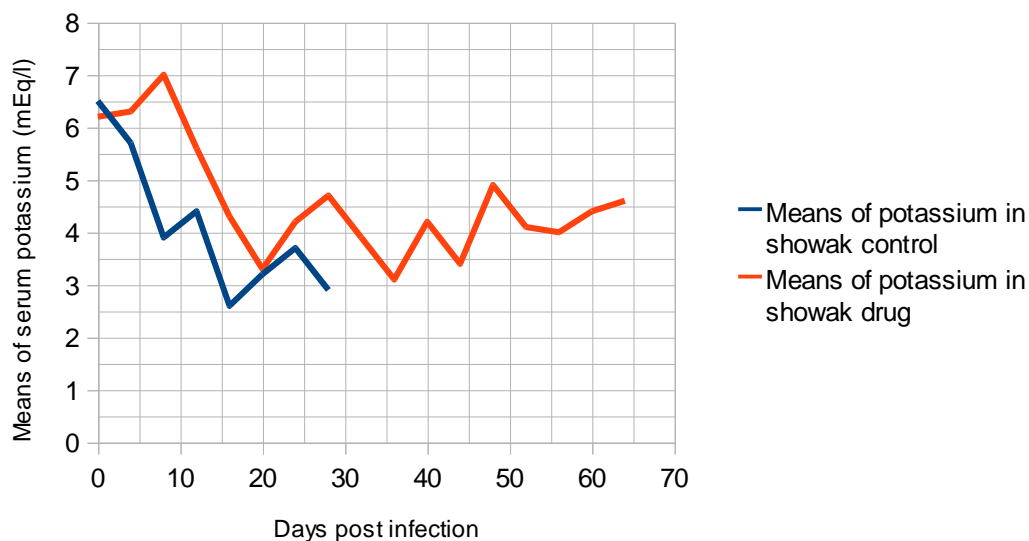


4.4.12. Serum potassium

4.4.12.1. Showak Stabilated

The mean serum values of potassium in the mice infected-not-treated (A group) were decreased during the study. This decrease has started at day 8 post infection with a mean of 3.9 mEq/l. The lowest mean level recorded was 2.6 mEq/l at day 16 post-infection. The statistical analysis showed a means of 4.1 ± 1.4 mEq/l which was significant (P-value = 0.026). While the mean serum values of potassium in the mice infected-treated (B group), were decreased during this study. This decrease has started at day 16 post infection with a mean of 4.3 mEq/l. The lowest mean level recorded was 3.1 mEq/l at day 36 post infection. The statistical analysis showed a means of 5.2 ± 1.3 mEq/l which was not significant (P-value = 0.077) (Table 18 and Fig. 26).

Fig. 26. Comparison of the means of serum potassium between mice infected with *T. evansi* (Showak Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.



4.4.12.2. Umbadir Stabilated

The mean serum values of potassium in the mice infected-not-treated (C group) were decreased during the study. This decrease was started at day 12 post infection with a mean of 3.2 mEq/l. The lowest mean level recorded was 2.6 mEq/l at day 44 post-infection. The statistical analysis showed a means of 4.4 ± 1.3 mEq/l which was significant (P-value = 0.040). While the mean serum values of potassium in the mice infected-treated (D group) were normal at all days of the experiment. The statistical analysis showed a means of 6 ± 0.62 mEq/l which was not significant (P-value = 0.832) (Table 18 and Fig. 27).

Fig. 27. Comparison of the means of serum potassium between mice infected with *T. evansi* (Umbadir Stabilate) treated by Quinapyramine Sulphate a dose rate of 20mg/kgbw and mice infected-not-treated control.

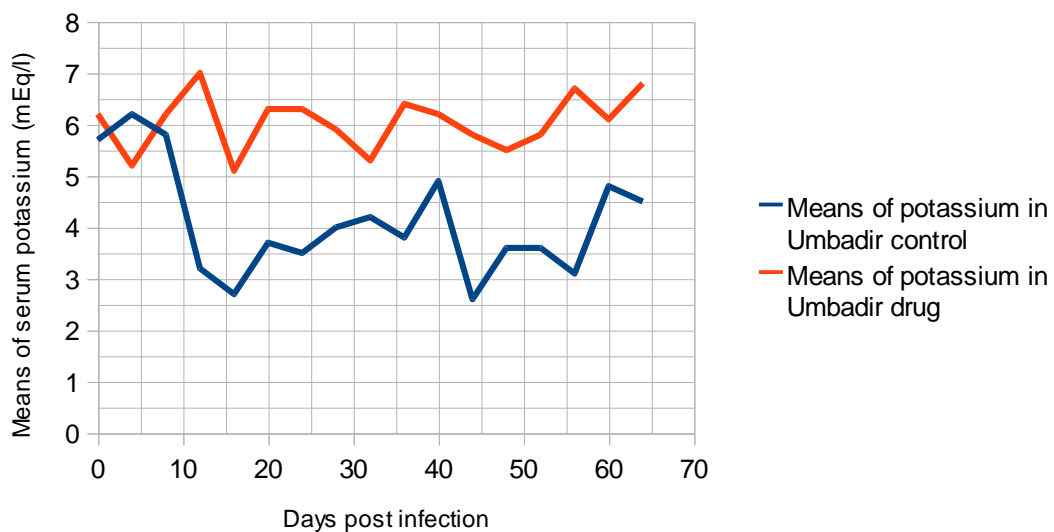


Table 18. Means serum levels of biochemical changes in mice infected experimentally with *T. evansi* mice infected-not-treated control and treated with Quinapyramine Sulphate at a dose rate of 20mg/kgbw.

