

**EFFECTS OF ACUTE EXPERIMENTAL *TRYPANOSOMA BRUCEI BRUCEI*
INFECTION ON SERUM PROFILES OF ENDOGENOUS ANTI-OXIDANTS
IN RELATION TO TISSUE AND HAEMATOLOGICAL CHANGES IN
WISTAR RATS.**

BY

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SEPTEMBER, 2018

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WISTAR RATS.**

BY

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**A DISSERTATION SUBMITTED TO THE SCHOOL OF
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**DEPARTMENT OF VETERINARY PATHOLOGY
FACULTY OF VETERINARY MEDICINE
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA**

SEPTEMBER, 2018

DECLARATION

I declare that the work reported in this dissertation entitled “**Effects of acute experimental *Trypanosoma brucei brucei* infection on serum profiles of endogenous anti-oxidants in relation to tissue and haematological changes in *Wistar* rats**” was performed by me in the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria – Nigeria, under the supervision of Professor K.A.N. Esievo and Professor S. Adamu. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation has been previously presented for another degree or diploma at any institution.

Juwon Pius ERIN
Name of Student

.....
Date

CERTIFICATION

This dissertation, entitled **‘EFFECTS OF ACUTE EXPERIMENTAL *TRYPANOSOMA BRUCEI BRUCEI* INFECTION ON SERUM PROFILES OF ENDOGENOUS ANTI-OXIDANTS IN RELATION TO TISSUE AND HAEMATOLOGICAL CHANGES IN *WISTAR* RATS’**, carried out by Juwon Pius ERIN meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to God, my dad (Late Elder L.A Erin) of blessed memory and my mum (Dcns Patricia Erin) for holding on and being a flawless source of support and inspiration for my advancement in life and career.

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ABSTRACT

The alterations in serum profiles of endogenous antioxidants in relation to sequential tissue and haematological changes were determined to assess the impacts of oxidative stress in *Wistar* rats experimentally infected with *Trypanosoma brucei brucei*. A total of 40 adult male rats, used for this research were acclimatized and allocated based on their mean body weight into infected and control groups, of 20 rats each. Blood was obtained from an infected donor rat at peak parasitaemia and reconstituted with physiological saline to give 1×10^6 trypanosomes/ml, 1 ml was then injected intraperitoneally to each of the rats in the infected group. On the day of infection (day 0 post-infection (pi)), before the infection of the rats with the trypanosomes, 4 rats each from the infected and control groups were sacrificed; blood was collected into plain and EDTA sample bottles; to obtain serum and for determinations of haematological parameters, respectively. Post-mortem examination was carried out while tissues samples were collected and fixed in 10 % formalin for histopathology and representative portion of the liver and kidney were also stored in phosphate buffered saline solution. Following the same modality as on day 0 pi, blood and tissue samples collection were repeated on days 3, 5 and 7 pi. The sera were assayed for activities of super oxide dismutase (SOD) and glutathione peroxidase (GPx), serum biochemicals and enzymes and the liver and kidney homogenate were assayed for reduced glutathione (GSH) concentrations. The pre-patent period was 3.75 ± 0.11 days and the levels of parasitaemia increased throughout the study. Clinical manifestations were anorexia, pale ocular mucous membrane, polyuria, respiratory distress, unthriftiness, raised hair coat, and weight loss. There was a significant ($p < 0.05$) reduction in the packed cell volume (PCV) and total white blood cell count (TWBC), 33.00 ± 2.89 % and $6.93 \pm 0.48 \times 10^9/L$, respectively on day 7 pi when compared to the control and pre-

infection values. Serum SOD activity had a significant ($p < 0.05$) negative correlation ($r = -0.9$) with the levels of parasitaemia in the *T. b. brucei*-infected rats. All the other markers of antioxidation; serum GPx activity and organ (liver and kidney) GSH concentrations presented a decrease with increase in the levels of parasitaemia, although not statistically significant ($p > 0.05$). Serum GPx activity had a significant ($p < 0.05$) positive correlations with liver ($r = 0.96$) and kidney ($r = 0.93$) GSH concentrations, respectively. All the measured serum biochemicals and enzymes had a significant ($p < 0.05$) negative correlation with serum GPx activity; aspartate aminotransferase (AST) ($r = -0.99$), alanine aminotransferase (ALT) ($r = -0.97$), alkaline phosphatase (ALP) ($r = -0.96$) and urea ($r = -0.99$) except creatinine. The activity of AST were significantly ($p < 0.05$) higher on days 5 and 7 (101.70 and 104.60 U/L, respectively) pi while ALT activity (57.20 ± 3.26 U/L) and concentrations of urea (9.32 ± 0.98 mmol/L) and creatinine (111.10 ± 6.09 μ mol/L) were significantly ($p < 0.05$) higher on day 7 pi. Hepatic and renal lesions observed were mainly congestion, mononuclear cells infiltrations and erythrophagocytosis which were most severe on day 7 pi. There were also degeneration and necrosis of hepatocytes and epithelial cells of the renal tubules. In conclusion, infection of rats with *T. b. brucei* caused a decrease in the serum antioxidant enzymes activities proportional to the level of parasitaemia and duration of the infection is believed to be responsible for sequential tissue damage.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

African trypanosomosis is caused by flagellate protozoan parasites of the genus, *Trypanosoma* (Hoffman *et al.*, 2013). The pathogenic trypanosome species in domestic animals are *Trypanosoma vivax* (*T. vivax*), *T. congolense* and *T. brucei* in cattle, sheep and goats, and *T. simiae* in pigs (Nantulya, 1990; Adamu *et al.*, 2009). In humans, the trypanosome species responsible for the disease are *T. brucei gambiense* and *T. brucei rhodesiense* (Smith *et al.*, 1998; Dumas and Bisser, 1999). The parasites inhabit the blood plasma, intercellular tissues and body cavity fluid of an infected animal precipitating anaemia and tissue damage (Sharma *et al.*, 2000). African trypanosomosis is a serious threat to human and animal health in sub-Saharan Africa. It was estimated that 60 million people and over 30% of cattle population in the region are at risk of the infection (WHO, 1998; 2005; Sachs, 2010).

The clinical manifestations of the disease includes anaemia, weight loss, weakness, anorexia, jaundice, abortion, low milk production, decrease in reproductive capability and geophagia (Radostitis *et al.*, 2003). Anaemia which is a major cardinal sign of trypanosomosis, had been incriminated in the tissue degenerative changes and consequently most of the tissue disorders observed in affected animals (Ekanem and Yusuf, 2008; Akanji *et al.*, 2009). Several pathophysiological mechanisms had been put forward to explain the development of anaemia in trypanosome-infected animals (Esievo *et al.*, 1982; Igbokwe, 1994; Sharma *et al.*, 2000). Generation of reactive oxygen species, during infection with trypanosomes, had been reported to play a

contributory role in the development of the anaemia (Igbokwe, 1994; Ogunsanmi and Taiwo, 2007; Akanji *et al.*, 2009). There is, however, dearth of information on the possible effect of trypanosomosis on serum antioxidants profile in affected animal or human host, hence the need for a research in this direction.

1.2 Statement of Research Problems

African trypanosomosis has been a global concern and biomedical scientists have been striving to develop integrated approach to control both the human and animal forms of the disease, by application of all findings in natural hosts and laboratory animal models (Abenga, 2014). Many research findings had demonstrated the ameliorative effects of different antioxidant sources in experimental trypanosomal infections. For example the ameliorative effects of Vitamin C and E (Umar *et al.*, 1999, 2000, 2001), methanolic extract of *Calotropis gigantea leaves* (Shaba *et al.*, 2011), crude extract of *Solanum nigrum* (Serem *et al.*, 2013), methanolic extract of ginger (Kobo *et al.*, 2014a) and flavonoid mixture (Kobo *et al.*, 2014b) on oxidative stress strongly suggested that oxidative stress is a contributory pathophysiologic mechanism of trypanosomosis. However, little work had been done to evaluate the levels of serum endogenous antioxidants in relation to tissue damage in affected hosts. Information on the nature of alterations in these serum factors may provide clues in estimating the degree of tissue damage in trypanosome-infected animals and, thus, aid in management of the disease.

Trypanosomosis was described as one of the most neglected diseases especially in terms of drug development (WHO, 1998; Truc, 2003). So far, treatment had been focused on specific causative agents of the disease, the trypanosomes, while supportive treatment via identification of specific plasma biochemical alterations had not been given due consideration. Over two decades ago, the need to renew interest in organ-

related changes in trypanosome-infected animals was emphasized (Nok *et al.*, 1995). Research efforts should therefore be geared towards unraveling the effects of generated free radicals (reactive oxygen species) on tissues and antioxidant arsenal of the host, which may become a prerequisite for strategic research towards drug development in the management of the disease (Abenga, 2014).

1.3 Justification of the Study

In spite of the intensive researches carried out on trypanosomosis, the disease has continued to be an impediment to profitable livestock production (Esievo and Saror, 1991; Omotainse *et al.*, 2000). An in depth knowledge of the mechanisms of tissue damage and serum biochemical alterations could pave way to development of more effective treatment approaches to manage the disease in trypanosome-infected animals especially in this period of cutting edge biotechnology. Since oxidative stress had been identified as one of the contributory mechanisms of tissue damage in trypanosomosis (Igbokwe, 1994; Ogunsanmi and Taiwo, 2007; Umar *et al.*, 2007), understanding of the specific alterations in serum levels of antioxidants may provide not only additional clues in diagnosis of the disease, but could also guide in estimating degree of tissue damage and in the administration of supportive treatments.

Since free radicals are normally scavenged by the antioxidant system of the organism, which comprises an enzymatic component (glutathione peroxidase, catalase and superoxide dismutase as the major antioxidant enzymes) and a non-enzymatic component (glutathione, selenium, vitamin C and E) (Cadenas and Davies, 2000; Lu *et al.*, 2010); knowledge of the magnitude of alterations in their serum profiles may prove vital in further understanding of the mechanism of development of the disease in affected animals.

1.4 Aim of the Study

To determine the serum endogenous antioxidants responses and relate them to sequential tissue and haematological changes in *Wistar* rats experimentally infected with *Trypanosoma brucei*.

1.5 Objectives of the Study

The objectives of the study are to determine the:

- a. changes in the serum profiles of endogenous antioxidants in rats experimentally infected with *T. brucei*.
- b. changes in level of organ (liver and kidney) reduced glutathione (GSH) concentrations in rats experimentally infected with *T. brucei*
- c. relationship between the alterations in serum levels of endogenous antioxidants and organ reduced glutathione concentration and level of parasitaemia, haematologic parameters and tissue pathology (gross and histopathology).

1.6 Statement of Research Hypothesis (Ho)

- a. There is no alteration in the serum levels of endogenous antioxidants in *Wistar* rats experimentally infected with *T. brucei*.
- b. There is no change in the level of organ reduced glutathione concentration in *Wistar* rats experimentally infected with *T. brucei*.

c. There is no relationship between the alterations in the levels of endogenous antioxidants and organ glutathione concentrations and the levels of parasitaemia, haematologic parameters and tissue pathology in Wistar rats experimentally infected with *T. brucei*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Trypanosomosis

Trypanosomosis is a parasitic disease caused by infection with haemoprotozoan parasite of the genus *Trypanosoma*, which belongs to the phylum; Sarcomastigophora, order; Kinetoplastida and family; Trypanosomatidae. Trypanosomes multiply in the blood stream, intercellular tissues and body cavity fluid (Stevens and Brisse, 2004). Transmission of the disease is by inoculation of the parasites but not contagious (except dourine, a venereal trypanosomosis of equines) (Ugochukwu, 2008).

Trypanosomes are microscopic, unicellular, elongated and motile parasites; with a single flagellum located at their base around a structure, called kinetoplast that house the mitochondrial DNA of the parasite (Vickerman, 1985; Stevens and Brisse, 2004). The *Trypanosoma* genus is further subdivided into two sections, namely the Stercoraria and Salivaria, based on how the parasites are transmitted from the insect vector to the mammalian host as the parasite completes their cyclic development (Uilenberg *et al.*, 1998).

In the stercoraria type, the infective trypanosomes develop in the hindgut and are transmitted via the faeces of the insect vector, while the salivarian parasites develop into the infective form in the anterior part of the digestive tract of the tsetse fly and are inoculated via the saliva into the mammalian host (Stevens and Brisse, 2004). A striking feature of the salivarian species is that they possess variable surface glycoproteins (VSGs), which enable them to change their surface coats by a process known as antigenic variation (Donelson, 2003; Stevens and Brisse, 2004). The

salivarian species are further divided into four subgenera; Duttonella, Nannomonas, Trypanozoon and Pycnomonas (Stevens and Brisse, 2004).

Trypanosomes are pathogenic, not only to animals but also affect humans where they cause sleeping sickness. The trypanosomes of veterinary and medical importance had been described (Stevens and Brisse, 2004; Abenga *et al.*, 2005). Most mammalian species are to some degree susceptible to trypanosomosis transmitted by various haematophagous insects, mainly *Glossina* species commonly known as tsetse flies, which are considered to be the true intermediate hosts of trypanosomes (Esievo and Saror, 1991; Ugochukwu, 2008). The American trypanosomosis (Chagas disease, due to *Trypanosoma cruzi*) was reported, in the 1980s, to infect over 24 million people with another 100 million people at risk of the infection (Walsh, 1984), although these estimates have since reduced. Chagas disease is also considered the most important parasitic disease affecting Latin America, with a social and economic impact in excess of the combined impact of other diseases as malaria, leishmaniasis and schistosomiasis. The World Health Organization as at 2006 estimated 300,000 new cases of human African trypanosomosis yearly, while the economic cost of animal trypanosomosis as at the year 2000 was estimated at US\$4.75 billion per year (Swallow, 2000). The tsetse transmitted African animal trypanosomosis (including *T. b. brucei*, *T. vivax*, *T. congolense*, and *T. simiae*) has a greater impact, denying livestock vast areas of grazing, and affecting agricultural production both directly and indirectly by limiting the use of draught animals.

2.1.1 Trypanosomes strain variation

Trypanosome strain variation has been acknowledged to influence the disease process, and has also been incriminated for driving the disease towards severity or otherwise in

the affected host (Garcia *et al.*, 2006; Sternberg and MacLean, 2010). Recent research has shown that the virulence of trypanosome is selective and some selected traits have been identified in their genes, as virulence factors, which are determinant of the disease outcome (Morrison 2011). They have also been suggested to be responsible for the difference in disease expression of the two human infective *Trypanosoma brucei* subspecies; *T. b. gambiense* causes a chronic infection while *T. b. rhodesiense* results in an acute and severe disease (Garcia *et al.*, 2006; Morrison 2011).

The term ‘virulent trypanosome’ according to Morrison (2011) has been used to qualify different trypanosomes, based on the disease outcome;

- (i.) the level of parasitaemia and the prepatent period, with virulent strains being those that give a high parasitaemia and short prepatent period,
- (ii.) transmission efficiency by the vector, with virulent strains being those that are transmitted rapidly and give high levels of vector infection, and
- (iii.) the level of pathology in the affected host, with virulent strains causing a more severe pathology.

The level of genetic variation and differentiation has been described as contributory to disease expression and outcome. Further research is needed to know how this genetic variation translates into or affects the disease process (Garcia *et al.*, 2006; Morrison 2011). The use of post-genomic tools seems promising, offering an incredible opportunity to identifying regulatory genes and visualize *in vivo* the host–parasite interactions as it influence disease process (Garside and Brewer, 2008; Morrison 2011).

2.2 African Trypanosomosis

The African trypanosomosis is an infectious disease of humans and animals with similar aetiology and epidemiology. The distribution of the disease corresponds to the tsetse flies belt, which cover an estimated area of about 8 million km² between 14⁰ North and 20⁰ South of the latitude (Steverding, 2008). During the rainy season the tsetse fly populations further encroach into more livestock herds and settlements, increasing the risk of trypanosomal infection (Ahmed, 2005; Odeyemi *et al.*, 2015). Throughout history, African trypanosomosis has greatly affected the economic, social and cultural development of the region (Anene *et al.*, 2000; Steverding, 2008).

2.2.1 African animal trypanosomosis

African animal trypanosomosis (or nagana) is caused by *T. brucei spp*, *T. congolense* and *T. vivax*. In wild animals, they cause a rather mild infection, but cause a severe, often fatal disease in our domestic animals. All domestic animals can be affected by nagana and the symptoms, includes; fever, listlessness, emaciation, hair loss, discharges from the eyes, oedema, anaemia, and paralysis (Steverding, 2008). As the illness progresses, infected animals also invariably become weak, till they eventually become unfit for work, a probable reason why the natives named the disease "Nagana" meaning "powerless or useless" in the Zulu language (Steverding, 2008).

2.2.2 Human African trypanosomosis

Human African trypanosomosis or sleeping sickness is caused by two subspecies of *T. brucei*, *T. brucei gambiense* and *T. brucei rhodesiense*, while the third subspecies, *T. brucei brucei*, is only infectious to animals. *T. brucei gambiense* is responsible for the chronic form of sleeping sickness in West and Central Africa, whereas *T. b.*

rhodesiense gives rise to the acute form of the disease in East and Southern Africa. There are two distinct stages during the course of sleeping sickness. The first or early stage of the disease, also known as the haemolympathic phase, is defined by the restriction of the trypanosomes to the blood and lymph system (WHO, 2005). The symptoms of this stage are fever, headaches, joint pains and itching. The second or late stage of the disease, also known as the neurological phase, is characterized by the presence of the parasites in the cerebrospinal fluid (WHO, 2005). In general, this is when the typical signs of the disease occur: confusion, disturbed sleep pattern, sensory disturbances, extreme lethargy, poor condition and coma. If left untreated, sleeping sickness patients die within months when infected with *T. b. rhodesiense* or within years when infected with *T. b. gambiense* (WHO, 2005; Simo *et al.*, 2006;). Wild and domestic animals may play a major role as parasite reservoirs for human infections with trypanosomes (Njiokou *et al.*, 2006).

2.3 Transmission of African Animal Trypanosomosis

Arthropod vectors are the intermediate host responsible for the transmission of trypanosomes either cyclical or non-cyclical (Urquhart *et al.*, 2002). In cyclical transmission, the trypanosomes multiply in the arthropod, where they undergo series of morphological transformation into the infective form of the parasite. When the multiplication occurs in the digestive tract and proboscis and the infection is transmitted during feeding, it is termed as anterior station development. On the other hand, when multiplication and transformation occur in the gut and the infective forms migrate to the rectum and are passed in the faeces is posterior station development. Trypanosomes (except *T. equiperdium*, and *T. evansi*) are transmitted cyclically by tsetse fly. In non-cyclical transmission, trypanosomes are transferred from one

mammalian host to another by the interrupted feeding of biting insects, notably Tabanids and Stomoxys (Maxie *et al.*, 1979; Soulsby, 1982). This activity is responsible for the persistence of *T. vivax* in areas of Africa free from tsetse flies as well as in several South American countries like Brazil, Colombia, and Guyana (Maxie *et al.*, 1979).

2.3.1 Life cycle

The vector, tsetse flies ingest trypanosomes during their feeding on an infected host. These trypanosomes lose their glycoprotein surface coat and in the case of *T. brucei* and *T. congolense*, become elongated and multiply in the midgut, migrating forward to the salivary gland (*T. brucei*) and the proboscis (*T. congolense*) (Urquhart *et al.*, 2002). They, subsequently, undergo a transformation losing their typical trypanosome or trypomastigote form and acquire an epimastigote form, characterized by the fact that the kinetoplast lies just in front of the nucleus. After further multiplication of the epimastigotes, they transform again into small typically trypomastigote forms with a glycoprotein surface coat. These are the infective forms of the parasite; known as the metacyclic trypanosomes, which are required to transmit the infection to the next host. The entire process takes at least two to three weeks and the metacyclic trypanosomes are inoculated into the new host when the tsetse fly feeds on them (Hoare, 1972; ILRAD, 1990).

2.3.2 Clinical manifestation of Trypanosomosis

Animals suffering from trypanosomosis may manifest syndromes ranging from subclinical, mild or chronic infections to acute or fatal form of the disease (Stephen, 1986; Holmes *et al.*, 2000). The severity of the disease is dependent on the species and breed of affected animals and the dose and virulence of the infecting trypanosomes.

Other factors which play prominent roles in the disease process include stress, poor nutrition and concurrent diseases (Holmes *et al.*, 2000). Dog and cat are susceptible to *T. brucei* and *T. congolense* (Nfon *et al.*, 2000). *Trypanosoma congolense* infection may result in peracute, acute or chronic disease in domestic animal species (cattle, sheep, goat, horse and camel). Dogs commonly suffer chronic *T. congolense* infection even though there have been reports of acute form of the disease in experimental infections (Ezeokonkwo, 2009). *Trypanosoma brucei* manifests mild, chronic or subclinical infection, except in horse, camel, dog and cat where it causes severe to fatal infection which if untreated almost invariably cause mortality (Ezeokonkwo, 2009). The prepatent period (PP) is shorter for *T. brucei* (5-10 days) than *T. congolense* (7-24 days) (Ezeokonkwo, 2009). The PP is followed by intermittent fever, depression, lethargy, weakness and anorexia. The heartbeat and respiration may be increased and there is progressive anaemia (paleness of mucous membrane), loss of body condition and generalized enlargement of superficial lymph glands. Abortion is common in females (Anene and Omamegbe, 1984; Anene *et al.*, 1991). Animals become extremely weak at the terminal stage of the disease and death is associated with congestive heart failure due to anaemia and myocarditis or secondary bacterial or viral infections. The secondary infections are believed to develop because immune defense mechanisms are compromised in trypanosome infected animals (Ikeme *et al.*, 1984; Anene *et al.*, 1989a).

2.3.3 Diagnosis

Trypanosomal infections are often diagnosed based on clinical manifestations of the disease and use of parasitological techniques. The clinical signs of the animal trypanosomosis are indicative but not pathognomonic, a reason for which further

laboratory confirmation is required in its diagnosis (Elnasri, 2005). The classical direct parasitological methods for the diagnosis of trypanosomosis, namely microscopic examination of blood or lymph node material, are not highly sensitive, but a number of techniques, including enrichment of the sample, rodent inoculation and DNA methods may increase the sensitivity (Tran *et al.*, 2009). In a review by Dagnachew and Bezie, 2015 the following were listed, as the other diagnostic techniques aside the use of clinical presentation;

2.3.3.1 Parasitological diagnosis

The basic techniques include the use of fresh blood samples (wet mount) or stained blood film (thin or thick smear). Modification has since been made to improve diagnostic sensitivity by concentrating the blood through centrifugation in a haematocrit tube by the haematocrit centrifuge technique (HCT) or the dark ground buffy coat technique (DG) (Woo, 1970). The HCT is one of the most widely used parasitological techniques. Motile trypanosomes can be viewed between the leucocyte layer and plasma, enabling the detection of trypanosomes six to ten days before they can be detected in either thin or thick smear (Woo, 1970; Molyneux, 1975). In order of decreasing sensitivity, the results were as follows: DG>HCT> thick smear > thin smear > wet mount (Paris *et al.*, 1982). Suggested modification although not routinely used, involves the separation or removal of blood cells prior to centrifugation by miniature anion exchange chromatography or hypotonic lysis (Nantulya, 1990). Freshly collected blood can also be inoculated into laboratory rodents which can then be examined for periods of 30 to 60 days for the development of trypanosome infection (Paris *et al.*, 1982).

2.3.3.2 Immunological Techniques:

By serological techniques like immunofluorescence (IFA) and enzyme linked immunosorbent assay (ELISA), direct and indirect diagnosis of trypanosomes in the infected host can be carried out. There are a good number of serological tests available for detection of trypanosomal antibodies but they have a rather poor specificity, because of cross-reaction from the different species of the genus *Trypanosoma*. The species specificity of the assay was improved following the development of monoclonal antibodies as capture antibodies that recognized the specific circulating antigens in the infected host (Nantulya and Lindqvist, 1989; Nantulya, 1990).

2.3.3.3 Molecular techniques

These are used for the demonstration of nucleotides sequences, which are specific for all trypanosome subgenus, species or even type or strain. Nucleotides are the constituents of DNA, the molecules which constitute the genes on the chromosomes in the cell nucleus. A positive result indicates active infection with the trypanosome for which the sequences are specific, as parasite DNA will not persist for long in the host after all live parasites have been eliminated. These tests are not only suitable for detecting parasites in the mammalian host, but also in the insect vector (Masake *et al.*, 1996).

2.3.3.4 Isolation and purification of blood trypanosomes

For isolation of blood trypanosomes, different biochemical, biological and physical methods have been used. Like the ion-exchange chromatography which is a biochemical based procedure, described by Lanham and Godfrey (1970), based on differences in electrical charges between erythrocytes and trypanosome cell

membranes. This technique is widely used in parasite separation and antigen preparation for ELISA antibody detection tests (Desquesnes, 2001).

2.3.4 Pathogenesis

Animals become infected with trypanosomes when they are bitten by tsetse fly. In the process of taking a blood meal from an animal, infected tse tse fly deposits saliva laden with trypanosomes in the connective tissue of the animal's skin. At this site of inoculation, the metacyclic forms multiply locally and differentiate to the blood stream form, which is specially adapted to live in mammalian blood, producing within a few days raised cutaneous inflammatory swelling called chancre (Urquhart *et al.*, 2002). Thereafter, they enter the blood stream, multiply by binary fission and a parasitaemia detectable in the peripheral blood usually becomes, apparent 1-3 weeks later. Once in the blood, the parasites have access to most other major organs. With the appearance of parasites in the blood, susceptible animals develop intermittent fever and anaemia (ILRAD, 1990). Subsequently, the parasitaemia may persist for many months, although its levels may wax and wane due to immune response of the host. The fever is highest at the first peak of parasitaemia and fluctuates thereafter with parasitaemic waves (Taylor and Authie, 2004).

Anaemia develops with the onset of parasitaemia and it is the cardinal feature of the disease (Anosa, 1988; Urquhart *et al.*, 2002; Naessens *et al.*, 2005). The anaemia that occurs during acute trypanosomosis is due primarily to accelerated destruction of red blood cells (Jennings *et al.*, 1974, 1977; Valli and Forsberg, 1979; ILRAD, 1990). Red cells are phagocytosed by activated macrophages and the haemoglobin of red cells is digested and stored in the macrophages as iron complexes. Early in a trypanosome infection, the number of macrophages increases throughout the body. This expanded

pool of macrophages actively remove red cells within vessels and tissues in many sites, including the spleen, liver, lungs, lymph nodes and bone marrow, thereby greatly reducing the half-lives of red cells (Murray and Dexter, 1998).

Physical alterations in the surface membrane of red cells are responsible for their early removal by macrophages. Febrile responses lead to decreased erythrocyte half-life, due to increased osmotic fragility, decreased plasticity and increased membrane permeability. In infections that cause extremely high parasitaemia, disseminated intravascular coagulation (DIC) may occur, resulting in an accelerated destruction of red cells. This coagulation causes fibrin thrombi to be deposited in small vessels. Red cells are damaged by these partially blocked capillaries, and such damaged red cells may then be phagocytosed by macrophages (ILRAD, 1990). Aberrant antibodies may bind to the hosts own blood cells, thus facilitating their removal by macrophages (Assouku and Gardiner, 1992).

Microcytosis and low plasma-iron turnover rates have been observed during chronic trypanosomosis, suggesting impaired erythropoiesis (Tratour and Idris, 1973; Dargie *et al.*, 1979). In addition, the presence of massive haemosiderin deposits within the mononuclear phagocyte system may be indicative of defective iron utilization (Murray and Dexter, 1988; Valli and Forsberg, 1979). Trypanosomes have been found in the bone marrow, where it is possible that they damage precursor cells by signaling for their early removal by macrophages (ILRAD, 1990). Increase in numbers of cells of the monocytic lineage in bone marrow with a resulting destruction of immature red blood cells has been observed. These observations suggest that trypanosomal infection may cause defective blood cell production (Anosa, 1980).

2.3.5 Gross pathology

The gross post-mortem lesions observed in sacrificed *T. congolense* infected rabbits include varying degrees of emaciation, dehydration, mucopurulent oculonasal discharges and pasted perineum. The lungs would be congested and there would be serous atrophy of the perirenal, pericardiac and abdominal fats. Also, splenomegaly and hepatomegaly would be observed. The liver would have greyish depressed focal areas of necrosis. The skeletal muscles would be pale (Takeet and Fagbemi, 2009). Similarly, study on comparative pathology of the lymph nodes, spleen, liver and kidney in experimental ovine infection with *T. brucei*, *T. congolense* and *T. vivax* revealed cachexia and serous atrophy of fat, which were more pronounced in chronic infections. There was enlargement of the prescapular and prefemoral lymph nodes, spleen, liver and kidneys (Omotainse and Anosa, 2009).

2.3.6 Histopathology

The interstitial activities of trypanosomes in tissues attract severe inflammatory reaction in various organs (Ormerod, 1970; Losos and Ikede, 1972; Poltera 1985; Iliyasu *et al.*, 2015). According to the report of Losos and Ikede (1972), the lesions were characterized by interstitial and perivascular mononuclear cell infiltrates associated with extravascular localization of trypanosomes in connective tissues, though the parasites were not seen. The lesions observed in chronic experimental *T. brucei*-infected rabbits were severe granulomatous inflammation with predominance of macrophages, epithelial giant cells and polymorphonuclear leucocytes (Van den Ingh, 1976; Iliyasu *et al.*, 2015).

Hepatic lesions reported by Omotainse and Anosa (2009), were vascular congestion, perivascular cuffing of mononuclear cells, disorganisation of the hepatic cord with

hepatocellular degeneration and erythrophagocytosis. Consistent with an earlier report by Van den Ingh (1976), of granulomatous reaction in a chronic *T. b. brucei* infection in rabbits, causing centrilobular degeneration, hypercellular sinuses with accumulation of plasma cells, lymphocytes and activated Kupffer cells, (Iliyasu *et al.*, 2015). In donkeys infected with *T. b. brucei*, the liver was reported to show marked haemosiderosis, centrilobular congestion and fatty change (Ikede, *et al.*, 1977; Iliyasu *et al.*, 2015).

Omotainse and Anosa (2009), reported renal pathologies (similar to the hepatic changes), congestion, perivascular and interstitial mononuclear cells infiltration, thickening of glomerular capsules, desquamation of tubular cells and protein casts in the tubules. This was consistent with the glomerulonephritis earlier reported by Anosa and Kaneko (1984), characterized by thickened glomerular capsules, thickened intertubular and perivascular spaces by lymphocytes, plasma cells and macrophages, in *T. brucei*- infected rodents.

Histopathology lesion on the spleen and lymph nodes reported in canine *T. brucei* infection were diffuse inflammatory reaction with haemorrhages, necrosis and cellular infiltrations with polymorphonuclear cell, lymphocyte and plasma (Kaggwa *et al.*, 1984; Iliyasu *et al.*, 2015). The red pulp of the spleen was hypercellular due to mononuclear and polymorphonuclear cell infiltration and generalized follicular hyperplasia (Iliyasu *et al.*, 2015). The spleen, lymph nodes and liver were reported to have activated macrophages, erythrophagocytosis and haemosiderosis indicating massive destruction of red blood cells in these organs (liver with the lowest severity), and that is the reason why these organs were proposed as the sites of erythrocyte destructions in trypanosomiasis (Omotainse and Anosa, 2009).

2.3.7 Haematological changes

The infective trypanosomes have been subdivided into two groups, the haematic group (*Trypanosoma congolense* and *T. vivax*) which remains in the plasma and the tissue invading group (*T. brucei*, *T. evansi*, *T. gambiense*, *T. rhodesiense* and *T. equiperdum*) found in extra and intra vascular spaces (Ngure *et al.*, 2008). Their presence in the blood is accompanied with numerous changes in the cellular and biochemical constituents of blood (Taiwo *et al.*, 2003).

Trypanosome infection may cause anaemia associated with decrease in packed cells volume (PCV), haemoglobin and red blood cell (RBC) counts as reported by many authors in different animal species, as a result of massive erythrophagocytosis by an expanded and active mononuclear phagocytic system of the host (Igbokwe and Nwosu 1997; Lukins 1999). The mechanism or pathophysiology of anaemia in trypanosomosis is complex and multifactorial in origin (Naessens *et al.*, 2005). Anaemia which is regarded as the most consistent finding in trypanosomosis has been reported in *T. vivax* infected cattle and goats and *T. congolense* infected sheep (Bisalla *et al.*, 2007), *T. congolense* infected dogs (Gow *et al.*, 2007), and *T. brucei* infected goats, sheep and rabbits (Seed, 1969; Taiwo *et al.*, 2003). Study on experimentally infected Nubian goats with *T. vivax* resulted in significant decrease in packed cell volume (PCV), total red blood cells counts (RBCs) and haemoglobin concentration (Hb) values. Significant increases were encountered in mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) values. No significant change was observed in mean corpuscular haemoglobin concentration (MCHC). Total white blood cells (WBCs) counts were at normal range (Osman *et al.*, 2012).

Other important changes in the blood during the acute phase of the disease involved white blood cells, platelets and plasma factors and occurred simultaneously with the anaemia of trypanosomosis. Thrombocytopaenia have been reported during the infection (Wellde *et al.*, 1978; Logan-Henfrey *et al.*, 2000) with decreased number of circulating blood platelets in early infection due to a shortened platelet life span (Esien and Ikede, 1978). The total leucocyte counts were usually depressed during the early acute or sub-acute phase of trypanosome infections (Anosa, 1980; Anosa and Isoun 1980) but elevated leucocyte values were present in *T. brucei* and *T. congolense* infected dogs (Anene *et al.*, 1989) and *T. brucei* infections in highly tolerant deer mice (Anosa and Kaneko, 1983). During the chronic phase of infections, the blood leucocyte values recovered gradually and sometimes attained pre-infection values (Anosa, 1980; Anosa and Isoun, 1980).

The decrease in lymphocyte count has been reported to occur in the acute phase of trypanosome infection (Maxie *et al.*, 1979; Anosa, 1980; Anosa and Isoun, 1980), although increased numbers were associated with *T. brucei* infection of highly tolerant deer mice (Anosa and Kaneko, 1983) and with human trypanosomosis (Anosa, 1988). Lymphopaenia occurred partly because of depletion of lymphocytes from lymphoid follicles which occurred in acute *T. vivax* infection (Anosa, 1977; Anosa and Isoun, 1980) and partly because of the sequestration of many lymphocytes in the inflammatory reactions in *T. vivax* infections of ruminants and *T. brucei* infection in mice (Anosa and Kaneko, 1984). Monocytosis is not unusual in trypanosomosis (Isoun, 1975; Anosa, 1980; Anosa and Isoun, 1980), usually coexisting with marked proliferation of macrophages in the tissues of infected animals (Odeyemi *et al.*, 2015).

2.3.8 Biochemical changes

Biochemical changes have been observed to be associated with trypanosome infection in animals and several factors have been found to influence the nature and severity of these changes. These include the strain of the infecting agent and host variability in susceptibility to infection. Biochemical evaluation of the body fluids gives an indication of the functional state of the 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). Varying observations of biochemical changes have been reported in studies of trypanosomosis in animals (Awobode, 2006).

Significant increase in the serum activities of ALP, ALT, and AST on single or mixed experimental infection in twenty mongrel dogs with *T. b. brucei* and *T. congolense* had been reported by Ezeokonkwo *et al.*, (2012). The serum activities of ALP and AST became noticeable from day seven post infection, in all the infected groups whereas that of ALT became noticeable from day 14 and increased continuously until the experiment was terminated. These increases however did not differ significantly between the infected groups in most cases (Ezeokonkwo *et al.*, 2012). This observation was substantially consistent with the experiment on albino rats and mongrel dogs which showed significant increases in the serum activities of ALP, ALT, and AST respectively following trypanosome infections (Obidike *et al.*, 2005; Akpa *et al.*, 2008).

These significant elevations in ALP, ALT, and AST indicated that the integrity of the vital organs like the liver was compromised following infection of the dogs with the trypanosomes. It has been well established that increased destruction of hepatocytes, skeletal muscle cells and other cells of the body, gave rise to increased release of these substances in circulation, hence their elevation in the serum. Specifically in dogs, it has

been reported that the ALP and ALT levels are relatively narrow in range under normal circumstances and hence a rise in the level in serum is an indicator of hepatic malfunction (Ezeokonkwo *et al.*, 2012).

The rise in aspartate aminotransferase (AST) activity can be attributed partly to cellular damage caused by the trypanosomes lysis, while the increase in alanine aminotransferase (ALT) activity probably results from host destruction of trypanosomes (Enwezor and Sackey, 2005). The general causes of the elevation of AST levels in the serum of animals are necrosis of the liver, skeletal muscles and kidneys (Lording and Friend, 1991). Raised level of alkaline phosphatase (ALP) can be seen in inflammatory conditions of the gut and liver, while active hepatocellular damage is reflected by increases in plasma levels of AST and ALT (Lording and Friend, 1991).

Serum total protein has being reported as either increased, decreased or within normal range by different researchers on African trypanosomosis (Anosa, 1988). Increase in serum globulin (chiefly gamma-globulin) have been consistent and implicated with the overall increase in total protein, the serum albumin however decreased leading to a fall in albumin/globulin ratio of the infected animal (Abenga and Anosa, 2005). Adejinmi and Akinboade, (2000) work on *T. brucei* infected goats reported a decreased in serum total proteins with also a lower level of albumin and albumin/globulin ratio. Hypoalbuminaemia in trypanosomosis is probably due to plasma dilution or decreased protein synthesis due to hepatic dysfunction while the hypergammaglobulinaemias have been associated with an increase in immunoglobulin M (IgM) (Abenga and Anosa, 2005).

Elevation in serum creatinine during trypanosomal infection has been linked to either damage to host tissues or renal disorder as a result of the infection (Abenga and Anosa, 2005). Creatinine elevation is often corroborated with high levels of serum urea to

confirm renal disorder by different researchers on trypanosomosis. Joint increases in the levels of serum creatinine and urea have been reported in both experimental and natural trypanosomal infection in different parallel studies on different animal species and human subject (Bakari, *et al.*, 2017).

2.4 Mechanisms of Tissue Damage in Animal Trypanosomosis

Many factors are believed to act in concert to cause cellular injury and ultimately tissue damage in animal trypanosomosis. These factors may emanate from the physical and metabolic activities of the *Trypanosoma spp*, trypanosome autolysates and oxidative lipoperoxidation, among others (Igbokwe, 1994). The extent of tissue invasion varies with the *Trypanosoma spp* involved, with the *T. brucei* group being the most tissue invasive, followed by the *T. vivax* and *T. congolense* which are rather more restricted to the blood circulation (Igbokwe, 1994). Trypanosomes may cause direct mechanical injury to erythrocytes and other cells by the lashing action of their locomotory flagella and their microtubule reinforced bodies (Vickerman and Tetley, 1978; Igbokwe, 1994).

The trypanosomes also produce numerous biochemical substances that are toxic and further worsen the severity of the tissue damage suffered by the affected host. These substances include protease, neuraminidase, phospholipase and some other toxic metabolites Igbokwe (1994).

- i. Proteases: Circulating proteolytic enzymes have been reported in trypanosomes infected host (Vickerman and Tetley, 1978), probably causing tissue inflammation and ultimately tissue damage (Cook *et al.*, 1966; Seed and Hall, 1985).

- ii. Neuraminidases: Esievo *et al.*, (1982) suggested that the trypanosomes may be producing neuraminidase *in vivo* during the infection, which may cleave off the surface sialic acids of blood cell and render them more prone to phagocytosis (Esievo, 1979; Esievo, 1983).
- iii. Phospholipases and free fatty acids: The trypanosomes which generate phospholipases have been reported to cause cell destruction or haemolysis (Tizard *et al.*, 1978). The trypanosomes generated free fatty acids were considered relatively safe since they merely bound to plasma, thus believed to only causing damage when they are in excess of their binding to plasma. But further research show that bound FFA may also be cytotoxic due to the activities of its oxidized product (Assoku *et al.*, 1977; Assoku and Tizard 1978).
- iv. Pyruvate: Pyruvate is the end product of glucose metabolism by the trypanosomes, it is readily metabolized by the host for energy. However, pyruvate has been shown to accumulate in the blood of trypanosome infected animals in amount directly proportional to their level of parasitaemia (Tizard *et al.*, 1978). It was thought that the pyruvate in the blood may cause acidosis and probably lower the affinity of haemoglobin for oxygen, hence contributing to the development of tissue pathologies, in the infected host (Newton, 1978).
- v. Aromatic by-products; Trypanosomes are capable of metabolizing aromatic amino acids to form toxic by-products (Seed and Hall, 1985). Phenylpyruvate (catabolized from phenylalanine) possesses proteolytic activity and may inhibit glycogenesis and mitochondrial function, while indole-ethanol (tryptophol), a by-product of tryptophan catabolism acts on the cell membranes causing osmotic fragility and lysis (Igbokwe, 1994).