parameters	units	Group A	Group B	Group C	Group D
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Total proteins	g/dL	8.2±1.3 [*]	8.7±1.3 [*]	8.2±1.9 [*]	6.4±0.84 ^{ns}
Glucose	mg/dL	37.9±13.9 [*]	46.2±12.6 [*]	45±18.4 [*]	74.9±19.8 ^{ns}
Urea	mg/dL	55.5±21.1 [*]	41±23.8 [*]	29.8±8.3 [*]	19.6±1.7 ^{ns}
Albumin	g/dL	5.9±0.97 [*]	5.8±1.7 [*]	4.7±0.88 ^{ns}	4.3±0.54 ^{ns}
Calcium	mg/dL	10.7±5.7 ^{ns}	15.3±6.8 [*]	10.7±5.8 ^{ns}	8.7±3.3 ^{ns}
Cholesterol	mg/dL	133.4±19.5 [*]	135.4±18.1 [*]	118.6±21.6 ^{ns}	98.8±24.8 ^{ns}
Creatinine	mg/dL	2.8±1.1 [*]	2.1±1.2 [*]	2.4±0.98 ^{ns}	0.54±0.35 ^{ns}
Phosphorus	mg/dL	3.8±2 ^{ns}	5.4±1.9 ^{ns}	3.3±1 ^{ns}	6.9±2.4 ^{ns}
GOT	U/L	105.2±36.1 [*]	83.6±0.35 [*]	84±27.3 ^{ns}	62.9±10.2 ^{ns}
GPT	U/L	39.8±9.2 [*]	34.8±7.9 [*]	33.5±8.1 [*]	24.2±4.2 ^{ns}
Sodium	mEq/L	148.2±3.7 ^{ns}	150±3.2 ^{ns}	147.6±4.7 ^{ns}	148.8±3.5 ^{ns}
Potassium	mEq/L	4.1±1.4 ^{ns}	5.2±1.3 ^{ns}	4.4±1.3 [*]	6±0.62 ^{ns}

GOT= Glutamate Oxaloacetate Transaminase; GPT= Glutamate Pyruvate Transaminase.

Values were expressed as Mean ±SD, ns= not significant, * = significant (P<0.05).

4.5. Histopathological changes

Representative tissue sections of the liver, kidney, heart, spleen, lungs and brain from the three groups (A, B and C) showed the followings; all tissues obtained showed exactly the same histopathological changes. No significant histopathological alterations were observed in the liver and heart. The most consistent histopathological changes were seen in the brain, lungs, spleen and kidneys.

4.5.1. Brain

Brain revealed acute congestion of meningeal capillaries, perivascular oedema, occluded capillaries parasitic emboli, neuronocrosis (vaculation), gliosis and trypomastigotes in dilated capillaries were also seen. Trypanosomes were observed in congested blood vessels of brain in rat died of teaming parasitaemia (Fig. 28).

4.5.2. Lungs

Lungs revealed oedema, congestion, multifocal alveolar emphysema, focal areas of atelectasis, increased cellularity of the alveolar wall, hyperplasia of the peri-bronchiolar lymphoid tissues, perivascular infiltration of lymphocytes around small blood vessels (venules and arterioles) and haemorrhages were also seen (Fig. 29).

4.5.3. Spleen

Spleen exhibited extensive haemorrhages and acute congestion along with segregation of lymphoid follicles, hyperplasia, reticuloendothelial cells and hypertrophy. Considerable amount of amorphous haemosiderin granules was evident in most of the sections of spleen (Fig. 30).

4.5.4. Heart

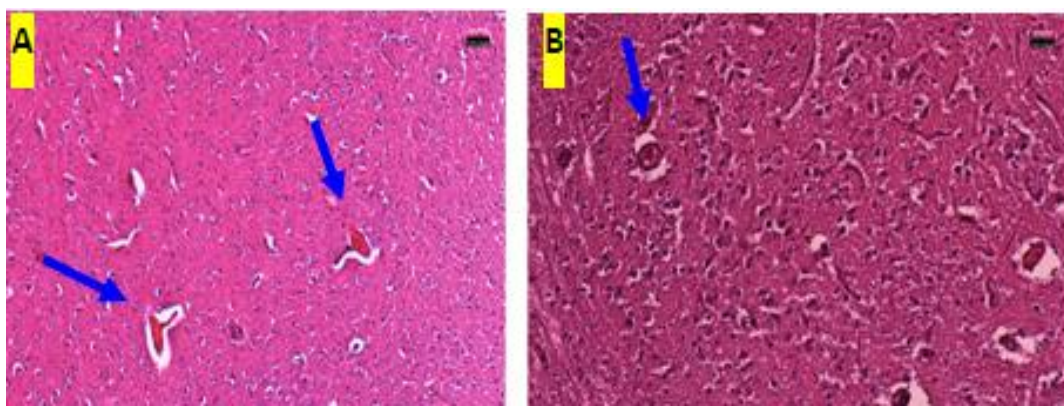
No significant histopathological alterations were observed in the heart (Fig. 31).

4.5.5. Kidneys

Acute congestion of the glomerular tuft (Fig. 32 and 33).

4.5.6. Liver

No significant histopathological alterations were observed in the liver (Fig. 34).



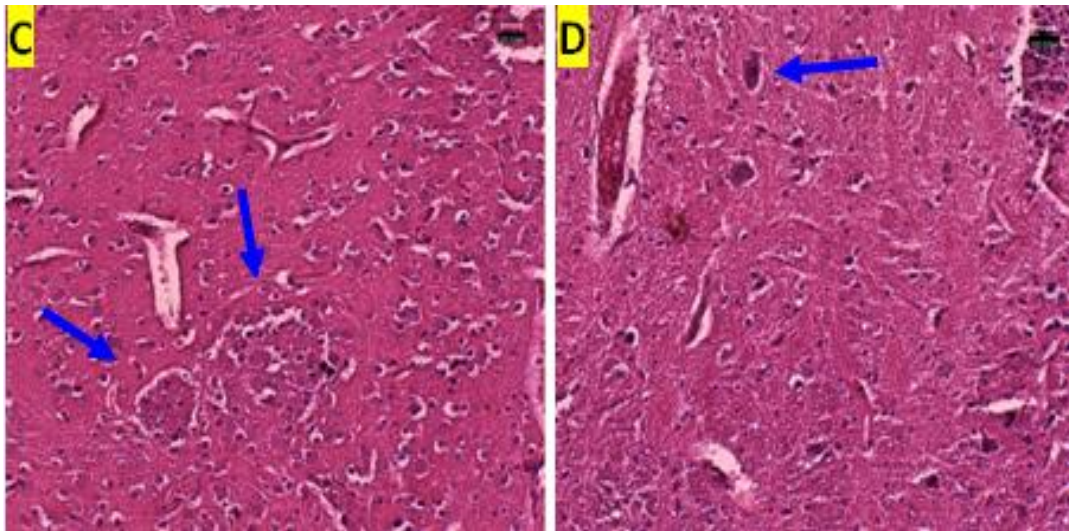


Fig. 28. Brain sections from mice infected with *T. evansi* isolated from (showak and Umbadir area) and treated with Q.S : showing congestion, perivascular edema (A:arrows), occluded capillaries: parasitic emboli (B:arrows), neuroncrosis (vaculation) and gliosis (C: arrows) and Trypomastigotes in dilated capillaries (D: arrow) (H&E stain).

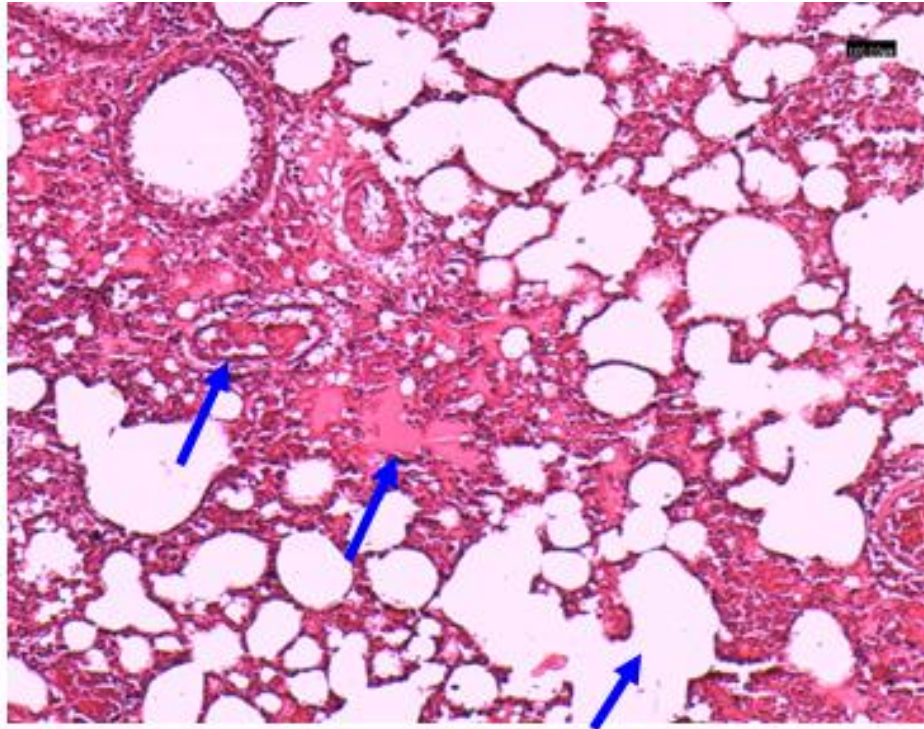


Fig. 29. Lung from mice infected with *T. evansi* isolated from (showak and Umbadir area) and treated with Q.S section showing congestion, oedema, hemorrhages and emphysema (H & E stain).

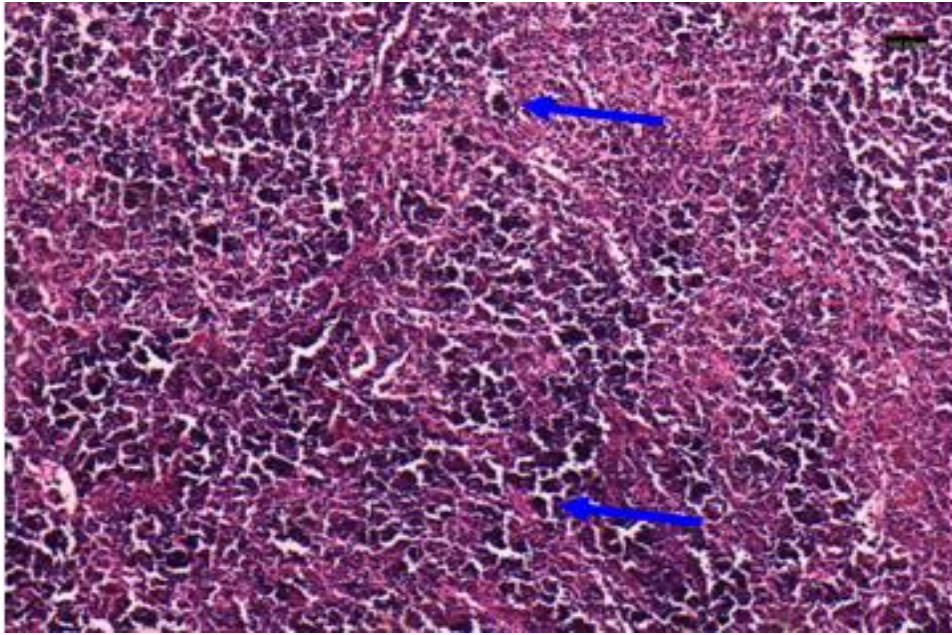


Fig. 30. Spleen from mice infected with *T. evansi* isolated from (showak and Umbadir area) and treated with Q.S: Histopathological section showing amorphous haemosiderin granules (arrows) (H & E stain).