These varied factors and mechanisms of cellular injury in trypanosomosis vary in their capacity to cause tissue damage and are dependent on the phases of the disease (Igbokwe, 1994).

2.4.1 Trypanosomal infection-induced oxidative stress and tissue damage

Oxidative stress results from an imbalance between formation and neutralization of free radicals. Various pathologic processes disrupt this balance by increasing the formation of free radicals or decreasing the level of available antioxidants or both. Free radical induced oxidative stress has been reported by different researchers to play an important role in the pathogenesis of African trypanosomosis (Igbokwe, 1994; Ogunsanmi and Taiwo, 2007; Umar *et al.*, 2007; Akanji *et al.*, 2009).

Large amounts of reactive oxidative products are produced by *T. b. brucei* group (Omer *et al.*, 2007) and host animal activated macrophages and monocytes during trypanosomosis (Igbokwe *et al.*, 1996). Beside the elevated generation of oxidative substances by the parasite, have also been implicated to weakening the host's antioxidant defense system (Igbokwe *et al.*, 1996; Omer *et al.*, 2007; Umar *et al.*, 2007), hence increasing the severity of the oxidative damage suffered by the infected host.

Oxidative stress has also been reported to be alleviated in experimental trypanosomal infections by administration of various exogenous antioxidants sources, such as flavonoid mixture (Kobo *et al.*, 2014a); vitamins C and E (Umar *et al.*, 1999, 2000, 2001), methanolic extract of *Calotropis gigantea leaves* (Shaba *et al.*, 2011), crude extract of *Solanum nigrum* (Serems *et al.*, 2013), methanolic extract of ginger (Kobo *et al.*, 2014b) using different animal models. These antioxidant therapy considerably reduced the degree and rate of degeneration of the tissues and organs, though they do

not have any significant effect on the onset and level of parasitaemia, these exogenous antioxidants possibly reinforce the hosts' antioxidant reserves against trypanosomes generated ROS (Igbokwe *et al.*, 1996; Omer *et al.*, 2007; Ogunsanmi and Taiwo, 2007; Yusuf *et al.*, 2012).

Lipids especially polyunsaturated fatty acids are sensitive to oxidation, leading to the term lipid peroxidation, of which, malondialdehyde (MDA) is the most abundant (Igbokwe *et al.*, 1996). The accumulation of MDA in tissues or biological fluids is indicative of the extent of free radical generation, oxidative stress and tissue damage suffered by an individual (Gutteridge, 1995). Elevated levels of MDA in the serum, plasma and organ homogenates have been reported in various experimental trypanosomal infections (Igbokwe *et al.*, 1996; Omer *et al.*, 2007; Ogunsanmi and Taiwo, 2007). This, among many other reasons, is why oxidative stress is considered to play a major role in the pathophysiology of African trypanosomosis.

2.5 Concept of Oxidative Stress

Oxidative stress is defined as a disruption in the oxidant-antioxidant balance in excess of the former with the potential of causing oxidative damage (Costantini and Verhulst, 2009). Damage induced by oxidative stress primarily occurs through production of reactive oxygen species (ROS). Antioxidants are present in low concentrations compared to oxidizable cellular components (DNA, proteins, lipids or carbohydrates) that they protect from oxidative damage caused by ROS (Bouayed and Bohn, 2010; Bhattacharya, 2015). At high doses ROS become deleterious and the exogenous antioxidants also play a key role to maintain or restore this delicate homeostasis in living systems. The body's endogenous defense against oxidative damage includes enzymatic antioxidants (superoxide dismutase, glutathione peroxidase and catalase)

which are the most effective that play a critical role in maintaining optimal cellular functions and thus systemic health and well-being (Gad and Sayd, 2015).

However, under conditions which support oxidative stress (as trypanosomosis), endogenous antioxidants may not be sufficient, thus exogenous antioxidants may be required to maintain optimal cellular functions. Non-enzymatic antioxidants include vitamins E and C, thiol antioxidants (glutathione, thioredoxin and lipoic acid), melatonin, carotenoids, natural flavonoids, and other compounds. Endogenous and exogenous antioxidants act synergistically to maintain or re-establish the normal redox equilibrium of the host (Valko *et al.*, 2007).

2.5.1 Reactive oxygen species (ROS)

The ROS can be defined as an intermediate oxygen carrying metabolites with or without an unpaired electron, comprising oxyradicals (i.e.; oxygen-centered free radicals) such as superoxide anions ($O_2^{\bullet-}$), hydroxyl radicals (OH^{\bullet}), alkoxy radicals (RO^{\bullet}) and peroxyradicals (ROO^{\bullet}) and non-radicals such as hydrogen peroxide (H_2O_2) (Valko *et al.*, 2007; Ayla and Metin, 2015). They are able to oxidize other components and turn them into free radicals usually by a chain reaction leading to the formation of numerous new radicals (Valko *et al.*, 2007; Bouayed and Bohn, 2010; Bhattacharya, 2015).

The radical chain reaction typically continues until the system becomes anaerobic or the substrates (e.g.; membrane polyunsaturated fatty acids) are depleted (Rice-Evans *et al.*, 1996; Bouayed and Bohn, 2010). This chain reaction can however be stopped when two radicals form non-radical products or by the presence of chain-breaking antioxidants (Rice-Evans *et al.*, 1996; Surai and Fisinin, 2014). The superoxide radical ($O_2^{\bullet-}$) resulting from the reduction of a single electron from oxygen, have been

considered as the precursor of the other ROS ($\text{OH}\cdot$, $\text{RO}\cdot$, $\text{ROO}\cdot$ and H_2O_2) (Bouayed and Bohn, 2010).

Oxygen can undergo one electron transfer to form $\text{O}_2^{\bullet-}$, which itself can then undergo a one-electron transfer to form H_2O_2 . Which in turn, can undergo transfer of an electron to form H_2O (Ayla and Metin, 2015). The $\text{O}_2^{\bullet-}$ can also react with nitric oxide ($\cdot\text{NO}$) a nitrogen-centered radical, to generate a highly reactive molecule, peroxynitrite anion (ONOO^-), also termed a reactive oxygen and nitrogen species (RONS), which is capable of causing DNA fragmentation and lipid oxidation among other damage to the cellular component of an organism (Lee *et al.*, 2004; Bouayed and Bohn, 2010; Ayla and Metin, 2015). Superoxide, peroxide, and the hydroxyl radical are considered the primary ROS and have been considered in most researches on the role of free radicals in biology and medicine (Bhattacharya, 2015).

2.5.2 Antioxidant defense system

Living organisms have developed specific antioxidant defense mechanisms to protect against oxidative stress, to adapt to the highly oxidative life on earth with over 23% of atmospheric oxygen (Surai and Fisinin, 2014). This antioxidant defense system is an integrated system which contains numerous protective antioxidant compounds to enable maximum cellular protection, in three major levels of defense (Fotina *et al.*, 2013; Surai and Fisinin, 2010, 2014). The first level of defense is the antioxidant enzymes, namely superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) plus their metal-binding proteins, that mitigate against free radical formation by inhibiting the precursors of free radicals (Fotina *et al.*, 2013). Unfortunately, some radicals can escape these antioxidant enzymes, to cause oxidative damage (Nasir *et al.*, 2015).

The second level of defense consists of the non-enzymatic antioxidant which include thiol antioxidants (glutathione, thioredoxin and lipoic acid), vitamins E and C, melatonin, carotenoids, natural flavonoids, and other compounds. They are sometimes referred to as “chain-breaking antioxidants” because they prevent the propagation step of lipid peroxidation by scavenging peroxy radical intermediates in the oxidative damage propagation chain reaction (Nasir *et al.*, 2015). However, even this second level of antioxidant defense cannot sufficiently prevent oxidative damage. Thus, a third level of defense is required to eliminate damaged molecules or repair them. The antioxidant in this level includes lipases, peptidases or proteases and other enzymes (DNA repair enzymes, ligases, nucleases, polymerases, proteinases, phospholipases and various transferases) (Narra *et al.*, 2015). All these antioxidants operate in the body in association with each other, forming an integrated antioxidant system and are vital for maximum protection from the deleterious effects of free radicals and their toxic metabolism (Surai and Fisinin, 2010; Fotina *et al.*, 2013).

2.5.3 Endogenous antioxidants

The endogenous antioxidants in the animal body comprise a complex network of enzymatic and non-enzymatic component, which work synergistically with each other to protect the cells and organ systems from free radical and oxidative damage. The most efficient enzymatic antioxidants contain superoxide dismutase, glutathione peroxidase and catalase and non-enzymatic thiol antioxidants glutathione (Fotina *et al.*, 2013; Surai and Fisinin, 2010, 2014)

2.5.3.1 Superoxide dismutase

In 1967, two biochemists, Irwin Fridovitch and Joe McCord discovered the antioxidant enzyme, superoxide dismutase (SOD), which provides an important means of cellular

defense against free radical damage (Chitra and Pillai, 2002). Generally, SOD destroys O_2^- by successive oxidation and reduction of the transition metal ion at the active site in a “ping pong” type mechanism, with remarkably high reaction rates (Meier *et al.*, 1998). In humans, there are three forms of SOD: cytosolic Cu/Zn-SOD (requires copper or zinc as cofactor and is located mainly in the cytoplasm), mitochondrial Mn-SOD (requires manganese as cofactor, and is primarily located within the mitochondria matrix); and extracellular SOD (EC-SOD) also requires copper or zinc as cofactor, but is mainly found in the extracellular space (Sandstrom *et al.*, 1994; Sun *et al.*, 1995).

2.5.3.2 *Glutathione peroxidase*

Glutathione peroxidase (GPx) is an enzyme, which protects the cells from damage by free radicals like hydrogen and lipid peroxides (Sperenza *et al.*, 1993). It catalyses the reduction of hydroperoxides with glutathione as the substrate, thus, protecting the mammalian cells against possible oxidative damage (Sperenza *et al.*, 1993).

There are five GPx isoenzymes present in all mammals (Imai *et al.*, 1998). Although they are ubiquitous, they are either present in the mitochondria or cytosol of cells, the levels of the individual isoform vary depending on the tissue type (Tappel, 1978). These isoforms include GPx1, GPx2, GPx3, GPx4 and GPx5. The GPx5 is the most recent, which was first expressed specifically in the epididymis of a mouse, and found to be selenium-independent (Imai *et al.*, 1998). Although GPx shares the degradation of H_2O_2 , with catalase, it can also react effectively with lipid and other organic hydroperoxides, making GPx a major antioxidant against low levels oxidative challenge (Tappel, 1978).

2.5.3.3 Catalase

Catalase is mainly responsible for the elimination of hydrogen peroxide. It is present in nearly all animal cells, where it breaks down excessive H_2O_2 into H_2O and O_2 , leading to its elimination (Aebi, 1980; Buschfort *et al.*, 1997).

2.5.3.4 Glutathione

Glutathione is the most abundant intracellular antioxidant and indeed a multifunctional protector of the cell against ROS. It is the major soluble antioxidant of the cell present in the cytosol, nuclei, and mitochondria (Meister, 1991). The reduced form of glutathione is GSH while the oxidized form is GSSG (glutathione disulphide). GSH, owes its antioxidant capacity to the sulphur atom in its moiety that can accommodate the loss of a single electron to form GSSG and the ratio of GSH/GSSG is a determinant of oxidative stress suffered by an individual (Kurutas, 2016).

Glutathione protects against oxidative damage in several ways, by being a co-factor in the enzymatic degradations of some ROS, participating in the transport of amino acid across plasma membrane, the direct non enzymatic degradation of hydroxyl radical and singlet oxygen and the reactivation of inactive Vitamins C and E, to their active form (Awasthi *et al.*, 1980; Wink and Mitchell, 1998; Kurutas, 2016). Decrease in serum or organ GSH concentration, have been reported to have a linkage with chronic, degenerative or age related disease (Birket *et al.*, 2013; Kurutas, 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Venue of the Experiment

The experiment was carried out at the Laboratory Animal Unit, Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

3.2 Experimental Design

A total of 40 adult male rats were obtained from the Animal House, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The rats were kept in rat cages with wood shavings used as bedding, which was changed weekly. They were fed on pelleted grower mash and water was provided *ad libitum*. A period of 14 days was allowed for the rats to get acclimatized prior to the commencement of the experiment. During this period, the rats were exposed to the routine handling conditions they were subjected to during the experiment. On the day of commencement of the experiment, the rats were allocated, on basis of mean body weight, to two groups (infected and control) of 20 rats each.

3.2.1 The trypanosome parasite

Trypanosoma. brucei brucei (Federi strain) was sourced from the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. The parasite was maintained by serial passages in donor rats until the day of inoculation in the experimental animals. Parasitaemia was monitored daily by preparing a wet mount

of the blood collected from the tail vein of the infected rats according to the method of Woo (1969).

3.2.2 Inoculation of rats

Inoculations of rats in the infected group were carried out as described by Ekanem and Yusuf (2005). In brief, when the donor rat was at swarming parasitaemia, blood was obtained and diluted with physiological saline to obtain a trypanosome concentration of 1×10^6 trypanosomes/ml. One milliliter of this diluted blood was injected intraperitoneally to each rat in the infected group. This day was termed day 0 post-infection (day 0 pi).

3.2.3 Clinical observations in the infected rats

Following infection of the rats with *T. b. brucei*, the experimental animals were monitored closely for clinical signs of trypanosomosis.

3.2.4 Sample collection

Before infection, on the day of inoculation (day 0) of the rats with *T. b. brucei* infected blood, 4 rats each from the infected and control groups were euthanized to obtain sufficient blood from each rat for haematological and biochemical analysis; whole blood in EDTA coated sample bottle and in plain sample bottle for serum respectively. Also, on the day 3 at the onset of parasitaemia in the infected group and subsequently at days 5 and 7, 4 rats each from the two groups were euthanized and blood collected and used as described earlier. Each time, post-mortem examination was carried out while tissues samples were collected and fixed in 10 % buffered formalin for histopathological studies and representative samples of the liver and kidney were also stored in phosphate buffered saline solution, they were homogenized by macerating 1g

of the tissue with 5ml of phosphate buffered saline solution. The homogenate were stored at 4°C in the refrigerator before they were used to determine the organ GSH concentrations.

3.2.5 Determination of parasitaemia in *T. b. brucei* infected Rats

Beginning from day 1 post infection (pi) and throughout the course of the experiment, blood was collected from the tail directly unto a clean glass slide and covered with cover slip. The wet mount was viewed under the light microscope at x40 magnification to monitor and score parasitaemia according to the rapid matching method described by Herbert and Lumsden (1976).

3.2.6 Evaluation of haematologic parameters

3.2.6.1 Determination of packed cell volume

Packed cell volume was determined using microhaematocrit method according to Dacie and Lewis (1991). Briefly, the blood samples were mixed well by gentle inversion of the sample bottles several times. Heparinized capillary tubes (John Poulten Limited[®], Essex, England) were filled to 75% of their length by capillary action. The empty ends of the tubes were sealed with flames. The tubes were then loaded onto microhaematocrit centrifuge (Hawksley[®], Lancing, England) and centrifuged at 3000g for 10 minutes. The PCV values were read with the aid of microhaematocrit reader.

3.2.6.2 Determination of erythrocyte count

The erythrocyte count was evaluated using improved Neubauer haemocytometer method. Blood was drawn to 0.5 mark of the haemocytometer and diluted with 0.9 % normal saline, which was drawn to the 101 mark. After gentle mixing of the blood in

the pipette, the diluted blood was discharged into haemocytometer counting chamber after discarding six drops which were mostly diluents and allowed to settle. The high dry objective lens of the microscope was employed in determining the total erythrocyte count. The total number of cells in five squares in the centre of counting chamber was determined and multiplied by 10,000. This value represented the total number of erythrocytes per microlitre (Dacie and Lewis, 1991).

3.2.6.3 Determination of haemoglobin concentration

The haemoglobin concentration was determined using cyanmethaemoglobin method of Baker and Silverton (1985). Briefly, 0.02 ml of blood was obtained into clean cuvette and then diluted with 5 ml of cyanmethaemoglobin reagent (modified Drabkin fluid). After the diluent was added, it was stoppered and inverted 2-3 times and allowed to stand for 10 minutes for maximum conversion of Hb to cyanmethaemoglobin. The absorbance of the resulting mixture was read using spectrophotometer (Beckham Coulter, Model B U520, Austria) at wavelength of 540 nm against reagent blank. The optical density at 540 nm was recorded and compared with the reading, obtained using a standard solution of cyanmethaemoglobin.

3.2.6.4 Calculation of erythrocytic indices

The mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated from Hb, PCV and erythrocyte counts as described by Schalm *et al.* (1975) using the following formulae, respectively:

$$\text{MCV (fl)} = \frac{\text{PCV} \times 10}{\text{Number of erythrocytes per } \mu\text{l blood} \times 10^{-6}}$$

$$\text{MCHC (g/dl)} = \frac{\text{Haemoglobin (g/dl)} \times 100}{\text{PCV (\%)}}$$

3.2.6.5 Determination of total leucocyte count

Haemocytometer method, as described by Dacie and Lewis (1991), was also employed for determination of the total leucocyte count. Briefly, the pipette was filled with blood up to 0.5 mark with the aid of a plastic mouth piece that was attached to the stem end of the pipette above the bulb. Excess blood clinging to the exterior of the pipette was removed by wiping with a piece of tissue paper. The diluting fluid (2 % glacial acetic acid, coloured with two drops of gentian violet) was then drawn to the 11.0 mark, and the blood and the diluting fluid were mixed by shaking the pipette vigorously for two to three minutes. Six drops of the diluting fluid were removed by releasing the thumb intermittently. The haemocytometer was then filled by capillary action. This was examined under the microscope at x 40 magnification. The leucocytes in the four squares of the haemocytometer were counted, and the value obtained was multiplied by the factor 50 to obtain the total leucocyte count.

3.2.6.6 Determination of differential leucocyte count

The method used to determine the differential leucocyte count as described by Dacie and Lewis (1991). Briefly, a thin blood smear was made, air-dried and fixed in methanol for four minutes before staining with Giemsa for 30 minutes. Thereafter, the smear was rinsed with distilled water and dried with blotting paper. The stained and dried blood smear was later viewed under microscope in oil immersion at x 100 magnification. Examination of slide began at the thin end of the smear, where erythrocytes were well separated and leucocytes were thinly spread. From the thin end, systematic meandering system of slow, careful and detailed examination of the

leucocytes was made. For a differential leucocyte count, a minimum of 100 cells were differentiated and counted.

3.3 Biochemical Analyses

3.3.1 Determination of serum antioxidant enzymes activity

3.3.1.1 Superoxide dismutase activity

The activity of superoxide dismutase (SOD) was measured using the Northwest Life Science Specialties SOD kit (NWLSS™ NWK-SOD02), based on the method of monitoring the auto-oxidation rate of haematoxylin, originally described by Martin (Jr) *et al.* (1987) and modified to enhance reliability. Briefly, 230 µl of assay buffer was added to wells of the microplate. Then, 10 µL of assay buffer (for blank) and 10 µl of serum sample were added. The wells were properly shaken, mixed, and incubated for 2 minutes. A multi-channel pipette was used to add 10 µl of haematoxylin reagent to begin the reaction. The content of each well was quickly mixed using instrument's shaker function; and, immediately, the absorbance at 560 nm was recorded. The SOD activity was calculated as: $\text{SOD U/ml} = 1.25 \times \% \text{ inhibition}$.