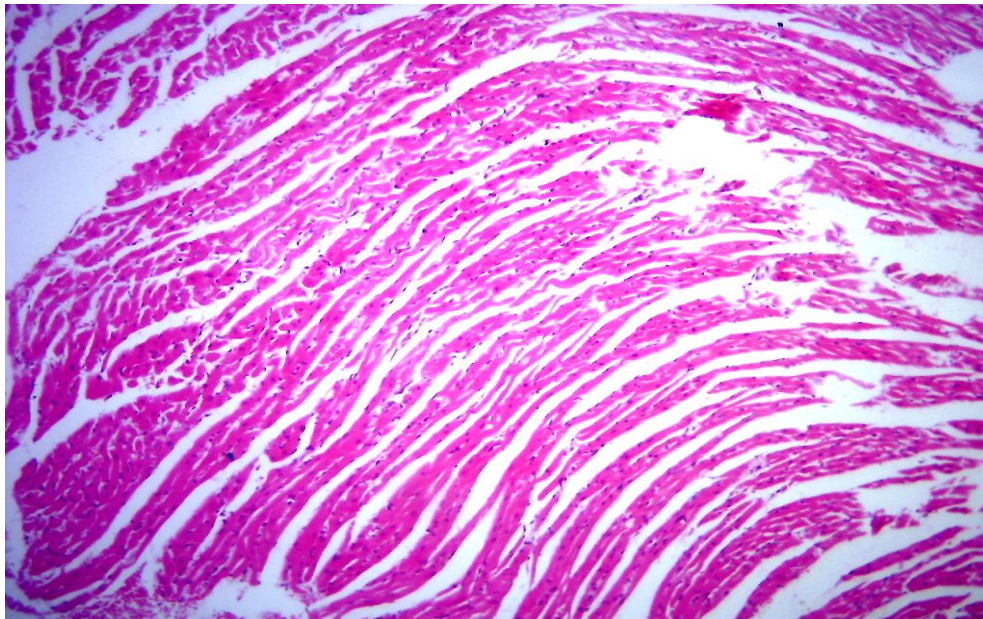


Fig. 31. Heart from mice infected with *T. evansi* isolated from (showak and Umbadir area) and treated with Q.S : No significant histopathological changes (H&E X100).

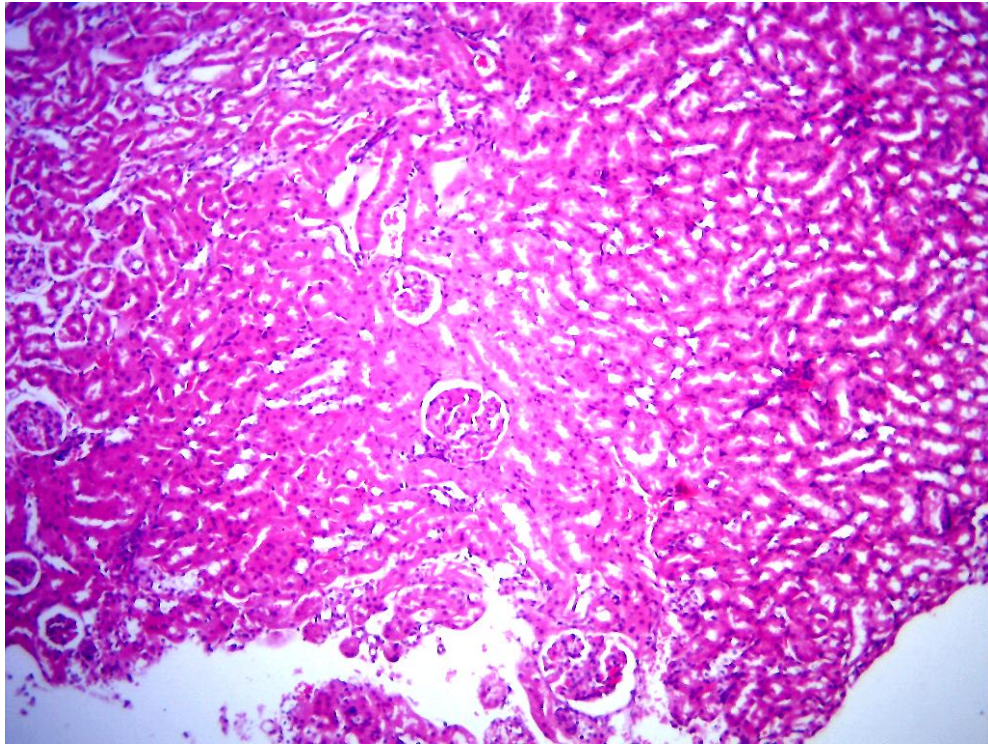


Fig. 32. Kidney from mice infected with *T. evansi* isolated from (showak and Umbadir area) and treated with Q.S: Acute congestion of the glomerular tuft (H&E X100)