3.3.1.2 Glutathione peroxidase activity

Glutathione peroxidase activity (GPx) was measured using the Northwest Life Science Specialist, Vancouver, Canada (NWLSS™) glutathione peroxidase assay kits protocol NWK-GPx01. Briefly, for standard procedure for microplate assay, all reagents were brought to room temperature (25°C). Diluted serum sample (50 µl) was added to the wells and then 50 µl of working nicotinamide adenine dinucleotide phosphate (NADPH) added to each well. Working H₂O₂ (50 µl) was also added to each well. After

waiting for 1 minute, microplate was placed in plate reader and read at 340 nm wavelength (Flohe and Gunzler, 1984).

To calculate GPx activity using the NADPH absorption coefficient, the GPx activity, expressed as mU/ml, was calculated using the GPx activity definition.

$$[\text{GPx}] = \frac{2(\text{mRate}_s - \text{mRate}_b) \cdot V_{\text{Rxm}}}{2.74 V_s} \cdot \text{df}$$

Where $\text{mRate}_s = -1,000 \Delta A_{340}/\text{min}$ of sample; $\text{mRate}_b = -1,000 \Delta A_{340}/\text{min}$ of blank; $2.74 = \text{NADPH } 340 \text{ nm millimolar absorption coefficient at } 1 \text{ cm path length}$; $V_{\text{Rxm}} = \text{volume of reaction mixture}$; $V_s = \text{volume of sample}$; $2 = \text{correction for } 2 \text{ moles reduced glutathione, oxidized to } 1 \text{ mole glutathione disulfide (oxidized glutathione) per mole NADPH oxidized}$; $\text{df} = \text{sample dilution factor}$.

3.3.2 Determination of serum biomarkers of hepatic and renal dysfunctions

3.3.2.1 Serum aspartate aminotransferase (AST)

Serum AST activity was determined using the standard colorimetric method as described by Reitman and Frankel (1957). This is an *in vitro* method of determining AST using Randox Glutamic-oxaloacetic transaminase test kit (Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY).

3.3.2.2 Serum alanine aminotransferase (ALT)

Serum ALT activity was determined using the standard colorimetric method of Reitman and Frankel (1957). This is an *in vitro* method of determining ALT in serum using Randox Glutamic-pyruvic transaminase test kit (Randox laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY).

3.3.2.3 Serum alkaline phosphatase (ALP)

Determination of serum ALP activity was done using the phenolphthalein monophosphate method (Klein *et al.*, 1960) for the *in vitro* determination of ALP in serum using Quimica Clinica test kit (QCA, CN-340km 1081- P.O. BOX 20-E43870 AMPOSTA/Spain).

3.3.2.4 Serum urea

Determination of serum urea was done using Urease Berthelot method (Fawcett and Scott, 1960) for quantitative *in vitro* determination of urea in serum using Randox urea colorimetric kit (Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY).

3.3.2.5 Serum creatinine

Determination of serum creatinine was based on the modified Jaffe method (Fossati *et al.*, 1983) for *in vitro* determination of creatinine in serum using Randox creatinine colorimetric method with depolarisation (Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY).

3.4 Pathologic Examination

Organs of every rat that was sacrificed in this work were examined for visible gross lesion and recorded. The liver and kidney were obtained and preserved in 10% buffered formalin for one week before embedding in paraffin. The tissues were then prepared for histopathological examination using the modified method described by Luna (1960).

3.5 Data Analysis

Summarized results were expressed as mean \pm standard error of mean (\pm SEM). Data obtained were subjected to student t-test and Pearson's correlation analysis. Values of P

< 0.05 were considered significant (Snedecor and Cochran, 1994). Graph pad prism version 4.2 was used for the analysis.

CHAPTER FOUR

4.0 RESULTS

4.1 Parasitaemia

Parasitaemia was detected in four of the *T. b. brucei*-infected rats on day 3 post infections (pi) and by day 4 pi, all the rats in the infected group were parasitaemic, thus, giving a pre-patent period of 3.75 ± 0.11 days.

The mean parasitaemia in the infected rats on days 3, 5 and 7 pi were $2.24 \pm 0.63 \times 10^6$ (mean $\log_{10}=6.30 \pm 0.13$), $107.50 \pm 10.62 \times 10^6$ (mean $\log_{10}=8.03 \pm 0.04$) and $403.60 \pm 48.79 \times 10^6$ (mean $\log_{10}=8.68 \pm 0.017$) parasites/ml of blood, respectively (Figure 4.1).

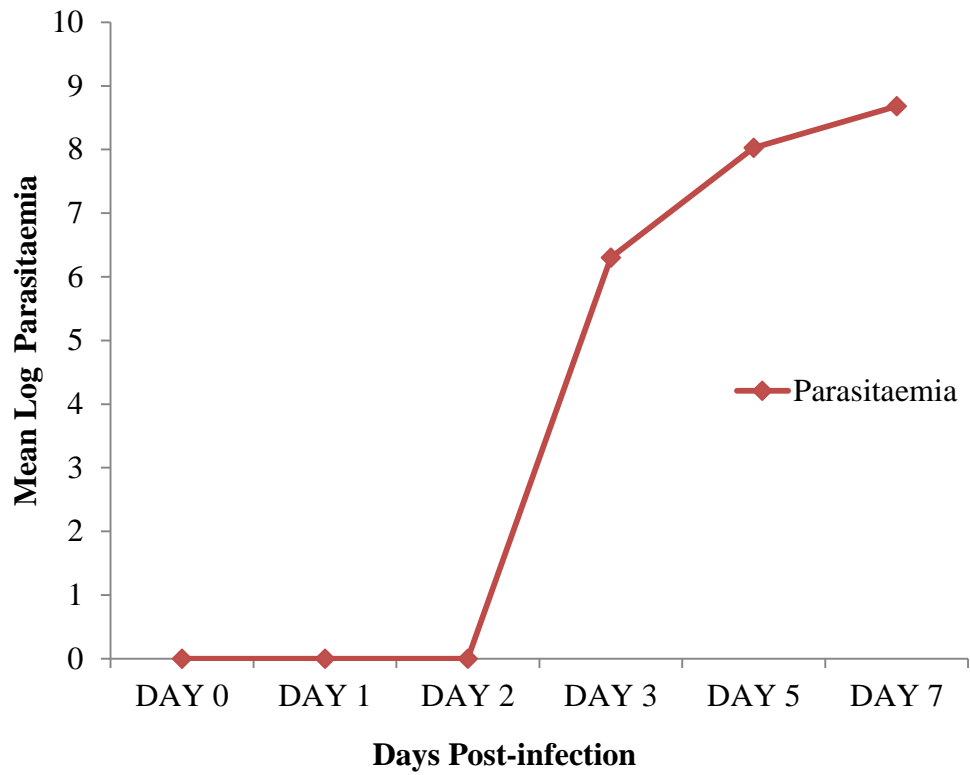


Figure 4.1: Mean Log₁₀ Parasitaemia in Rats Experimentally Infected with *T. b. brucei*

4.2 Haematologic Parameters

4.2.1 Packed cell volume (PCV)

The mean PCV values in the *T. brucei*-infected and control rats were as presented in Figure 4.2. The pre-infection mean PCV values in the *T. b. brucei*-infected and control groups were 52.00 ± 11.14 and 48.33 ± 5.24 %, respectively. Following the infection, the mean PCV values in the two groups remained fairly at the same level up to day 3 pi. Afterwards, there was a progressive decrease in mean PCV values of the *T. brucei*-infected rats, with the lowest value of 33.00 ± 2.89 %, which was significantly ($p < 0.05$) lower than the pre-infection or corresponding control group value, recorded on day 7 pi. The mean PCV values in the control group remained relatively unchanged up to termination of the experiment.

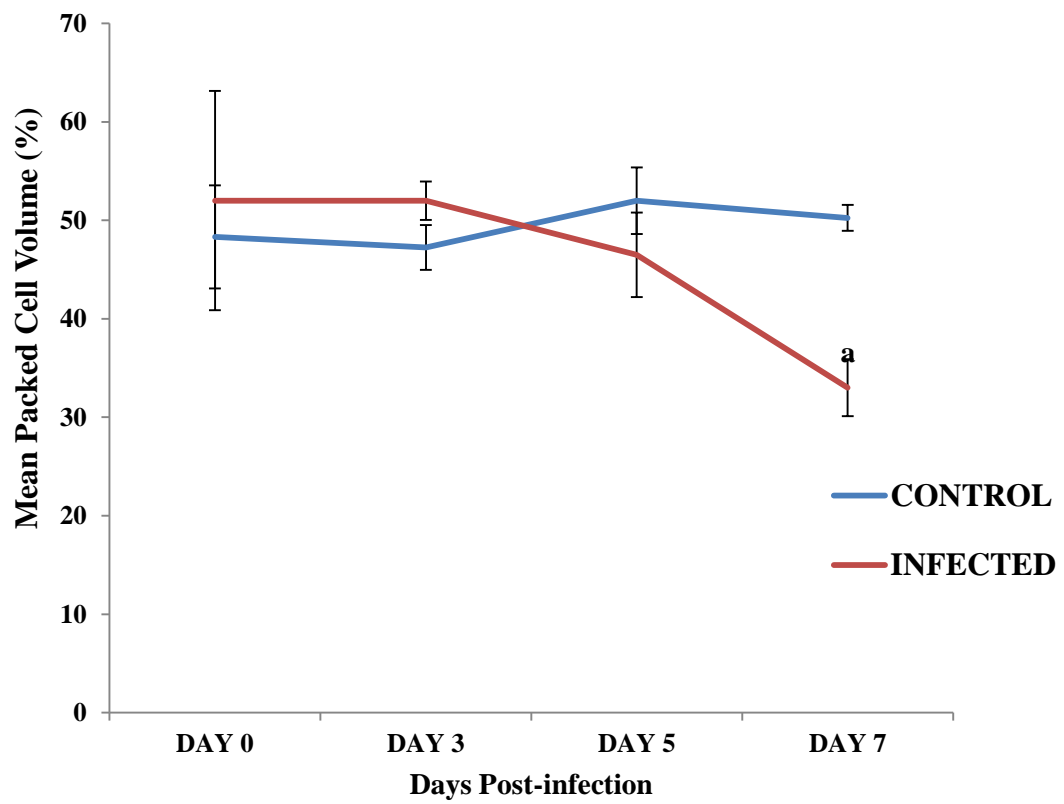


Figure 4.2: Mean \pm SEM Packed Cell Volume in *T. b. brucei*-infected and control rats.

a: Significantly ($P < 0.05$) lower when compared to the pre-infection and corresponding control values.

4.2.2 Total white blood cell count (TWBC)

The mean TWBCs in the *T. brucei*-infected and control rats were as presented in Figure 4.3. The pre-infection mean TWBC in the infected and control groups were 10.85 ± 2.09 and $10.03 \pm 1.16 \times 10^9/L$, respectively. Following infection the mean TWBC in the infected rats showed an initial decrease to 6.33 ± 0.47 on day 3 pi, before increasing to $7.58 \pm 0.66 \times 10^9/L$ on day 5 pi and later decreasing to $6.93 \pm 0.48 \times 10^9/L$ on days 7 pi which was significant lower than the corresponding control value on 3, 5 and 7 days pi. The mean TWBC of the control group only fluctuated within a narrow range throughout the experiment.

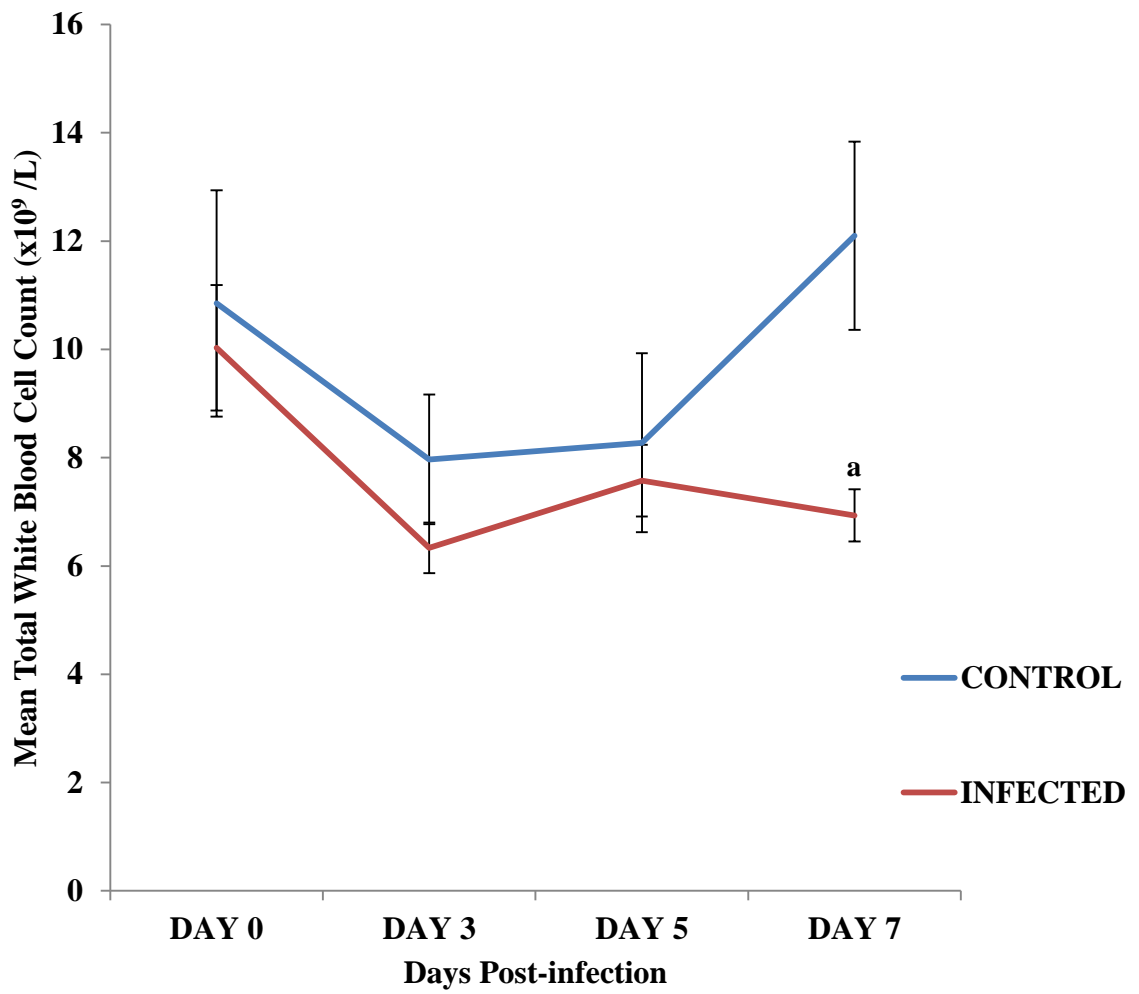


Figure 4.3: Mean \pm SEM Total White Blood Cell Count in *T. b. brucei*-infected and Control rats.

a: Significantly ($P < 0.05$) lower when compared to the corresponding control values.

4.3 Serum Antioxidant Enzymes Activity

4.3.1 Serum super oxide dismutase (SOD) activity

The mean serum SOD activities in the *T. brucei*-infected and control rats were as presented in Figure 4.4. The pre-infection mean serum SOD activities in the *T. b. brucei*-infected and control groups of rats were 7.23 ± 0.63 and 7.36 ± 0.53 U/ml, respectively. Following the infection, the mean serum SOD activity in the *T. b. brucei*-infected rats increased to 8.22 ± 0.83 U/ml on day 3 pi, after which a progressive decrease to 5.58 ± 0.86 U/ml was observed in day 7 pi. Although the mean serum SOD activities in the two groups post-infection were not significantly different ($p > 0.05$), the SOD activity in the infected group showed a pattern different from that in the control group which remained relatively unchanged throughout the experiment.

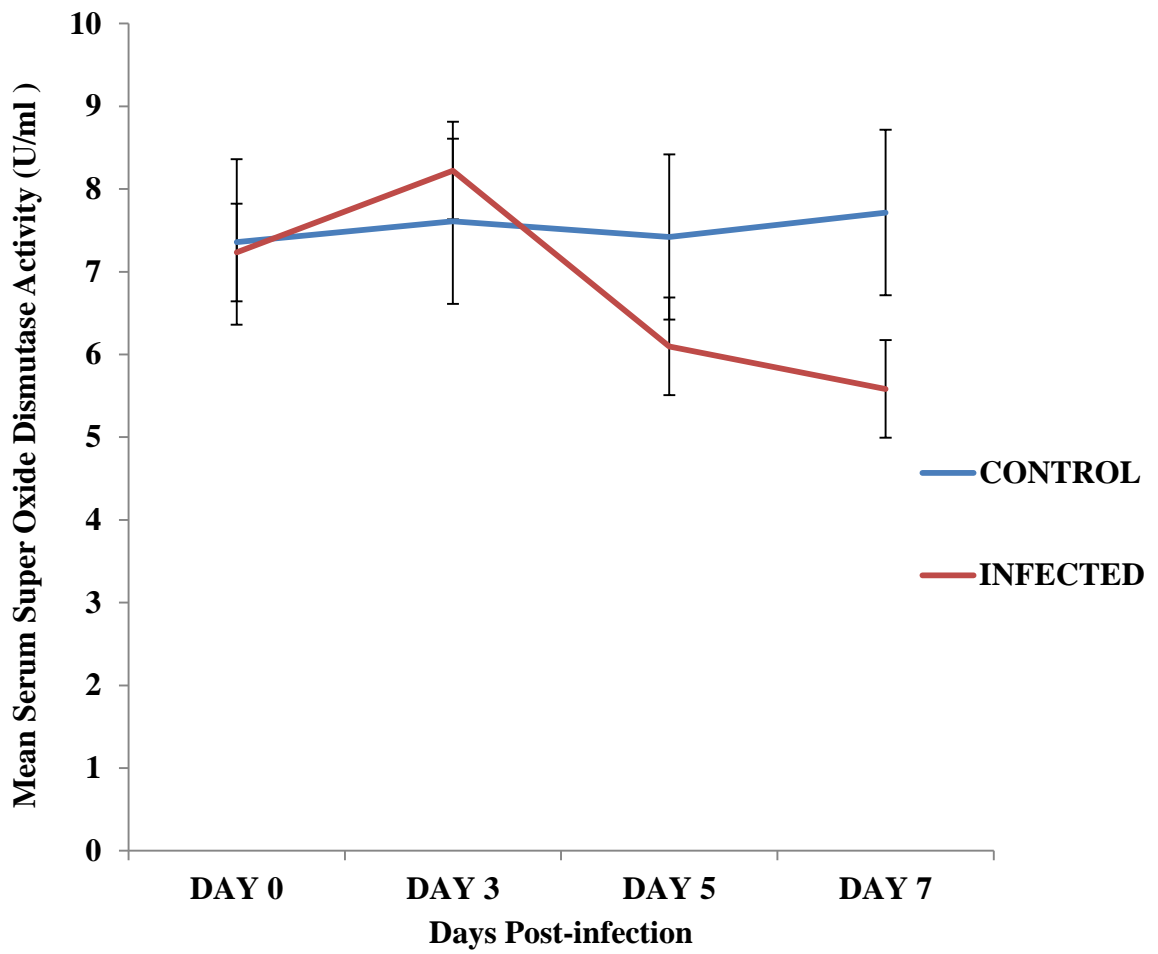


Figure 4.4: Mean \pm SEM Serum Super Oxide Dismutase Activity of *T. b. brucei*-infected and Control Rats.

4.3.2 Glutathione peroxidase (GPx) activity

The mean serum GPx activities in the *T. brucei*-infected and control rats were presented in Figure 4.5. The day 0 pre-infection mean serum GPx activities in the *T. b. brucei*-infected and control group of rats were 43.00 ± 2.14 and 39.66 ± 4.58 U/ml, respectively. Following the infection, the mean serum GPx activity in the *T. b. brucei*-infected rats progressively decreased to a lowest value of 32.59 ± 3.36 U/ml on day 7 pi which was significantly different ($P < 0.05$) from the corresponding value in the control group. However, the difference in the mean GPx activity in the two groups during the experiment were not significant ($p > 0.05$).