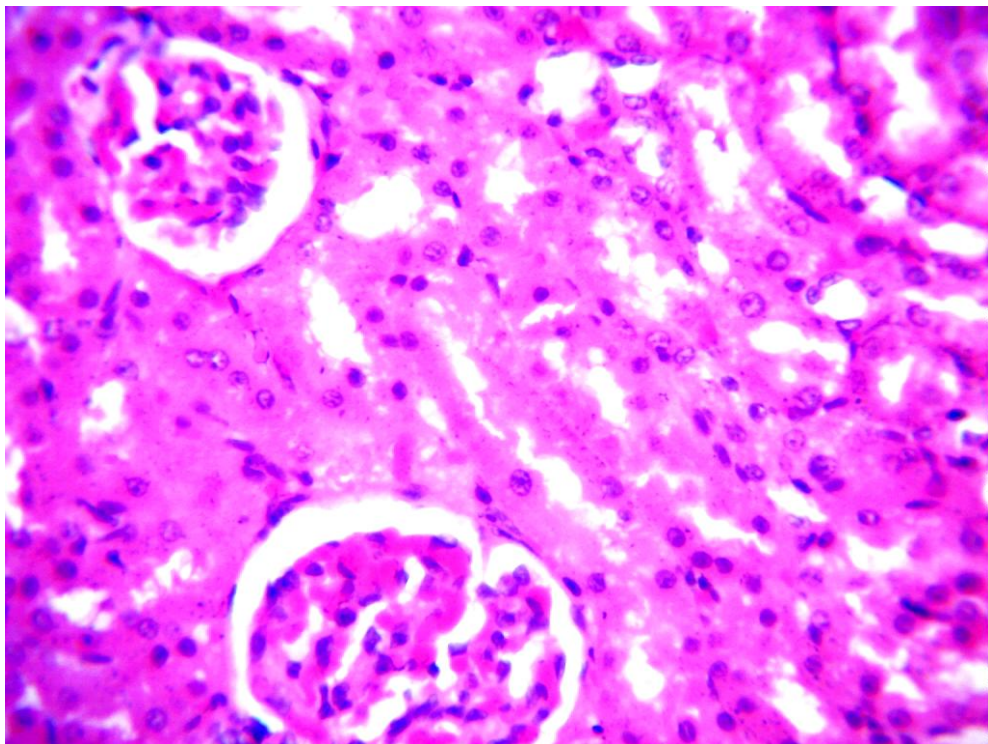


Fig. 33. Kidney from mice infected with *T. evansi* isolated from (showak and Umbadir area) and treated with Q.S : Higher magnification of the previous (H&E X400).

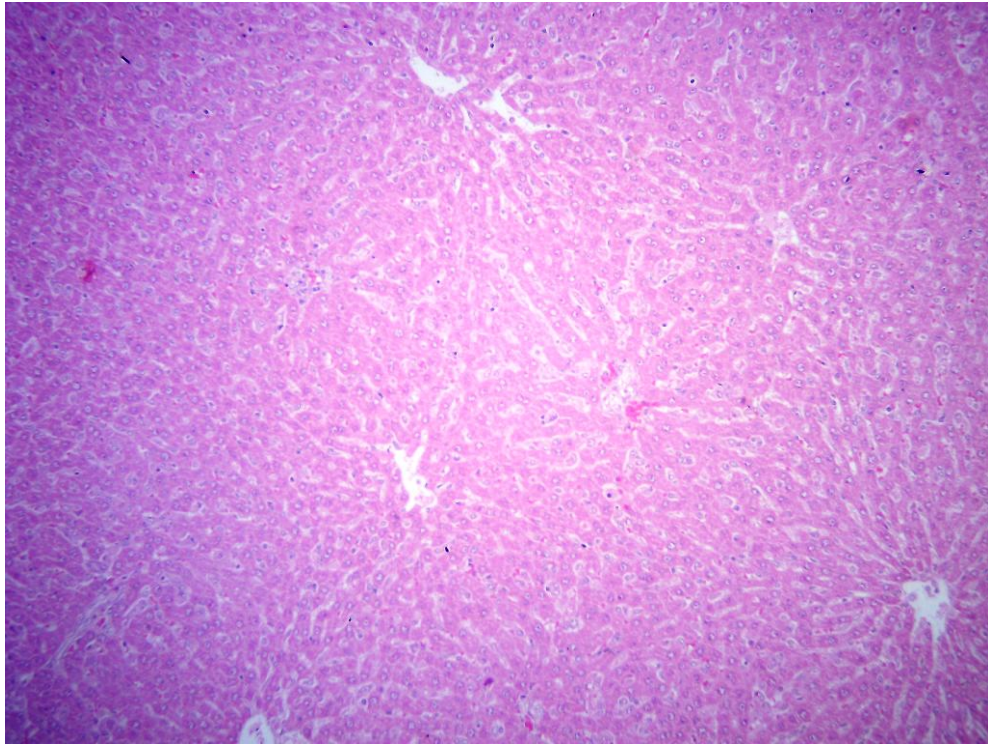


Fig. 34. Liver from mice infected with *T. evansi* isolated from (showak and Umbadir area) and treated with Q.S : No significant histopathological changes (H&E X100).

Chapter Five

Discussion

Camel trypanosomosis is a disease of major economic importance in many countries in Africa, Middle East and Asia. Although camel trypanosomosis has been recognized as the most important single cause of economic losses in camel rearing areas, yet there have been no planned campaigns to control *T. evansi* using chemotherapy and/or modern methods of fly control (Luckins, 1998). In most cases, control is limited to treating those animals that are considered to be infected on the basis of unreliable owners' clinical assessment.

In the present study, the pathogenesis of Showak stablited *T. evansi* which was isolated from a camel at a village within the vicinity of Showak area, Gedarif State, North eastern Sudan (named as Showak stock; resistant to Quinapyramine Sulphate) and Umbadir stablited *T. evansi* which was isolated from a camel at Umbadir, North Kordofan, Sudan (named as Umbadir stock; sensitive to Quinapyramine Sulphate) were investigated and studied. During this study, the pathogenicity of these two *T. evansi* stocks was compared in experimentally infected mice. The prepatent period of infection by *T. evansi* was found to be variable depending on the host and the parasite isolate. Mice inoculated by 1×10^4 of the Showak stabilate of *T. evansi* but not treated with Quinapyramine sulphate (A group) in this study, showed prepatent period of 4-6 days post infection which disagreed with the result reported by Da Silva *et al.* (2011). However, this result was in agreement with that reported by Garba *et al.* (2015) and Habila *et al.* (2012) who reported a prepatent period of 3-7 days in a donkey infected by *T. evansi*. Also a similar result was reported in a study conducted in goats (Ogbaje *et al.*, 2011). Group A has inflicted high mortalities during the experiment period which was similar to the results of Samah (2014); Hoare (1972); Silva *et al.* (2002) and Dargantes *et al.* (2005). In the mice infected-

treated with Quinapyramine Sulphate at a dose of 20 mg/kgbw (B group), the mice showed a prepatent period of 3-5 days post infection which was in agreement with the result reported by Da Silva *et al.* (2010) in cats experimentally infected with *T. evansi* as well as with rats infected by *T. evansi* (Wolkmer *et al.*, 2013) and with goats infected by *T. evansi* (Youssif *et al.*, 2008).

Mice inoculated by 1×10^4 of the Umbadir stabilate of *T. evansi* but were not treated with Quinapyramine sulphate (group C) in the present study showed a prepatent period of 3-4 days post infection which is similar to the result reported by Da Silva *et al.* (2011). The low mortalities recorded during the experiment in group C were in agreement with Faye *et al.* (2001) while Desquesnes (2013) reported that *T. evansi* (Sokoto isolate) was pathogenic to donkeys with low mortalities and high morbidity (100%). The result also was in agreement with that reported by Njiru *et al.* (2001) and Tekle and Abebe (2001) who encountered low mortality and high morbidity among camels infected with *T. evansi* in Ethiopia. In the mice infected-treated with Quinapyramine Sulphate after the patency at a dose of 20 mg/kgbw (D group), a prepatent period of 3-4 days post infection was recorded which is similar to the result reported by Da Silva *et al.* (2011); all mice remained negative and survived until the end of the study period which was attributed to the effect of the drug used.

The further confirmation of the identity of the candidate trypanosome by PCR through using primers that specifically targeted the ITS1 region of the rDNA gene of *T. evansi* that is performed in the present study, is similar to the result reported by Croof (2008) who used molecular method (PCR) in his study of 40 camels which were tested parasitologically and serologically where 90% of them were found to be positive. PCR has been used in detection of infection with *T. evansi* in buffaloes (Omanwar *et al.*, 1999; Holand *et al.*, 2000), in horses (Clausen *et al.*, 2003) and in camels

(Masiga and Nyang'ao, 2001). There was no comprehensive data on the use of PCR for detection of infection in Sudanese breed of dromedary camels (*Camelus dromedarius*). Hunter (1986) and Aradaib and Magid (2006) suggested the use of the reliable, easy to perform and less time-consuming PCR for accurate classification of trypanosome species in Sudan where the morphological feature of the trypanosome is the main tool used for its classification.

Biochemical evaluation of the body fluids gives an indication of the functional state of various body organs and biochemical changes in body fluids that result from infections depending on the species of the parasite and its virulence (Anosa, 1988).

The serum total proteins in the groups A, B and C, were increased progressively during the study which disagreed with the result reported by Hussain *et al.* (2016); Sivajothi *et al.* (2015); Biryomumaisho *et al.* (2003); Allam *et al.* (2011) and Megahed *et al.* (2012). Moreover, the result recorded in this study had contradicted the observations recorded in sheep infected with *T. brucei* studied by Taiwo *et al.* (2003). However, this increase of total protein was in agreement with the result reported by Arora and Pathok (1995) and Samia *et al.* (2004) who found that the concentration of total protein was increased in mice experimentally infected with *T. evansi*. Also, it is in agreement with the result reported by Orhue *et al.* (2005); Ekanem and Yusuf (2008) and Sow *et al.* (2014), who found that the concentration of total protein was increased in rats experimentally infected with *T. brucei* and *T. brucei*-infected rabbits. The increase in protein levels during the chronic phase of the infection is usually due to the increase in globulin levels, as a result of the immune response by the animals to the infection (Anosa and Isoun, 1976; Singh and Gaur, 1983; Rajora *et al.*, 1986). In the present study, the serum glucose in the groups A, B and C, has decreased during the study, which is similar to

the result reported by Sivajothi *et al.* (2015); Sinha *et al.* (2013); Arora and Pathok (1995) and Samia *et al.* (2004) who found that the concentration of glucose was decreased in rats experimentally infected with *T. evansi*. This situation could be explained by the parasites need for glucose for their cellular metabolism through their glycolytic pathway (Oppendoes *et al.*, 1986). However, this finding was not in agreement with that reported by Youssif *et al.* (2008) who found that goats infected by *T. evansi* had increased level of glucose.

The serum values of creatinine in the groups A, B and C, had not increased progressively during the study. This non-progressive increase of creatinine is in agreement with the results obtained in a *T. cruzi* infection in mice (Cano *et al.*, 2000), *T. brucei* infected animals (Sivajothi *et al.*, 2015; Ajakaiye *et al.*, 2014; Yusuf *et al.*, 2012) and *T. b. brucei* infected rats (Allam *et al.*, 2011; Ezeokonkwo *et al.*, 2012). However, these results were not in agreement with those obtained by Luckins (1992); Chaudhary and Iqbal (2000) and Youssif *et al.* (2008). The increase of creatinine is attributable to the increase in skeletal muscle disease, myocardial injury or necrosis and cerebral cortical necrosis; also, it could be due to destruction of kidney cells resulting in the inability of the kidneys to excrete creatinine (Ezeokonkwo *et al.*, 2012). The serum values of albumin in the groups (A, B and C), were increased during the study. This increase of albumin disagrees with the results reported by Arora and Pathok (1995) and Samia *et al.* (2004) who found that the concentration of albumin was depressed in rats experimentally infected with *T. evansi*. Also the result reported by Megahed *et al.* (2012) found that the concentration of albumin was decreased in pregnant camels infected with *T. evansi* compared with healthy pregnant camels and; also, a decrease of albumin in camels infected by *T. evansi* was further reported by Hussain *et al.* (2016).