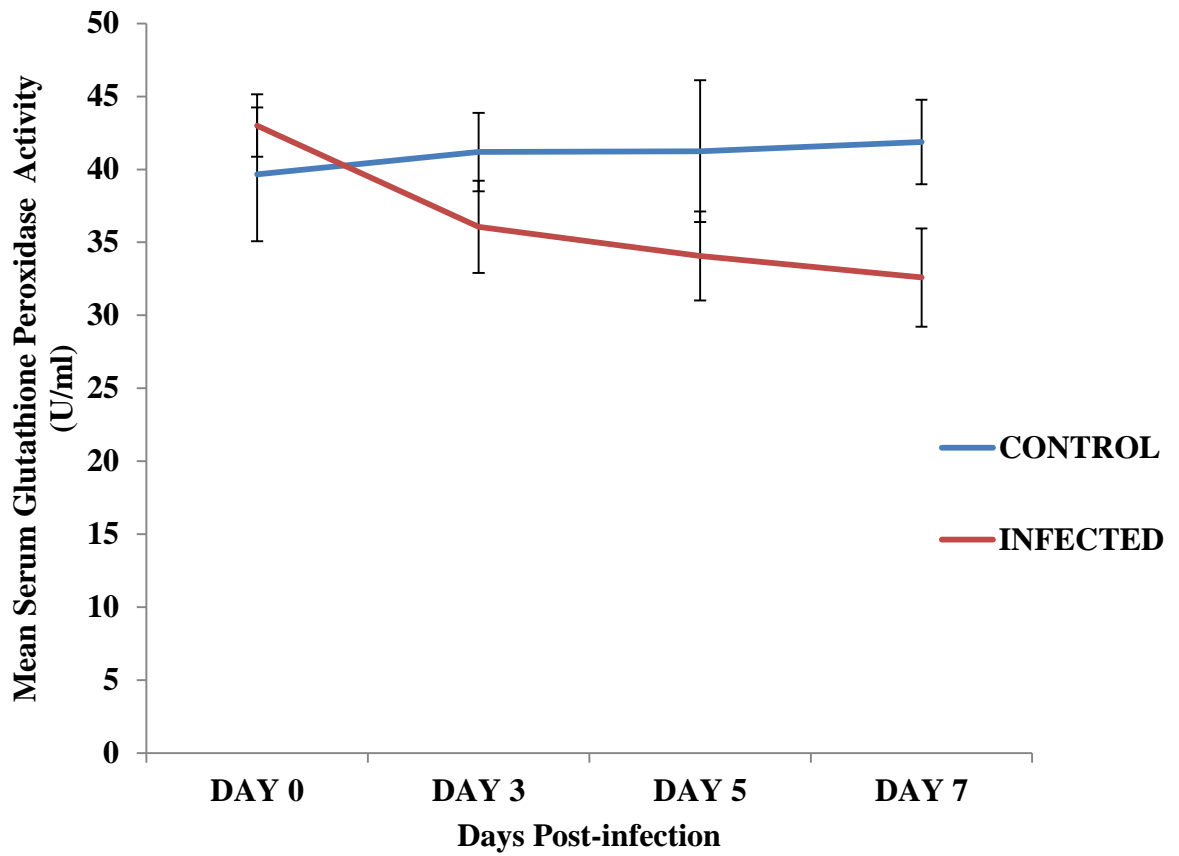


Figure 4.5: Mean \pm SEM Serum Glutathione Peroxidase Activity of *T. b. brucei*-infected and Control Rats.

4.4 Organ Concentration of Reduced Glutathione (GSH)

4.4.1 Liver GSH concentration

The mean liver GSH concentrations in the *T. brucei*-infected and control rats were as presented in Figure 4.6. The pre-infection mean liver GSH concentrations in the *T. b. brucei*-infected and control groups of rats were 19.53 ± 0.23 and $19.27 \pm 0.55 \mu\text{g/ml}$, respectively. Following the infection, the mean liver GSH concentration in the *T. b. brucei*-infected rats progressively decreased to a lowest value ($18.53 \pm 0.86 \mu\text{g/ml}$) on day 5 pi, and remained at this level until termination of the experiment. The hepatic GSH concentration in the *T. brucei*-infected group was only significantly ($P < 0.05$) lower than in the control group on day 5 pi.

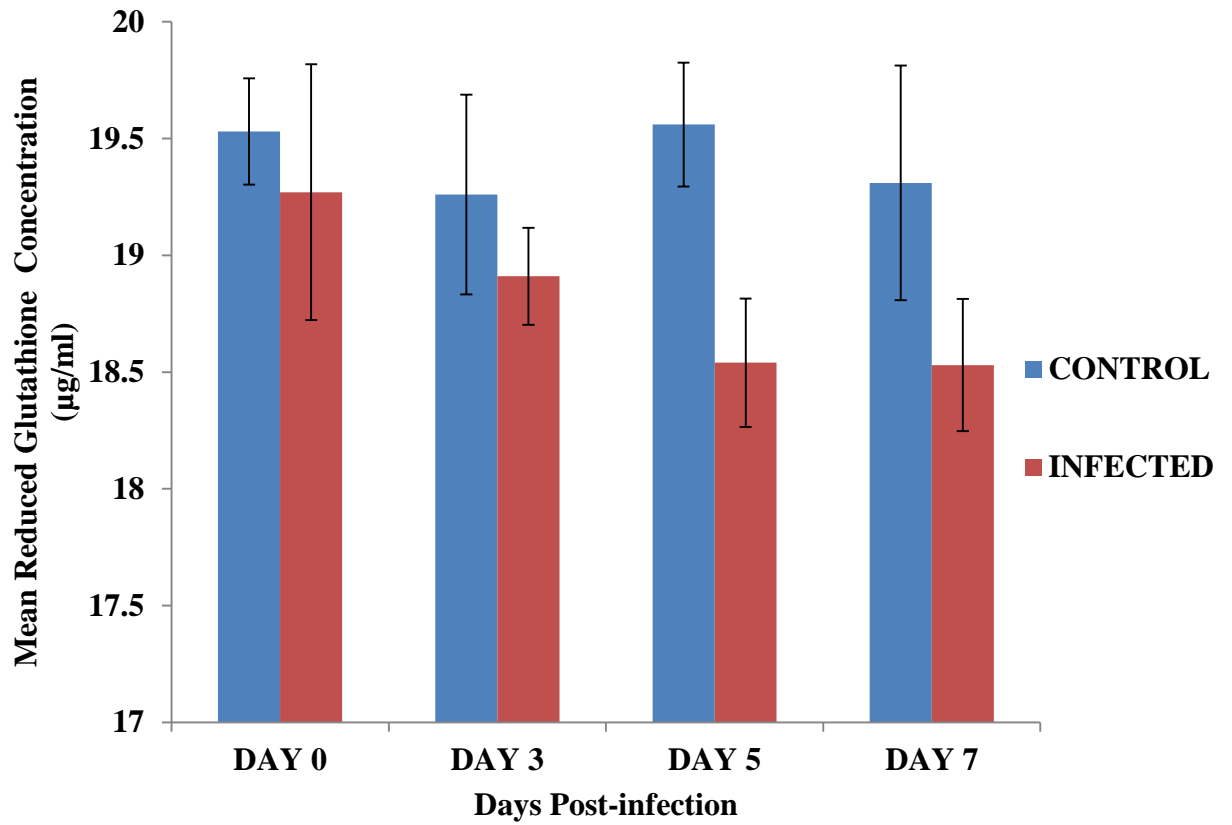


Figure 4.6: Mean \pm SEM Reduced Glutathione Concentrations in the Liver of *T. b. brucei* infected and Control Rats.

4.4.2 Kidney GSH concentration

The mean kidney GSH concentrations in the *T. brucei*-infected and control rats were as presented in Figure 4.7. The pre-infection mean kidney GSH concentrations in the *T. b. brucei*-infected and control group of rats were 24.34 ± 2.17 and 25.82 ± 1.031 $\mu\text{g/ml}$, respectively. Following infection with *T. brucei*, the mean kidney GSH concentration in the infected group decreased to the lowest value of 19.09 ± 0.70 $\mu\text{g/ml}$ at termination of the experiment. The mean kidney GSH concentration remained relatively unchanged in the control group with only insignificant fluctuations.

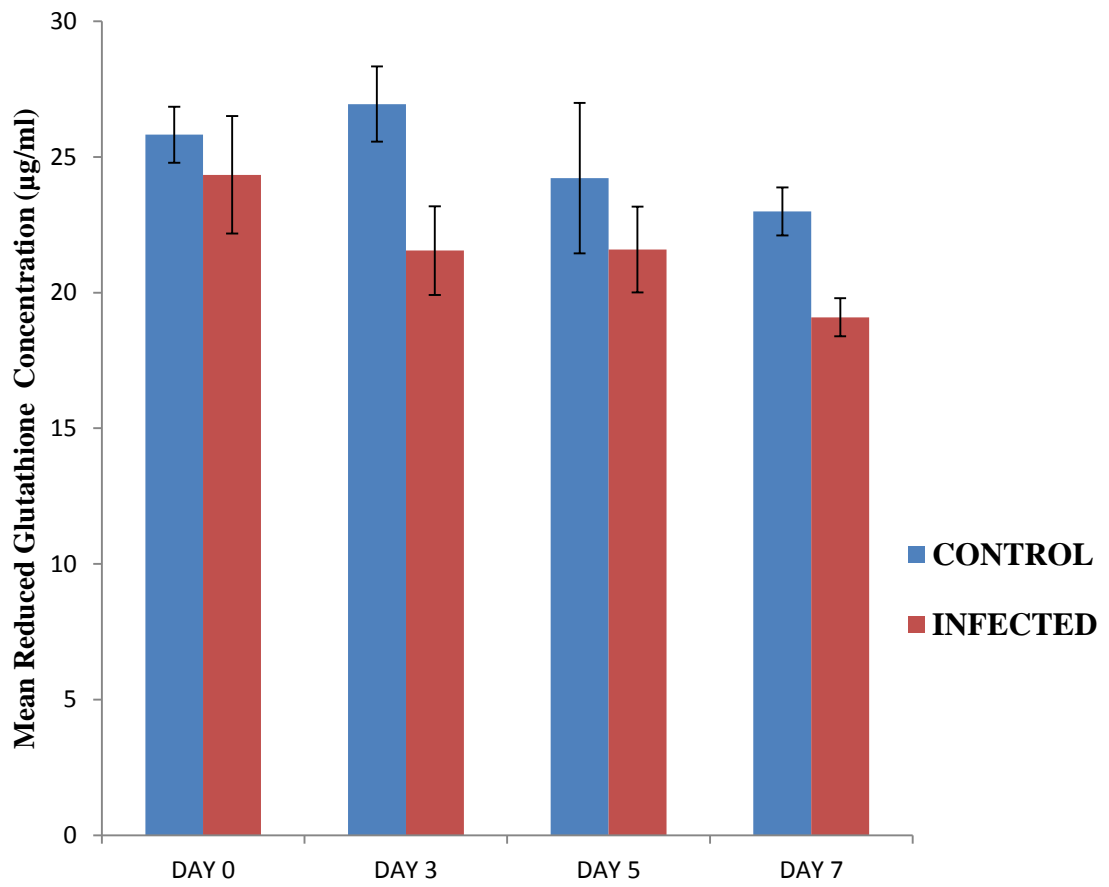


Figure 4.7: Mean \pm SEM Reduced Glutathione Concentrations in the Kidney of *T. b. brucei* infected and Control Rats.

4.5 Serum Hepatic Enzymes and Biochemicals

4.5.1 Serum aspartate aminotransferase (AST) activity

The mean serum AST activities in the *T. brucei*-infected and control rats were as presented in Figure 4.8. The pre-infection mean serum AST activities in the infected and control groups were 78.53 ± 5.04 and 83.18 ± 3.67 U/L, respectively. Following infection, the serum AST activity progressively increased in the *T. brucei*-infected group until termination of the experiment. The mean serum AST activity was significantly ($p < 0.05$) higher in the *T. brucei*-infected than in the control group on days 5 and 7 pi. The mean AST activity in the control group, on the other hand, remained relatively unchanged up to termination of the experiment.

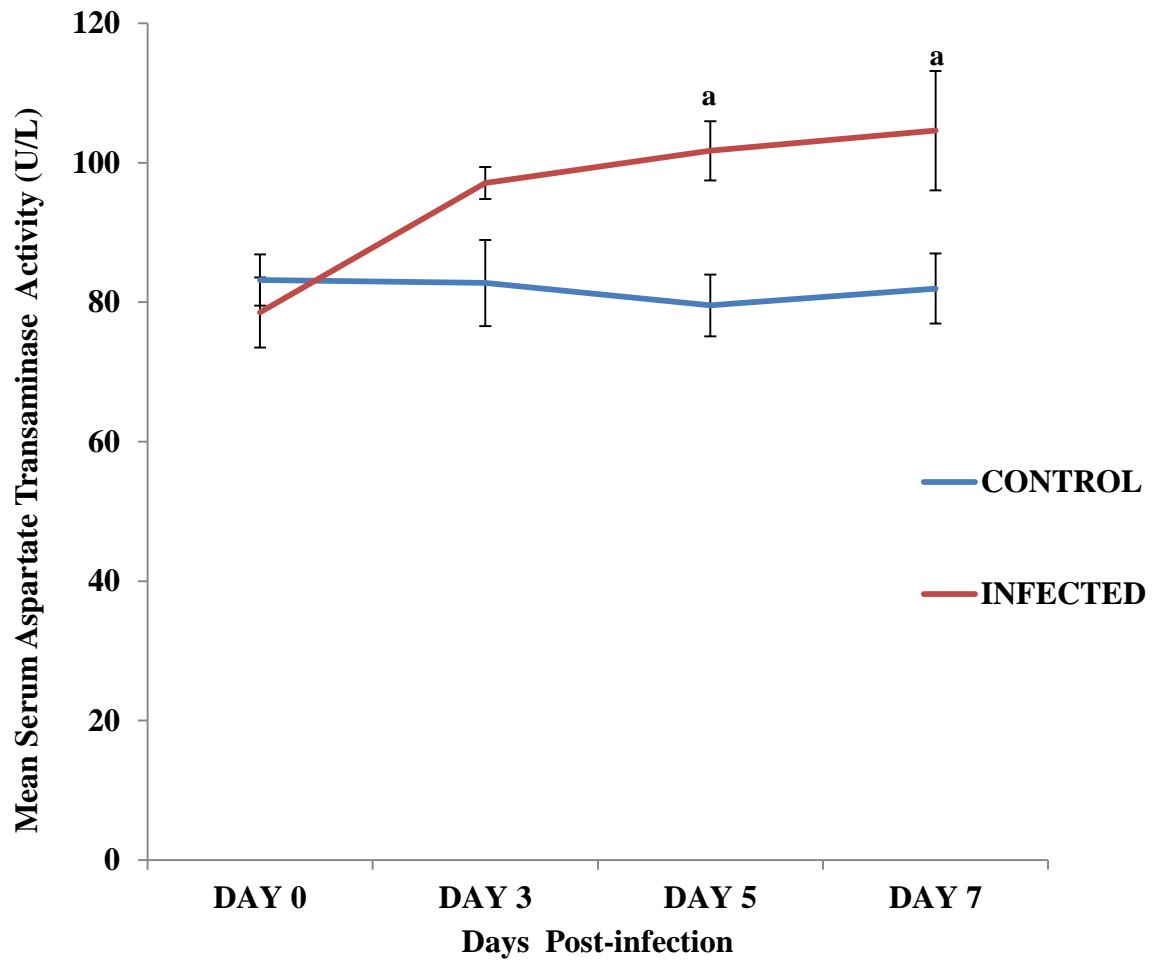


Figure 4.8: Mean \pm SEM Serum Aspartate Aminotransferase Activity of *T. b. brucei*-infected and Control Rats.

a: Significantly ($P < 0.05$) higher when compared to the pre-infection and controls.

4.5.2 Serum alanine aminotransferase (ALT) activity

The mean serum ALT activities in the *T. brucei*-infected and control rats were as presented in Figure 4.9. The pre-infection mean serum ALT activities of the infected and control groups were 39.35 ± 3.02 and 41.33 ± 2.60 U/L, respectively. Following the infection there was a progressive increase in the mean serum ALT activity in the infected rats, with the highest activity, 57.20 ± 3.26 U/L, recorded at termination of the experiment and was significantly ($p < 0.05$) higher than the pre-infection or corresponding control group value. The mean ALT activity in the control group remained relatively unchanged up to termination of the experiment.

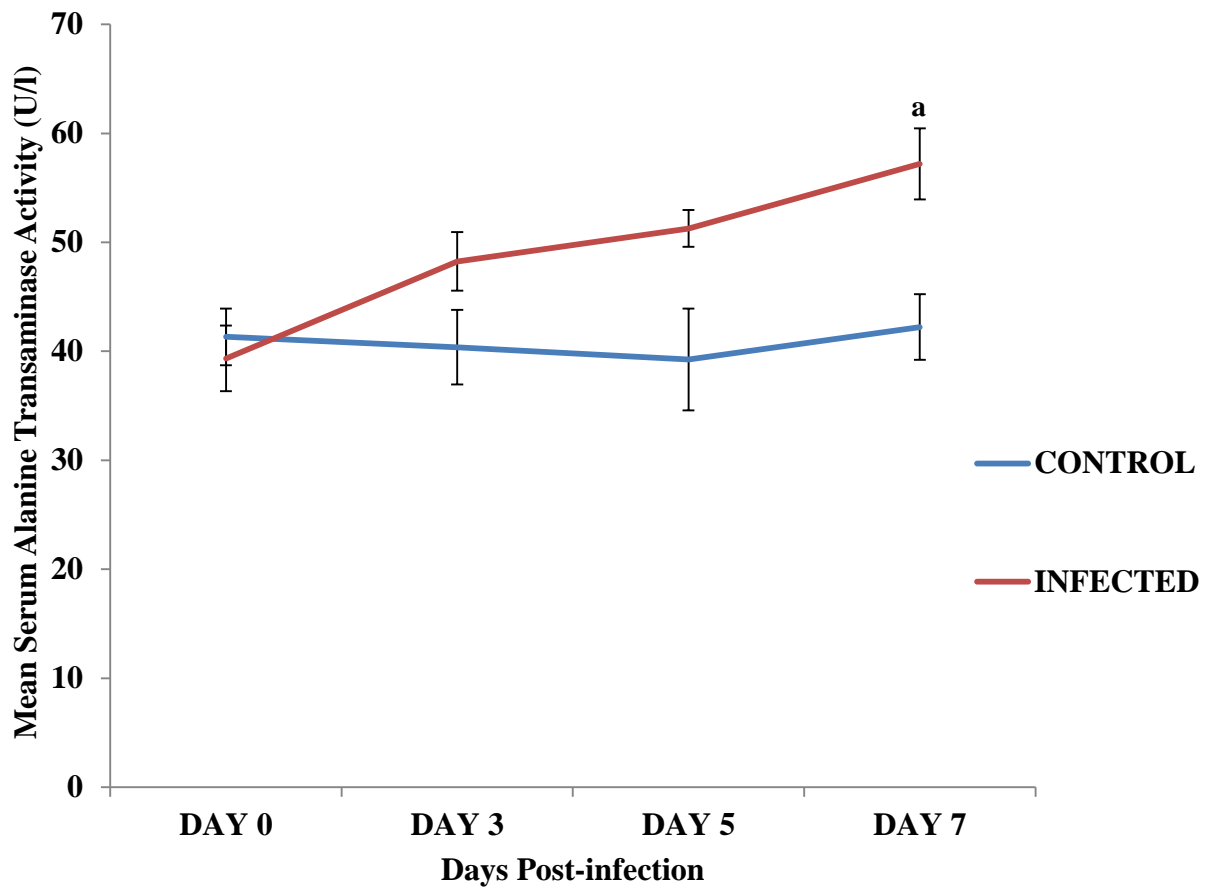


Figure 4.9: Mean \pm SEM Alanine Aminotransferase Activity of *T. b. brucei*-infected Rats and Control.

a: Significantly ($P < 0.05$) higher when compared to the pre-infection and controls.

4.5.3 Serum alkaline phosphatase (ALP) activity

The mean serum ALP activities in the *T. brucei*-infected and control rats were as presented in Figure 4.10. The mean pre-infection serum ALT activities of the infected and control groups were 104.80 ± 5.76 and 117.50 ± 2.10 U/L, respectively. Following infection there was a progressive increase in the mean serum ALP activity up to the end of the experiment. Although all the mean serum ALP activities obtained post-infection were not statistically different ($p > 0.05$) from the corresponding control values, they had a consistent and sequentially higher values than those of the control group, which remained relatively unchanged throughout the study.