In the present study the serum phosphorus in the groups A, B and C, were decreased, which is similar to the result reported by Youssif *et al.* (2008) in goats infected by *T. evansi* but is not similar with that reported in sheep infected with *T. congolense* (Schenk *et al.*, 2001; Neils *et al.*, 2006). This decrease of phosphorus might be due to renal excretion. The serum values of GOT and GPT in the groups A, B and C, were increased during the study. This increase was in agreement with the results obtained during an infection in sheep by *T. brucei* reported by Allam *et al.* (2011) and Taiwo *et al.* (2003), *T. vivax* infection of cattle and sheep (Gray, 1963), *T. congolense* infection of goats (Adah *et al.*, 1992), also in dogs infected with *T. brucei* (Omotainse *et al.*, 1994). Other studies have reported elevated serum enzymes (Arora and Pattok, 1995; Umar *et al.*, 2007; Ajakaiye *et al.*, 2014; Abd El-Baky and Salem, 2011). However, these findings contradict the observations made by Taiwo *et al.* (2003) during an infection of sheep with *T. congolense*. The causes of elevation of GOT and GPT levels in the serum were necrosis of the liver, skeletal muscles and kidneys (Lording and Friend, 1991) or, partly, due to cellular damage caused by lyses or destruction of the trypanosomes (Yusuf *et al.*, 2012).

The increase in serum urea in the groups A , B and C, is in agreement with the result reported by Sivajothi *et al.* (2015); Ajakaiye *et al.* (2014); Arora and Pattok (1995) and Samia *et al.* (2004) who found a similar increase in the concentration of urea in rats experimentaly infected with *T. evansi*. Megahed *et al.* (2012) reported similar results when they found that the concentration of urea was increased in pregnant camels infected with *T. evansi* compared with healthy pregnant camels. More studies had similar results among which those conducted in *T. b. brucei* infected rabbits (Arowolo *et al.*, 1988) and in goats (Adejinmi and Akinboade, 2000) and *T.b. gambiense* infected vervet monkeys (Abenga and Anosa, 2007). The elevated serum urea levels had been associated with kidney diseases such

as glomerulonephritis, urinary tract obstruction and excessive protein catabolism associated with severe toxic and febrile conditions (Anosa, 1988).

In the present study the serum calcium in the groups A , B and C, were increased during the study. Sheep infected with *T. congolense* showed increased levels of calcium (Neils *et al.*, 2006). In other studies, the levels of calcium did not change in camels infected by the protozoan (Chaudhary and Iqbal, 2000; Schenk, *et al.*, 2001], while the level of calcium was decreased in the rats infected with *T. congolense* (Egbe-Nwiyi *et al.*, 2005).

In the present study, the serum sodium in the groups A, B and C were found normal, which is not in line with the result reported in sheep infected with *T. congolense* where it increased as reported by Tella (2005). The results reported by Arora and Pathok (1995); Samia *et al.* (2004) and Wolkmer *et al.* (2013), however, revealed that the concentration of sodium was depressed in rats experimentally infected with *T. evansi*.

In the present study, the serum potassium in the groups A, B and C, were decreased which is in agreement with the result reported in sheep infected with *T. congolense* (Tella, 2005) and in sheep infected with *T. brucei* (Ogunsanmi *et al.*, 1994). Arora and Pathok (1995); Samia *et al.* (2004) and Wolkmer *et al.* (2013) also found that the concentration of potassium was depressed in rats experimentally infected with *T. evansi*, but was not in agreement with the result obtained by Ikejiani (1946) who found that serum potassium levels increased in *T. equiperdum* and *T.b. brucei* infection of rats. However, the result reported by Moon *et al.* (1968) in *T.b. rhodesiense* infected mice revealed normal level of potassium.

In the present study, the serum cholesterol in the three groups (A ,B and C), were increased which is in agreement with the findings reported by Megahed *et al.* (2012) in pregnant camels infected with *T. evansi* compared with healthy pregnant camels. Similar result was also reported by Sivajothi

et al. (2015) who found that cholesterol was increased in rats infected with *T. evansi*. However, the result reported by Egbe-Nwiyi *et al.* (2005) who showed that the level of cholesterol was decreased in rats infected with *T. congolense* and also those results reported by Barghash (2016) showed that the level of cholesterol was depressed in the rats infected with *T. evansi*, both were not in line with our findings in this study.

The main histopathological changes in the brain which revealed acute congestion of meningeal capillaries with perivascular oedema agree with the results reported by Aquino *et al.* (2002), Herrera *et al.* (2002), Dargantes *et al.* (2005), Rodrigues *et al.* (2005), Doyle *et al.* (2007) and Reham and Magdi (2013). Moreover, the presence of occluded capillaries, parasitic emboli, neuron necrosis (vaculation), gliosis and trypomastigotes in dilated capillaries were also reported by Biswas *et al.* (2010) in rats infected by *T. evansi*. The changes in the brain might be due to toxic substances released by the parasite. Also, the pathological changes in brain could be attributed to the constant irritation caused by the presence of parasites.

The main histopathological changes in the lungs revealed oedema, congestion and multifocal alveolar emphysema which is in agreement with the results reported by Takeet and Fagbemi (2009), Reham and Magdi (2013), Sivajothi *et al.* (2015b), Onah *et al.* (1996) and Auduo *et al.* (1999). The congestion and oedema in the lungs were mainly due to the inflammatory response to the parasite resulting in vasodilatation and exudation in the focal areas, atelectasis, increased cellularity of the alveolar wall, hyperplasia of the peri-bronchiolar lymphoid tissues and perivascular infiltration of lymphocytes around small blood vessels (venules and arterioles) and haemorrhages. Similar type of changes were also observed in the lungs of rats experimentally infected with *T. evansi* (Biswas *et al.*, 2001; Biswas *et al.*, 2010). However, these findings were not in line with

those reported by Nagle *et al.* (1980) who observed no changes in the lungs of *T. rhodesiense* infected rabbits.

The main histopathological changes in the spleen which included extensive haemorrhages and acute congestion along with segregation of lymphoid follicles, hyperplasia, reticuloendothelial cells and hypertrophy were similar to the results reported by Sivajothi *et al.* (2015b). Considerable amount of amorphous haemosiderin granules was evident in most of the sections of spleen which agrees with the findings of Reham and Magdi (2013) as well as with the findings reported by Bal *et al.* (2012) in the rats infected with *T. evansi*. Initial changes in the spleen may be due to immediate hypersensitivity to *T. evansi*.

The main histopathological changes in the kidneys which included acute congestion of the glomerular tuft agreed with the result reported by Bal *et al.* (2012) and Sivajothi *et al.* (2015b) in the rats infected with *T. evansi* and similar, also, with the result reported by Onah *et al.* (1996) and Auduo *et al.* (1999). It has been reported that changes in the kidneys are mainly due to the toxins produced by the parasite and the accumulation of immune complexes which impair the structure and function of the kidney (Morrison *et al.*, 1981; Ngeranwa *et al.*, 1993).

The lack of significant histopathological alterations observed in the liver in this study is similar to the result reported by Adewale *et al.* (2016); but, however, it is not similar to the result reported by Reham and Magdi (2013); Bal *et al.* (2012); Sivajothi *et al.* (2015b); Onah *et al.* (1996) and Audue *et al.* (1999). In the rats infected by *T. evansi*, where the liver revealed lesions varying from vacuolar degeneration, coagulative necrosis along with congestion and haemorrhages, these effects might be due to hypoglycemia leading to starvation of the cells.

The lack of significant histopathological alterations observed in the heart is similar to the result reported by Adewale *et al.* (2016); but is not similar to

the result reported by Reham and Magdi (2013); Sivajothi *et al.*, (2015b) and Bal *et al.* (2012) in the rats infected with *T. evansi*.

Conclusion

The identity of a local stabilate of *Trypanosoma evansi* was confirmed through adopting PCR where primers that target the internal transcribed spacer one (ITS1) of the ribosomal DNA were used.

In the present study, the pathogenesis of Showak stabilated *T.evansi* which was isolated from a camel at a village within the vicinity of Showak area, Gedarif State, North eastern Sudan (named as Showak stock; resistant to Quinapyramine Sulphate) and Umbadir stabilated *T.evansi* which was isolated from a camel at Umbadir, North Kordofan, Sudan (named as Umbadir stock; sensitive to Quinapyramine Sulphate) were investigated and studied. During this study, the pathogenicity of these two *T. evansi* stocks was compared in experimentally infected rats. Rats inoculated by 1×10^4 of the Showak stabilate of *T. evansi* but treated with Quinapyramine sulphate (A group) in this study, showed high mortalities during the experiment period. Rats inoculated by 1×10^4 of the Umbadir stabilate of *T. evansi* but were not treated with Quinapyramine sulphate (group C) in the present study showed a low mortalities recorded during the experiment in group C, In the rats infected-treated with Quinapyramine Sulphate after the patency at a dose of 20 mg/kgbw (D group), All rats survived until the end of the study period.

In the present study, the serum total proteins, creatinine, albumin, calcium, GOT, GPT, urea and cholesterol in the groups A, B and C were increased during the study. The serum glucose, phosphorus and potassium in the groups A, B and C were decreased during the study. The serum sodium in the groups (A, B and C) were found normal. All biochemical parameters in group D were normal. Histopathological changes Representative tissue sections of the liver, kidney, heart, spleen, lungs and brain from the three groups (A, B and C) showed the followings; all tissues obtained showed exactly the same histopathological changes. No significant

histopathological alterations were observed in the liver and heart. The most consistent histopathological changes were seen in the brain, lungs, spleen and kidneys.

Recommendations

Depending on the results of this study, the followings can be recommended:

- 1- To conduct similar research for *Trypanosoma evansi* camel isolates from Kordofan, Gadarif State and other areas in the Sudan in albino mice and other experimental animals.
- 2- To investigate the gross lesions and histopathological changes produced by the different isolates of *T. evansi* in mice.
- 3- To observe and investigate the drug resistance and susceptibility in rats experimentally infected with different *Trypanosoma evansi* isolates.

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Appendix

Appendix I:-

Trypanosome counting reagent:

Formalin	0.5 ml
Glacial acetic acid	2.0 ml
Carbol fuchsin	1.5 ml
Distilled water	21.5 ml

Appendix II:-

Mice Biochemical Reference Ranges:

parameters	Ranges Values	units
Total proteins	5.6 -7.6	g/dL
Glucose	50 – 135	mg/dL
Urea	15 – 21	mg/dL
Albumin	3.8 - 4.8	g/dL
Calcium	5.3-13 mg/dl	mg/dL
Cholesterol	40-130	mg/dL
Creatinine	0.2 – 0.8	mg/dL
Phosphorus	3.11-11 mg/dL	mg/dL
GOT	45.7 – 80.8	U/L
GPT	17.5 – 30.2	U/L
Sodium	143 - 156	mEq/L
Potassium	5.4-13 mg/dL	mEq/L