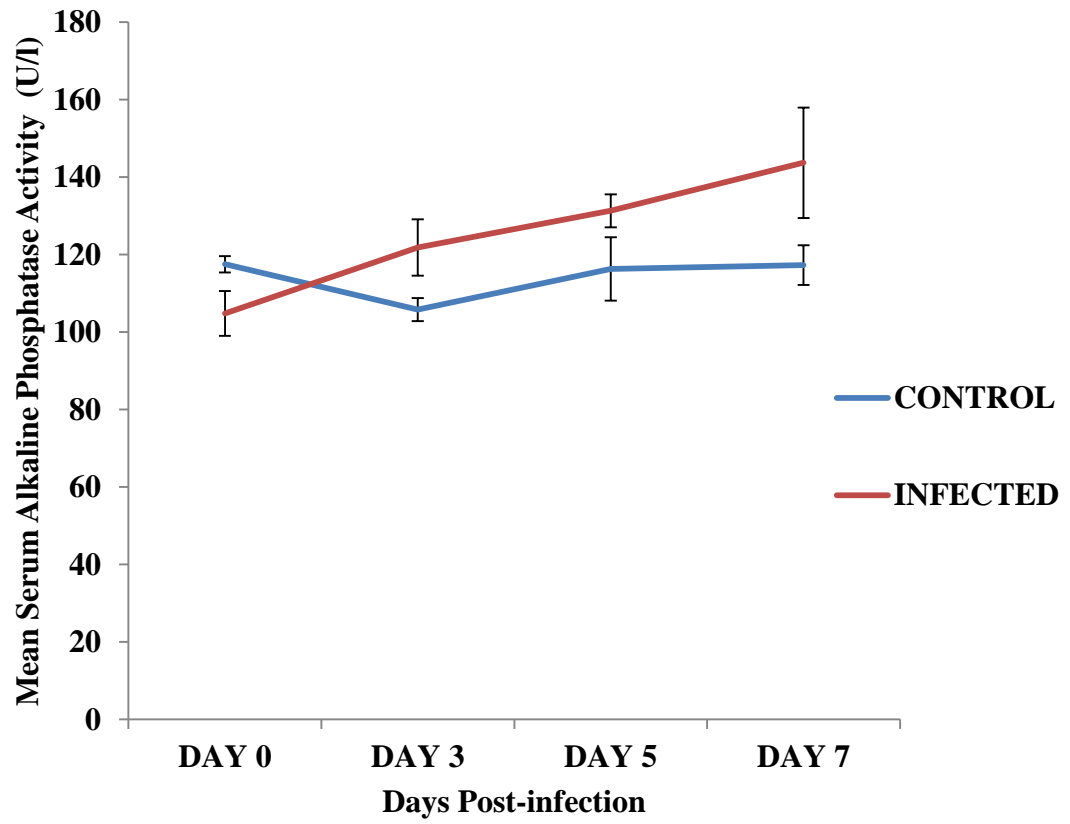


Figure 4.10: Mean \pm SEM Serum Alkaline Phosphatase Activity of *T. b. brucei*-infected and Control Rats.

4.5.4 Serum urea concentration

The mean serum urea concentrations in the *T. brucei*-infected and control rats were as presented in Figure 4.11. The mean pre-infection serum urea concentrations of the infected and control groups were 6.45 ± 0.47 and 6.77 ± 0.64 mmol/L, respectively. Following the infection there was a progressive increase in the mean serum urea concentration of the infected rats, with the highest concentration of 9.32 ± 0.98 mmol/L, attained on day 7 pi which was significantly ($p < 0.05$) higher than the corresponding control group value. The mean serum urea concentration of the control group remained relatively unchanged up to termination of the experiment.

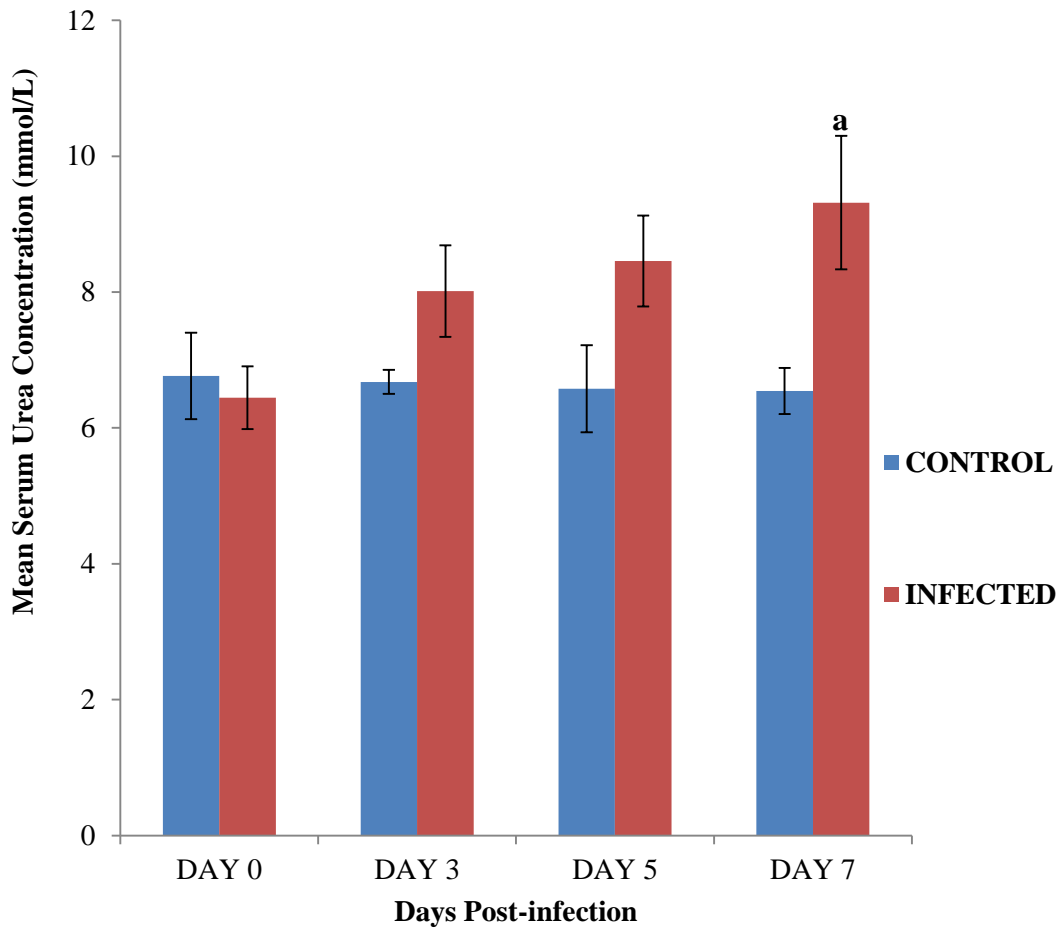


Figure 4.11: Mean \pm SEM Serum Urea Concentration of *T. b. brucei*-infected and Control Rats.

a: Significantly ($P < 0.05$) lower when compared to the pre-infection and control values.

4.5.5 Serum Creatinine Concentration

The mean serum creatinine concentrations in the *T. brucei*-infected and control rats were as presented in Figure 4.12. The mean pre-infection serum creatinine concentrations of the infected and control groups were 76.24 ± 3.28 and 74.92 ± 2.78 $\mu\text{mol/L}$, respectively. Following the infection, a progressive increase in the mean serum creatinine concentration was observed in the infected rats, with the highest level of 111.10 ± 6.09 $\mu\text{mol/L}$, recorded on day 7 pi, which was significantly ($p < 0.05$) higher than the corresponding control group value. The mean serum creatinine concentration of the control group remained relatively unchanged up to termination of the experiment.

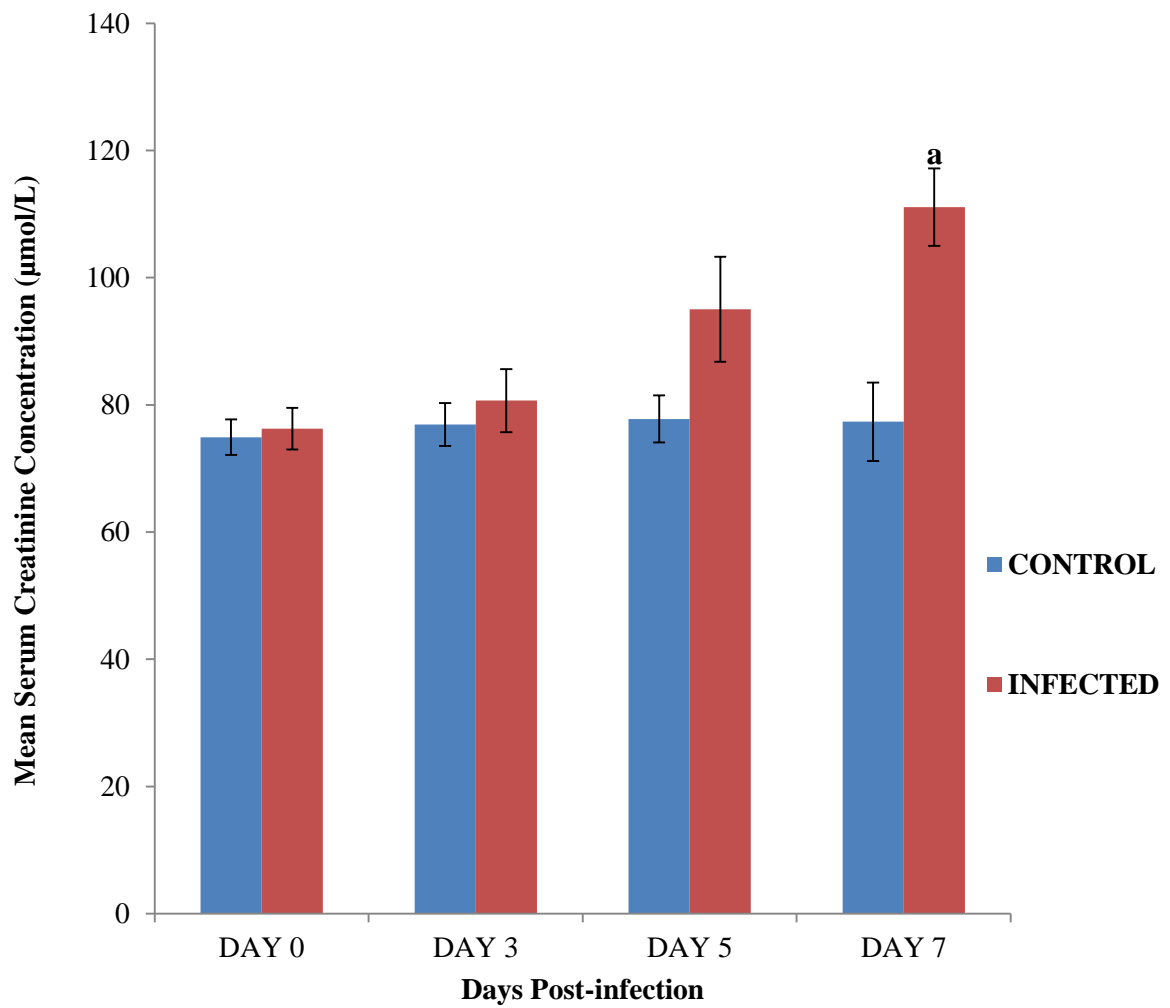


Figure 4.12: Mean \pm SEM Serum Creatinine Concentration of *T. b. brucei*-infected and Control Rats.

a: Significantly ($P < 0.05$) higher when compared to the pre-infection and controls.

4.6 Correlation of Parasitaemia with Serum Antioxidant Enzymes and Organ GSH

4.6.1 Parasitaemia and serum antioxidant enzymes activity

Serum SOD and GPx activities negatively correlated with parasitaemia ($r = -0.91$, $p = 0.04$; $r = -0.77$, $p = 0.12$, respectively), the former being significant with a $p < 0.05$ (Table 4.1).

4.6.2 Parasitaemia and organ GSH concentration

The concentration of GSH in the liver (r ; -0.84 and p ; 0.08) and kidney (r ; -0.84 and p ; 0.08) showed a negative correlation with parasitaemia, although both had no statistical significance ($p > 0.05$) (Table 4.1).

Table 4.1: Correlation of Parasitaemia with Serum Enzymes and Organ Reduced Glutathione Level in *T. b. brucei*-infected Rats

	Correlation Coefficient	P value
Serum Superoxide Dismutase and parasitaemia	-0.91*	0.04
Serum Glutathione Peroxidase and parasitaemia	-0.77	0.12
Liver Reduced Glutathione and parasitaemia	-0.84	0.08
Kidney Reduced Glutathione and parasitaemia	-0.84	0.08

*; Significant ($P < 0.05$)

4.7 Relationship between Parasitaemia and Haematological Parameters, Serum Biochemicals and Enzymes

4.7.1 Parasitaemia and haematological parameters

Correlation of parasitaemia with haematological parameters in *T. b. brucei*-infected rats is presented in Table 4.2. Both the PCV and TWBC had a negative correlation with parasitaemia, PCV ($r = -0.97$) was statistically significant with a p of 0.01 while TWBC ($r = -0.37$) had a $p > 0.05$, thus not statistically significant.

Table 4.2: Correlation of Parasitaemia with Haematological Parameters in *T. b. brucei*-infected Rats

	Correlation Coefficient	P value
Packed Cell Volume and parasitaemia	-0.97*	0.01
Total White Blood Cell Count and parasitaemia	-0.37	0.31

*; Significant (P < 0.05)

4.7.2 Parasitaemia and serum biochemicals and enzymes

All the assayed serum biochemicals and enzymes in the study had positive correlation with parasitaemia. The ALP ($r = 0.91$) and creatinine ($r = 0.99$) were statistically significant ($p < 0.05$) with p values of 0.047 and 0.003, respectively. The others (AST, ALT and urea) were not statistically significant ($p > 0.05$) (Table 4.3).

Table 4.3: Correlation of Parasitaemia with Serum Enzymes and Biochemicals in *T. b. brucei*-infected Rats.

	Correlation coefficient	P value
Aspartate aminotransferase and parasitaemia	0.735	0.13
Alanine Phosphatase and parasitaemia	0.91*	0.05
Alanine aminotransferase and parasitaemia	0.87	0.06
Urea and parasitaemia	0.83	0.09
Creatinine and parasitaemia	0.99**	0.00

*; Significant ($P < 0.05$), **; Very Significant ($P < 0.01$)

4.8 Correlations of some Parameters of the Infected Rats

4.8.1 Serum antioxidant activity with haematological parameters

4.8.1.1 Serum SOD activity with haematological parameters

Correlation of serum SOD activity with haematological parameters in *T. b. brucei*-infected rats is presented in Table 4.4. Both the PCV and TWBC had a positive correlation with SOD activity, although both had a $p > 0.05$, thus not statistically significant.

Table 4.4: Correlation of serum SOD activity with haematological parameters in *T. b. brucei*-infected Rats

	Correlation Coefficient (r)	P value
Packed Cell Volume and SOD	0.84	0.08
Total White Blood Cell Count and SOD	0.01	0.50

4.8.1.2 Serum GPx activity with haematological parameters

Correlation of serum GPx activity with haematological parameters in *T. b. brucei*-infected rats is presented in Table 4.5. Both the PCV and TWBC had a positive correlation with GPx activity, although both had a $p > 0.05$, thus not statistically significant.

Table 4.5: Correlation of serum GPx activity with haematological parameters in *T. brucei*-infected rats

	Correlation coefficient	P value
Packed Cell Volume and GPx	0.69	0.15
Total White Blood Cell Count and GPx	0.84	0.07

4.8.2 Serum antioxidant activity with organ GSH, serum biochemicals and enzymes

4.8.2.1 Serum SOD activity with organ GSH concentrations

The liver and kidney GSH concentrations positively correlated with the serum SOD activity in the *T. b. brucei*-infected rats, although both had $p > 0.05$, thus not statistically significant (Table 4.6).

4.8.2.2 Serum SOD activity with serum biochemicals and enzymes

All the considered serum biochemicals and enzymes negatively correlated with serum SOD activity in the *T. b. brucei*-infected rats, although they all (AST, ALT, ALP, urea and creatinine) had a $p > 0.05$, thus were not statistically significant (Table 4.6).

Table 4.6: Correlation of serum SOD activity with organ GSH, serum biochemicals and enzymes in *T. b. brucei*-infected Rats

	Correlation coefficient	P value
Liver Reduced Glutathione and SOD	0.70	0.15
Kidney Reduced Glutathione and SOD	0.55	0.23
Aspartate aminotransferase and SOD	-0.50	0.25
Alanine Phosphatase and SOD	-0.70	0.15
Alanine aminotransferase and SOD	-0.64	0.18
Urea and SOD	-0.60	0.21
Creatinine and SOD	-0.87	0.07

4.8.2.3 Serum GPx activity with organ GSH concentrations

The liver and kidney GSH concentrations positively correlated with serum GPx activity in the *T. b. brucei*-infected rats, both were statistically significant ($p < 0.05$) with p values of 0.02 and 0.03 for the liver GSH and kidney GSH, respectively (Table 4.7).

4.8.2.4 Serum GPx activity with serum biochemicals and enzymes

All the considered serum biochemicals and enzymes were negatively correlated with the serum GPx activity in the *T. b. brucei*-infected rats; AST, ALP, ALT and urea were all statistically significant ($p < 0.05$), except creatinine with a $p > 0.05$ was not statistically significant (Table 4.7).

Table 4.7: Correlation of serum GPx activity with organ GSH, serum biochemicals and Enzymes in *T. b. brucei*-infected Rats

	Correlation coefficient	P value
Liver GSH and GPx	0.96*	0.02
Kidney GSH and GPx	0.93*	0.03
AST and GPx	-1.00***	0.00
ALP and GPx	-0.96*	0.02
ALT and GPx	-0.97*	0.01
Urea and GPx	-0.99**	0.00
Creatinine and GPx	-0.83	0.09

*, Significant (P < 0.05), **, Very Significant (P < 0.01), ***, Highly Significant (P < 0.001)

4.9 Histopathological Findings

4.9.1 Liver

The liver of the *T. b. brucei*-infected group of rats at pre-infection showed no obvious pathology with normal hepatic architecture and cellular composition, same as the control rats on days 0, 3, 5 and 7 (Plate I). The infected group of rats showed tissues changes with increasing severity on days 3, 5 to 7.

On day 3 there were mild vascular congestion, erythrophagocytosis and mononuclear cellular infiltrations (Plate II). By day 5, there were increased vascular congestion, erythrophagocytosis, mononuclear cellular infiltrations with some level of hepatocyte degeneration (Plate III).

The hepatic lesions were most severe on day 7. There were marked congestion of the blood vessels and sinuses, severe distortion of the hepatic cord, increased number of degenerated and necrotized hepatocytes, mononuclear cellular infiltrations and erythrophagocytosis (Plate IV).

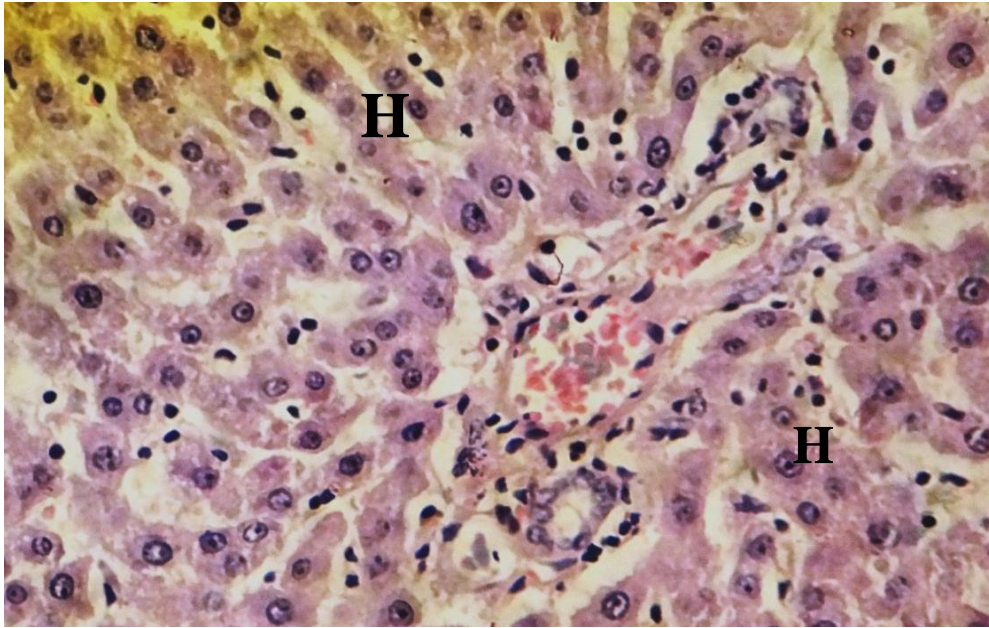


Plate I: Photomicrograph of liver of *T. b. brucei*-uninfected rats (control). Note the normal hepatocyte (H) in their usual cord-like arrangement with no apparent histopathological lesions. H & E x 400.

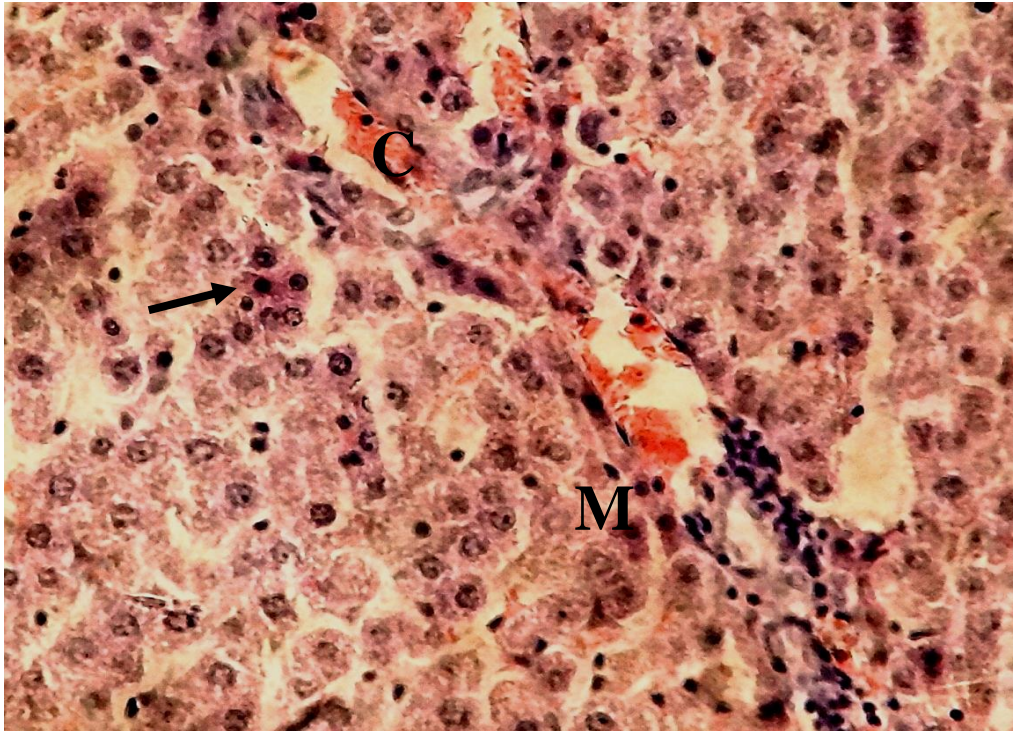


Plate II: Photomicrograph of liver of *T. b. brucei*-infected rats on Day 3 Post-Infection. Note the mononuclear cellular (M) infiltrations, mild vascular congestion (C) and erythrophagocytosis (black arrow). H & E x 400.

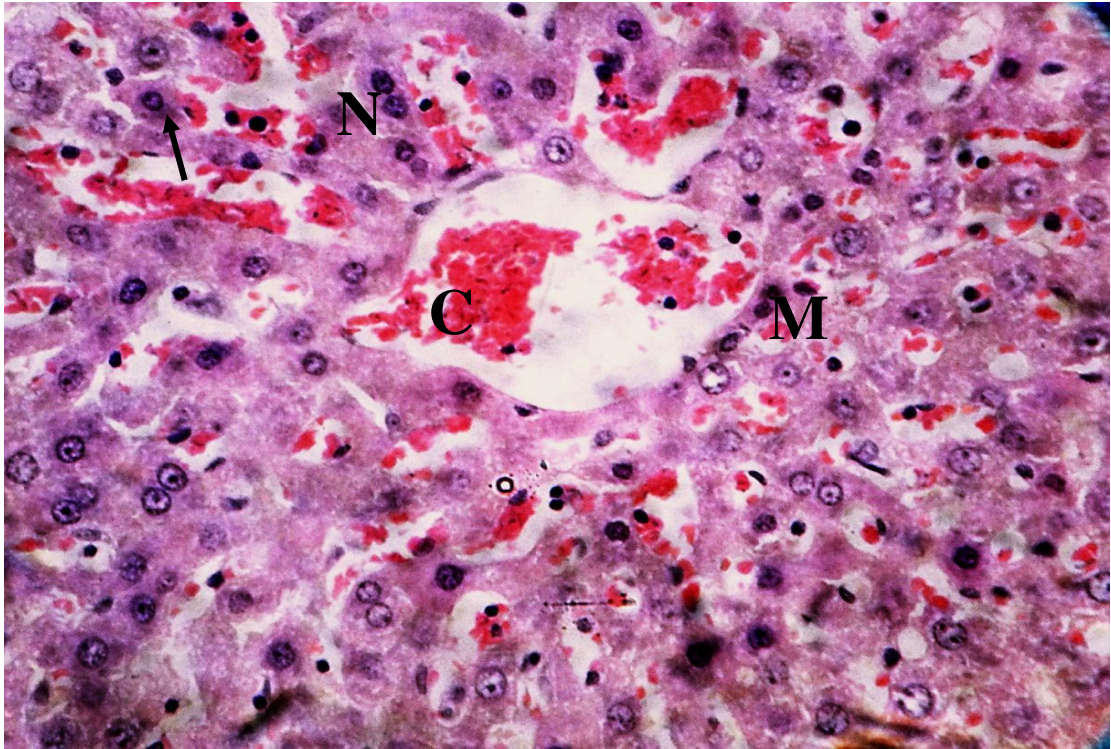


Plate III: Photomicrograph of liver of *T. b. brucei*-infected rats on Day 5 Post Infection. Note the congested vessels and sinuses (C), erythrophagocytosis (black arrow) mononuclear cells infiltrations (M) with hepatocyte clumping (N). H & E x 400.

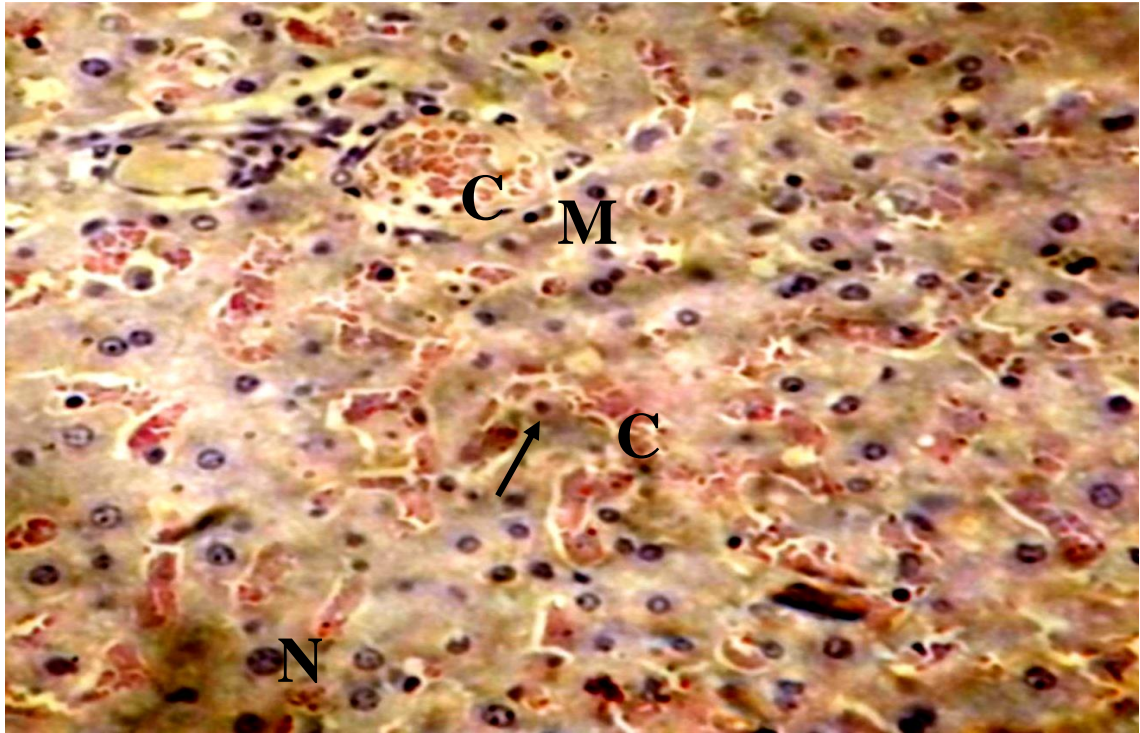


Plate IV: Photomicrograph of liver of *T. b. brucei*-infected rats on Day 7 Post Infection. Note the markedly congested vessels and sinuses (C), erythrophagocytosis (black arrow), clumping of the hepatocyte (N), mononuclear cellular infiltration (M). H & E x 400.

4.9.2 Kidney

The kidney of the *T. b. brucei*-infected and control groups of rats showed no obvious pathology with normal renal cellular components and structures. The rats in the control group also showed no renal lesions on days 3, 5 and 7 (Plate V).

The infected group of rats presented pathology with increasing severity, from days 3, 5 and 7. On days 3 (Plate VI) and 5 (Plate VII) there was congestion of the renal vessels and glomeruli and mononuclear cells infiltrations, which were more severe in day 5pi. Desquamation of the renal tubular epithelium was also observed in the infected group on day 7 pi (Plate VIII).

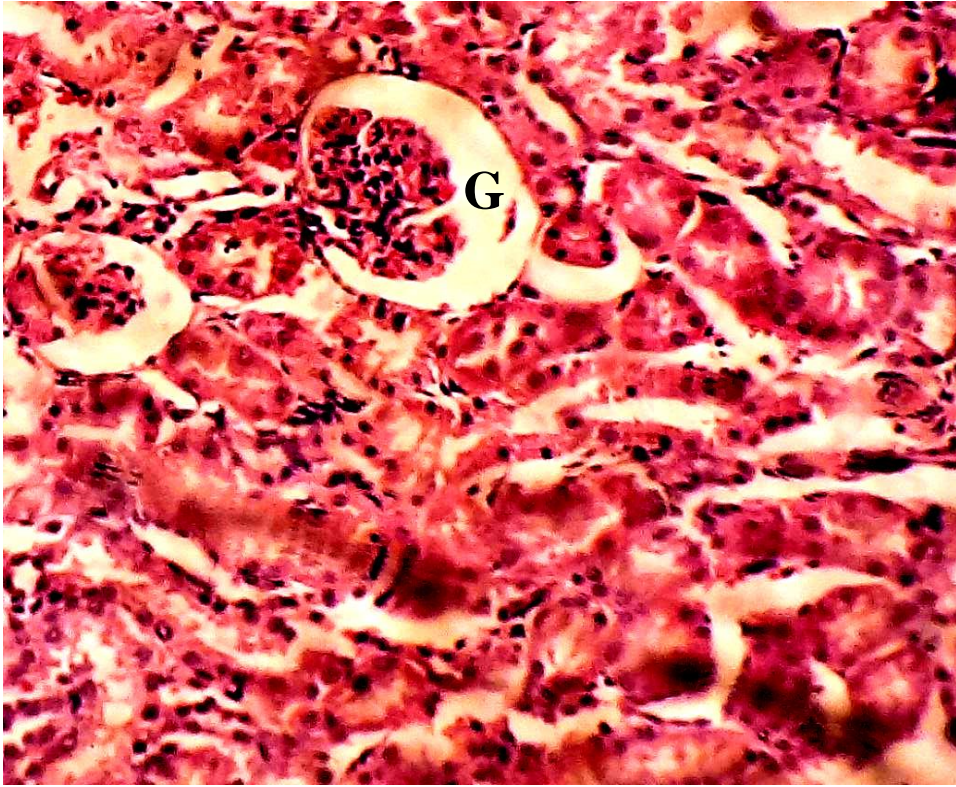


Plate V: Photomicrograph of kidney of *T. b. brucei* uninfected rats (control). Note the intact renal corpuscles (G) with no apparent histopathological lesions. H & E x 200.

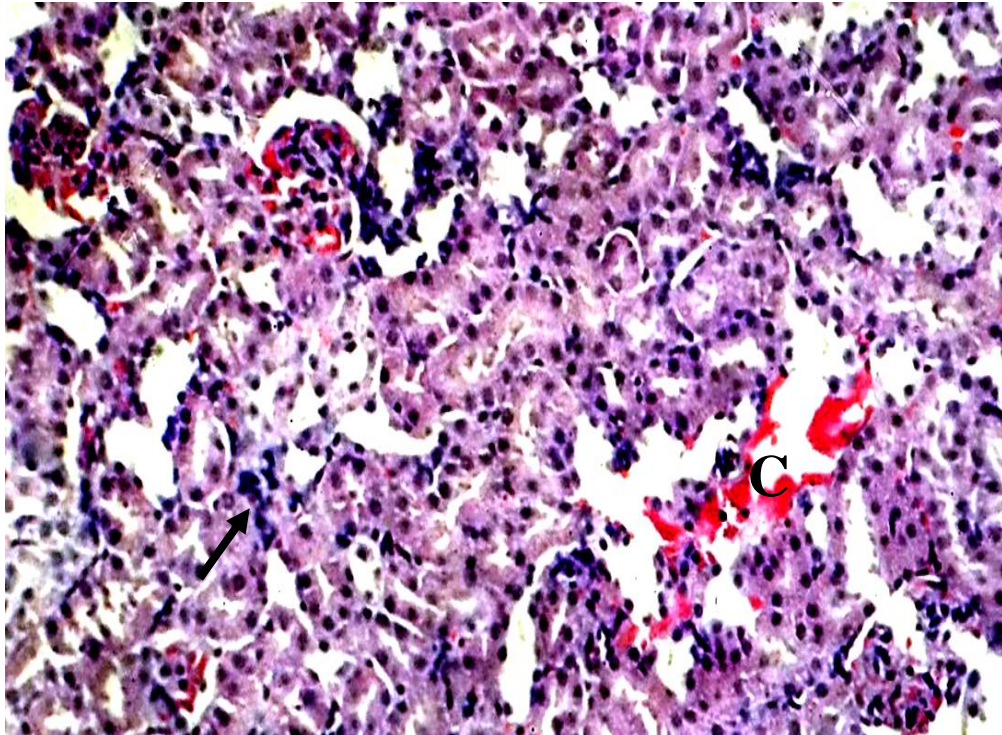


Plate VI: Photomicrograph of kidney of *T. b. brucei*-infected rats on Day 3 Post-Infection. Note the mild congestions (C) of the renal vessels and glomeruli and mononuclear cells infiltrations (black arrow). H & E x 200.

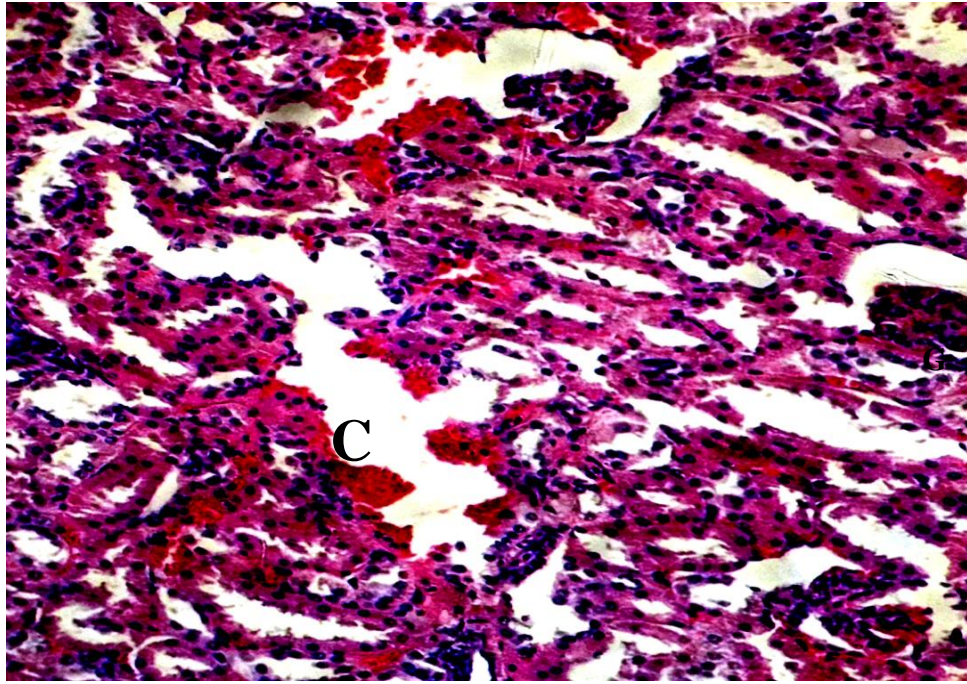


Plate VII: Photomicrograph of kidney of *T. b. brucei*-infected rats on Day 5 Post-Infection. Note the congestions (C) of the renal vessels and glomeruli with generalized mononuclear cellular infiltrations. H & E x 200.

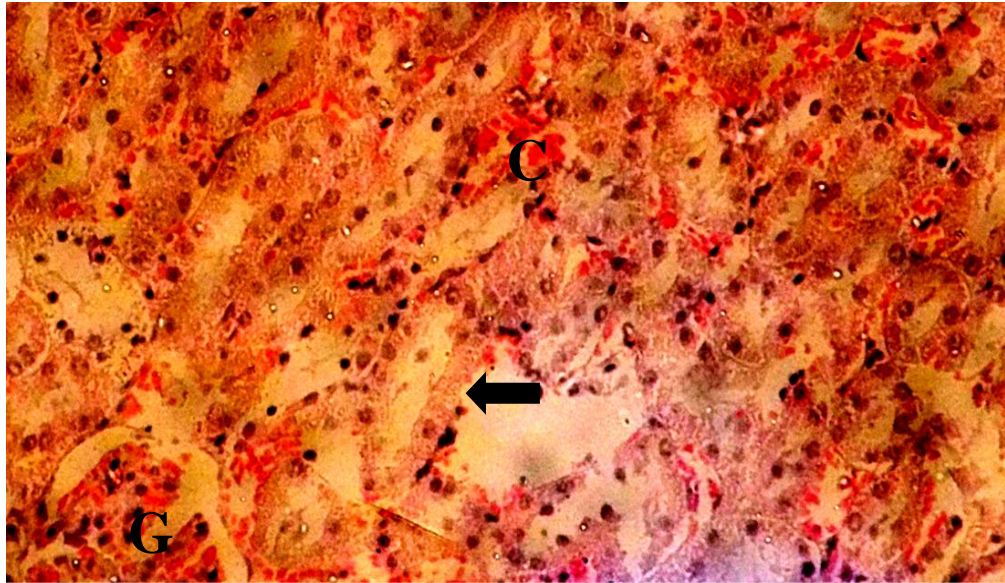


Plate VIII: Photomicrograph of kidney of *T. b. brucei*-infected rats on Day 7 Post-Infection. Note the marked congestions (C) of the renal vessels and glomeruli (G), generalized mononuclear cellular infiltrations and necrosis of the epithelial cells of the renal tubular (black arrow). H & E x 200.

CHAPTER FIVE

DISCUSSIONS

The findings in this study was consistent with earlier reports on the parasite in *T. brucei*-infected rats (Adeyemi *et al.*, 2009; Umar *et al.*, 2009) and mice (Mbutia *et al.*, 2011; Yusuf *et al.*, 2012). The mean prepatent period for the infection with *T. brucei* of 3.75 ± 0.11 days recorded in this study, with a progressive parasitaemia up to termination of the experiment was also similar to the ones in previous reports (Umar *et al.*, 1999; Umar *et al.*, 2007; Kobo *et al.*, 2014c). The clinical observations in the *T. b. brucei*-infected rats, recorded in this study, which include anorexia, pale ocular mucous membrane, frequent urination, respiratory distress, unthriftiness, raised hair coat, and weight loss were the classical symptoms in various reports of trypanosome infections in animals (Adeyemi *et al.*, 2009; Umar *et al.*, 2009; Kobo *et al.*, 2014c).

The significant reduction in packed cell volume in the *T. b. brucei*-infected rats, as observed in this study, which is progressive could result in anaemia (Esievo, 2017), which is consistent with the established clinical manifestations of animal trypanosomosis (Anosa 1988; Esievo and Saror, 1991). The reduction in packed cell volume coincided with onset of parasitaemia. This finding is in conformity with previous reports that correlated development of anaemia with onset and the degree of parasitaemia (Dargie *et al.*, 1979; Umar *et al.*, 2009). The significant reduction in total white blood cell count in the *T. b. brucei*-infected rats was in agreement with earlier reports (Itou *et al.*, 1996; Kagira *et al.*, 2006; Fialkow *et al.*, 2007; Kobo *et al.*, 2014a,c). In this study, the negative correlations of the levels of parasitaemia with PCV and total leucocyte count in the *T. b. brucei*-infected rats, with the former being statistically significant, indicated that the erythrocytes are either more sensitive or more

affected by the trypanosomes induced blood cell damage. Probably because leukocytes possess a relative more stable cell membrane, have a longer lifespan than the erythrocytes (Esievo, 2017) and partly because of the acute duration of this study.

There have been many reported pathophysiological mechanisms of anaemia in trypanosome-infected animals (Igbokwe, 1989; Mbaya *et al.*, 2012). Mechanical injury to erythrocytes caused by the lashing action of the trypanosome flagellae, undulating pyrexia, infecting trypanosome's released sialidase, the action of which subjected the erythrocytes to phagocytosis, the role of lipid peroxidation affecting erythrocyte membrane, malnutrition and bone marrow depressant effect of the infection are among such reported mechanisms (Morrison *et al.*, 1981; Igbokwe *et al.*, 1996; Adamu *et al.*, 2009; Mbaya 2012; Esievo, 2017).

Also worthy of note is the possession of ecto-phosphatase (as alkaline phosphatase which has been identified in some virulent trypanosomes (Meyer-Fernandes *et al.*, 1999). Dying trypanosomes release this acid phosphatase into circulation of the infected host, where it exerts its cytotoxic and hemolytic effects (Tizard *et al.*, 1978; Fernedes *et al.*, 1997; Mbaya *et al.*, 2012). This is usually restricted to microvasculature, where it provokes endothelial damage and erythrocyte destruction especially infection caused by *T. congolense*. In infections caused by *T. brucei* the enzyme is responsible for the autolysing of the connective tissue, increase vascular permeability and inflammation (Tizard *et al.*, 1978).

Thus, it is reasonable to adduce that lipid peroxidation effect on the erythrocyte membrane might have been responsible, at least in part, for the observed reduction in circulating erythrocyte mass in the *T. b. brucei*-infected group. Although findings in this study on SOD and GPx activities were contrary to previous reports by Ataley *et al.*

(2000), Ogunsanmi and Taiwo (2007) and Yusuf *et al.* (2012), who reported increased antioxidant enzymes activities. This discrepancy is only suggestive of perhaps, differences in the concentration of the released reactive oxygen or nitrogen species in the trypanosomal infected rats. This is because the more the reactive species released, the higher the tendency to overwhelm the antioxidant capacity of the host animal.

The finding in this study of reduction, though not significant statistically, in superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities following infection of the rats with *Trypanosoma b. brucei* supports the fact that reactive oxygen species were released during the infection and the reduction may have been a consequence of SOD and GPx utilization in the mopping up of the oxidant substance (Igbokwe *et al.*, 1996; Omer *et al.*, 2007; Yusuf *et al.*, 2012).

Lipoperoxidation of the polyunsaturated fatty acids of the erythrocytes membrane as a mechanism of erythrocyte destruction was attributed to disequilibrium between the host's antioxidant capacity and the generated ROS consequent to trypanosome infection, with the latter being in excess (Igbokwe *et al.*, 1998; Omer *et al.*, 2007; Ogunsanmi and Taiwo, 2007; Kobo *et al.*, 2014a,c). The disequilibrium could result from overwhelming of host's antioxidant defense system in trypanosome-infected animals (Igbokwe *et al.*, 1998; Omer *et al.*, 2007). Lipid peroxidation of erythrocyte membrane predisposed the erythrocytes to direct cellular lysis or phagocytosis (Slater 1984; Ngure *et al.*, 2009; Kobo *et al.*, 2014c).

The decrease in total leucocyte count observed in the infected rats, were attributed to factors such as variable surface antigen of the trypanosomes and bone marrow depression of their production (Kagira *et al.*, 2006; Kobo *et al.*, 2014c);

lipoperoxidation can also predispose them to direct cellular lysis or phagocytosis just as with the case of erythrocytes because of the polyunsaturated lipids in their membranes (Itou *et al.*, 1996; Fialkow *et al.*, 2007).

There is truly no consensus on the activities of antioxidant enzymes during trypanosomosis. Whereas some researchers reported a decreased antioxidant enzymes activity (Wen *et al.*, 2004; Saleh *et al.*, 2009), others reported increases in the antioxidant enzymes activity (Ataley *et al.*, 2000; Ogunsanmi and Taiwo, 2007; Yusuf *et al.*, 2012). In the present study, the serum level of SOD was higher in the infected when compared to the control with a lower level of GPx on day 3 pi but subsequently on days, 5 and 7pi both the levels of serum SOD and GPx progressively decreased in the infected animals. The higher level of serum SOD in the infected rats at the onset of the infection (day 3pi) is probably due to an up regulation of SOD being a first line of antioxidant defense involved with the conversion of superoxide anion (O_2^-) into hydrogen peroxide and other peroxides which are degraded by either GPx or catalase (Valko *et al.*, 2006). Increased SOD synthesis and activity following the up regulation, would concomitantly increase the formation of less toxic radicals scavenged by GPx and catalase, thus, lowering the level of GPx from their increased exhaustion. In a parallel study by Souza *et al.* (2014) with a different tissue parasite (canine visceral leishmaniasis), they found an increased SOD activity with lower levels of GPx and catalase in the blood of infected dogs corroborated by the work of Dimri *et al.* (2012) on dogs infected with *Dirofilaria immitis* with similar findings.

The progressive lower serum levels of SOD and GPx in the infected rats on days 5 and 7pi when compared to the controls could be attributed to possible exhaustion of the endogenous antioxidant enzymes embattled with the increasing generations of ROS

during the infection (Igbokwe *et al.*, 1996; Omer *et al.*, 2007). SOD is involved in the direct elimination of ROS, through dismutation of superoxide radicals (Punitha *et al.*, 2005). The GPx catalyzes the conversion of H₂O₂ to H₂O through the oxidation of GSH, through the glutathione redox cycle (Szkudelski, 2001; Duarte *et al.*, 2001). Under normal circumstances, sufficient amounts of ROS are removed by the antioxidant enzymes, but in excess generation of ROS, there is depletion of the endogenous antioxidant defense system (Igbokwe *et al.*, 1996; Baydas *et al.*, 2002; Yusuf *et al.*, 2012), and this could be the possible reason for the observed reduction in serum SOD and GPx activities in this study.

The negative correlation of the level of parasitaemia with serum antioxidant (SOD and GPx) activities and organ (liver and kidney) GSH levels, in the *T. b. brucei*-infected rats, further buttress the possible exhaustion of the host antioxidant defense, corroborated by earlier findings Igbokwe *et al.* 1996, Baydas *et al.* 2002, Yusuf *et al.* 2012. That only serum SOD activity had a statistically significant correlation with parasitaemia, among the endogenous antioxidants studied, could have been due to the modulating role of SOD on GPx activity. It has been reported that SOD is the most potent antioxidant enzyme which directly eliminates ROS, by dismutation of superoxide anions into H₂O₂ degraded by GPx, into H₂O with the oxidation of GSH (Szkudelski, 2001; Duarte *et al.*, 2001; Punitha *et al.*, 2005).

There was a decrease in the concentrations of GSH in the liver and kidney of the *T. b. brucei*-infected rats. The decrease was marginal and statistically insignificant, but followed similar patterns observed by earlier research where significant decrease in GSH concentrations of the tissues and serum of infected rats were reported (Igbokwe *et al.*, 1996; Saleh *et al.*, 2009; Yusuf *et al.*, 2012). Glutathione plays an important role in

the maintenance of intracellular redox homeostasis, hence ensure cellular and as well organ integrity. GSH being a major intracellular non-enzymatic antioxidant can readily lose an electron to neutralize ROS in the presence of GPx and convert to its oxidized state GSSG (Kurutas, 2016). The levels of GSH are tightly regulated by several regulatory mechanism (Lushchak, 2011), the GSH oxidized to GSSG during the degradation of H₂O₂ and lipid peroxides catalyzed by GPx, is reduced back to GSH by glutathione reductase at the expense of NADPH, in a continuous glutathione redox cycle (Dalton *et al.* 1999). It thus suggests that decreases in the concentrations of GSH in the liver and kidney were marginal and not statistically significant because of the tight regulations of GSH level and partly because of the sub-acute nature of the infection in this study.

The positive correlations of the liver and kidney GSH concentrations with serum antioxidant enzymes (SOD and GPx) activities in the *T. b. brucei*-infected rats, suggests that as the serum antioxidant enzymes activities progressively decreased with the infection there was also a proportionate decrease in the liver and kidney GSH concentrations. Serum SOD and GPx being enzymatic antioxidant and the body's first line of antioxidant defense against oxidative stress, that works in tandem with GSH, a non-enzymatic endogenous antioxidant, because of their synergistic relationship. A decrease in the serum antioxidants (SOD and GPx) activities would invariably cause a decrease in the GSH concentration in the liver and kidney. Serum GPx activity had a significant correlation with the liver and kidney GSH, whereas serum SOD activity had a non-significant correlation with the liver and kidney GSH concentration. This was not surprising, since GPx is an enzyme that GSH requires to participate in protection against oxidative stress in the organ, probably explains why the organ GSH concentration are more closely related to serum GPx than serum SOD.

In this study, there was an elevated serum level of biochemicals and enzymes activities in the infected group of rats following the onset of the infection, with progressive elevation from days 3, 5 to 7. While the control rats had concordant values within the reference value for the different serum enzymes and biochemicals considered, on days 0, 3, 5 and 7. The result suggests infiltration and inflammation in vital body organs particularly of the liver and kidney by the trypanosomes (Akpa *et al.*, 2008). It has been reported that *T. b. brucei* infection, like other trypanosomal infections precipitate increased serum biochemicals and enzymes in the affected host due to the tissue invasion by the trypanosomes, their increase could be due to the tissue damage, among other factors (Adeyemi and Sulaiman, 2010).

Thus, the elevated AST activity in the infected rats (significant on days 5 and 7) is a pointer to generalized tissue damage which is typical of the parasite *T. b. brucei* and the generated oxidative radicals which cause nonspecific oxidative damage in different tissue and organ of affected host (Lee *et al.*, 2004; Bouayed and Bohn, 2010; Ayla and Metin, 2015). Oxidative stress have been reported in trypanosomosis and have been implicated to play a contributory role in tissue damage and elevation of serum biochemicals and enzymes (Omer *et al.*, 2007; Umar *et al.*, 2007; Saleh *et al.*, 2009). The significantly elevated AST and ALT activities is probably due to hepatocellular damage, sequel to or worsened by hypoxia and severe oxidative stress caused by the trypanosome infection. Consistent earlier studies had also shown hepatocellular inflammation in trypanosomosis caused by oxidative stress, production of pro-inflammatory cytokines and activated mononuclear cells (Bosschaerts *et al.*, 2009; Mbuthia *et al.*, 2011).

The progressively increasing serum level of urea and creatinine in the *T. b. brucei*-infected rats which were both significantly elevated on day 7, as with the other serum enzymes, confirms the involvement of renal damages in this study. This give credence to the suggestion of Ekanem and Yusuf (2008), that trypanosomes cause a cumulative tissue and organ damage. Joint increase in serum urea and creatinine level is indicative of renal pathology (Esievo, 2017) which had been reported in other natural and experimental trypanosomal infections (Anosa, 1988; Abenga *et al.*, 2014). The kidney and liver are both involved in erythropoietin biosynthesis, hence regulates erythropoiesis (Abenga *et al.*, 2014), thus, will further complicate the development anaemia. Anaemia has been reported to worsen the severity of oxidative damage (Abenga *et al.*, 2014), thus, increasing the severity of the kidney and liver pathology.

The positive correlation of all the serum biochemicals and enzymes assayed in this study with the level of parasitaemia, impleed that as the level of parasitaemia increased there was a proportionate increase in their serum levels and activities, thus indicating progressive tissue pathology (Anosa, 1988; Ekanem and Yusuf, 2008). That only serum ALP activity and creatinine concentration had statistically significant correlations with parasitaemia, while the levels of the other correlations (urea, AST and ALT) were not statistically significant was noteworthy. In a way the *T. b. brucei*-infected rats could be said to have been able to contain the elevations of the serum enzymes and biochemicals in this study, probably because their serum level were tightly regulated and partly because of the sub-acute duration of this study.

The negative correlation of all the serum biochemicals and enzymes with both serum SOD and GPx activity in the *T. b. brucei*-infected rats, meaning that as the activities of the antioxidant enzymes diminished there was a proportionate increase in the serum

biochemicals and enzymes activities was also noteworthy. This was indicative of ongoing organ pathology (Anosa, 1988; Esievo, 2017), with depletion of the antioxidant defense during the infection. These negative correlations were all statistically significant with serum GPx activity except creatinine with a p value slightly greater than 0.05, but they all had a $p > 0.05$ with serum SOD. This suggests that the serum SOD activity does not truly represent the antioxidant status of the organs as serum GPx activity which also presented a statistically significant positive correlation with the liver and kidney GSH concentration.

The lesions observed in the liver and kidney were changes in the vascular beds, which are in consonance with earlier report on the acute phase of *T. b. brucei*-infection (Zwart, 1989; Omotainse and Anosa, 2009). The interstitial activities of the parasite, being tissue invasive attracted severe mononuclear inflammatory cell reaction in various organs, mainly lymphocyte and plasma cells (Omotainse and Anosa, 2009), presented in the liver and kidney sections of the infected rats on days 3, 5 and 7 pi. The liver and kidney lesions, started as mild to moderate congestion and mononuclear cellular infiltrations on day 3 pi, with progressive increase in the degree of congestion and infiltration on days 5 and 7 pi. Other specific lesions as necrosis of the hepatocyte and renal tubular epithelium, in the liver and kidney respectively, also occurred on day 7pi. Thus, it is logical to adduce that the durations and levels of parasitaemia was proportional to the degree of hepatic and renal damage suffered by the infected rats.

Hepatic lesions as erythrophagocytosis and congestion observed were consistent with the anaemia observed (Omotainse and Anosa, 2009). This have been reported to further increase the severity of the oxidative stress due to hypoxia, among other factors further worsened the organ pathology (Abenga *et al.*, 2014). The presence of activated

macrophages and erythrophagocytosis in the liver is suggestive of the liver playing a role in RBC destruction during trypanosomosis, in agreement with the earlier report of Omotainse and Anosa (2009).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, this study has shown that:

- i. There were decreases in the serum antioxidant enzymes activities (SOD, GPx) in the *T. b. brucei*-infected rats proportional to the level of parasitaemia and duration of the infection.
- ii. The parasitaemia was progressive and concurred with decreases in the serum antioxidants (SOD, GPx and organ GSH) levels of *T. b. brucei*-infected rats which correlated to the haematological and biochemical derangements and tissue pathology.
- iii. Increase in serum SOD activity corresponded closely with the onset of parasitaemia on day 3 pi, and this suggests its modulation of GPx activity during the infection.
- iv. GPx has strong correlations with organ GSH concentrations and their depletions are directly proportional to the haematological and biochemical derangement and tissue pathology. Thus diminishing GPx activity can be used to predict the possible damage suffered by the host.

6.2 Recommendations

- i. Determination of serum antioxidant enzymes activities is of clinical importance to determine the prognosis of patients with trypanosomosis and assessing their responses to treatment during management.
- ii. In the management of trypanosomosis, treatment should not be limited to the use of trypanocides and elimination of the parasite but effort should be made to determine and correct the antioxidant-oxidant imbalance caused by the infection.
- iii. Since the endogenous antioxidant defense is deficient to combat the excessive oxidative stress induced during trypanosomosis, exogenous antioxidant should be included in the management of trypanosomosis.
- iv. Further studies should be conducted at the molecular level to elaborate the possible interplay between this antioxidant enzymes and infection with trypanosomes.
- v. Research into the use of substances that can stimulate an upward regulation of the synthesis of endogenous antioxidant enzymes in the management of trypanosomosis is also recommended.

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APPENDICES

Appendix 1: Effects of *T. b brucei* Infection on Antioxidant, Haematologic and Serum Biochemical profiles of Male *Wistar* Rats (Mean \pm SEM, n=4)

PARAMETERS	DAY 0		DAY 3		DAY 5		DAY 7	
	INFECTED	CONTROL	INFECTED	CONTROL	INFECTED	CONTROL	INFECTED	CONTROL
SERUM SOD(U/ ml)	7.23 \pm 0.63	7.36 \pm 0.526	8.23 \pm 0.83	7.61 \pm 0.97	6.1 \pm 0.37	7.42 \pm 0.51	5.59 \pm 0.86	7.72 \pm 0.43
SERUM GPx(U/mg)	43.00 \pm 2.14	39.66 \pm 4.58	36.07 \pm 3.16	41.19 \pm 2.69	34.06 \pm 3.05	41.25 \pm 4.86	34.06 \pm 3.36	41.88 \pm 2.89
LIVER GSH(Ug /ml)	19.27 \pm 0.55	19.57 \pm 0.23	18.91 \pm 0.21	19.26 \pm 0.43	18.54 \pm 0.28	19.56 \pm 0.27	18.53 \pm 0.28	19.31 \pm 0.50
KIDNEY GSH(Ug/ml)	24.34 \pm 2.17	25.82 \pm 1.03	21.55 \pm 1.63	26.95 \pm 1.39	21.59 \pm 1.58	24.22 \pm 2.78	19.09 \pm 0.70	22.99 \pm 0.89
PCV (%)	52.00 \pm 11.14	48.33 \pm 5.24	52.00 \pm 1.96	47.25 \pm 2.29	46.5 \pm 4.29	52.00 \pm 3.39	33.00 \pm 2.89	50.25 \pm 1.32
TOTAL WBC($\times 10^{12}$ /L)	10.03 \pm 1.16	10.85 \pm 2.09	6.33 \pm 0.47	7.97 \pm 1.20	7.58 \pm 0.66	8.28 \pm 1.65	6.93 \pm 0.48	12.10 \pm 1.74
SERUM AST(U/l)	78.53 \pm 5.04	83.18 \pm 3.67	97.08 \pm 2.30	82.75 \pm 6.17	101.70 \pm 4.24	79.53 \pm 4.42	104.60 \pm 8.56	81.95 \pm 5.04
SERUM ALT(U/l)	39.35 \pm 3.02	41.33 \pm 2.60	48.25 \pm 2.70	40.38 \pm 3.42	51.28 \pm 1.70	39.25 \pm 4.68	57.20 \pm 3.26	42.23 \pm 3.02
SERUM ALP(U /l)	104.8 \pm 5.76	117.5 \pm 2.10	121.80 \pm 7.28	105.8 \pm 3.01	131.3 \pm 4.23	116.37 \pm 8.20	143.70 \pm 14.25	117.30 \pm 5.12
UREA (mmol/L)	6.45 \pm 0.46	6.77 \pm 0.64	8.01 \pm 0.67	6.68 \pm 0.18	8.46 \pm 0.67	6.58 \pm 0.64	9.32 \pm 0.98	6.54 \pm 0.34
CREATININE (mmol/L)	76.24 \pm 3.28	74.92 \pm 2.78	80.67 \pm 4.98	76.91 \pm 3.37	95.03 \pm 8.24	77.79 \pm 3.72	111.10 \pm 6.09	77.35 \pm 6.17

