EFFECT OF PRE AND POST-INFECTION ADMINISTRATION OF ZINC AND SELENIUM ON *Trypanosoma brucei brucei*INFECTIONIN WISTAR ALBINO RATS

BY

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ABSTRACT

The effect of pre and post-infection administration of zinc and selenium on *Trypanosoma brucei brucei* infection in Wistar albino rats was investigated. Rats (54) were divided into nine groups of six (6) rats each. Groups I, II and III served as normal, normal rats administered with combined zinc and selenium and *T. brucei brucei* infected untreated controls respectively. Rats in groups III-IX were infected intraperitoneally with $1x10^3$parasite load. Groups IV, V and VI were the pre-treated infected groups that were administered with daily dose of 50mg per kilogram body weight of zinc, 10mg per kilogram body weight of selenium and combination of zinc and selenium respectively for seven (7) days. Whereas groups VII, VIII and IX represented the post-infected treated groups that were administered with daily dose of 50mg per kilogram body weight of zinc, 10mg per kilogram body weight of selenium and combination of zinc and selenium respectively for seven (7) days. Administration of zinc and selenium significantly ($P<0.05$) kept parasitaemia lower in the pre and post-infected treated groups when compared to the infected untreated group. The level of parasitaemia in the pre-treated infected groups were significantly ($P<0.05$) lower than that observed in the post-infected treated groups. Infection with *T. b. brucei* significantly ($P<0.05$) lowered mean values of packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), total white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and differential leukocytes count in the infected untreated rats compared to the pre and post-infected treated groups. The results obtained for serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in the infected untreated group were significantly ($P<0.05$) higher when compared to the pre and post-infected treated groups. Zinc and selenium caused significantly ($P<0.05$) lower urea and creatinine

concentrations in the pre and post-infected treated groups when compared to the infected untreated group. Significantly ($P<0.05$) higher levels of serum total protein, albumin and total bilirubin were recorded in the pre and post infected treated groups when compared to the infected untreated group. Administration of zinc and selenium to the pre and post-infected groups caused significantly ($P<0.05$) lower values in the concentration of malondialdehyde (MDA), with significantly ($P<0.05$) higher activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in the liver and kidney homogenate. The relative organ weights obtained showed that the kidney, liver, spleen, lungs and heart weight lowered significantly ($P<0.05$) in the pre and post-infected treated compared to infected untreated group. Treatment with zinc and selenium may contribute in delaying the parasitaemia and protection against cellular damage during *Trypanosoma brucei brucei* infection in rats and control of trypanosomiasis.

CHAPTER 1

1.0 INTRODUCTION

Trypanosomiasis is one of the ancient and neglected tropical diseases (WHO, 2008) causing much trouble to man, mainly in sub-Saharan Africa and parts of South America. African trypanosomiasis is a complex disease caused by flagellated protozoan parasite of the genus *Trypanosoma* that affects humans, domestic and wild animals. The disease is transmitted by tsetse flies, a biting fly of the genus *Glossina*, endemic in 36 sub-Saharan African countries posing a serious setback to improved and profitable livestock production and mixed crop-livestock farming development in the African continent (Adamu *et al*., 2008; Stevens and Brisse, 2004; Swallow, 2002). The disease is called sleeping sickness in humans and Nagana or "Sammore" in cattle and Surra in Camels (Welburn *et al*., 2006). Among the important species that cause the disease in livestock are *Trypanosoma vivax, Trypanosoma congolense* and *Trypanosoma brucei* in cattle, sheep and goats, while *Trypanosoma evansi* in camels and *Trypanosoma simiae* in pigs (Maudlin *et al*., 2004; WHO, 2005).

Two species of trypanosome are infective to humans, namely *Trypanosoma brucei rhodesiense* (chronic type) in East Africa and *Trypanosoma brucei gambiense* (acute form) in West and Central Africa (Dumas *et al*., 1999). These subspecies of *Trypanosoma brucei* result in a disease known as sleeping sickness. *Trypanosoma b. rhodesiense* and *Trypanosoma b. gambiense*, however, also cause disease in animals. Although human African trypanosomiasis (HAT) was almost eradicated by the early 1960s, it has since re-emerged (Simarro *et al*., 2008) and now along with African animal trypanosomiasis

(AAT), results in severe health and social problems, as well as economic losses across a large extent of sub-Saharan Africa.

The characteristic clinical features of the disease include intermittent fever, rashes, anaemia, anorexia, poor hair coat, emaciation, lethargy, enlarged lymph nodes, abortion, infertility, decreased milk yield, sub mandibular oedema, ocular, nasal discharges and mortality, swelling around the eyes and hands, severe headaches, extreme fatigue, and aching muscles and joints (Adeiza *et al*., 2008). If the disease is not treated at this stage, it attacks the central nervous system, producing progressive confusion, slurred speech, personality changes, seizures and difficulty in walking and talking. Symptoms progress and lead to death if left untreated (Taylor and Authie, 2004). The course of the disease can be per-acute, acute and chronic depending on the host and parasite virulence (Adejinmi and Akinboade, 2000).

Trypanosoma brucei belongs to the order Kinetoplastida and is considered part of the earliest diverging eukaryotic lineages (Simpson *et al*., 2006). As such, they are regarded as a 'model organism' for the study of alternative mechanisms by which eukaryotes accomplish basic functions. During their life cycle, trypanosomes encounter the vastly different environments of the mammalian bloodstream and various tissues within the tsetse vector. They respond to these by dramatic morphological and metabolic changes, including adaptation of their lipid and energy metabolism (Hannaert *et al*., 2003).

Zinc, one of the most abundant trace elements in the body has been recognized as a micronutrient of diverse biological, clinical and global public health importance (Dalla Rosa *et al*., 2012; Hambidge *et al*., 2010; Zhou *et al*., 2007). It is an essential trace element for all forms of life, and important component of biological antioxidant systems (Debjit *et*

al., 2010; Sahin and Kucuk, 2003). It is contained in hundreds of enzymes and involved in numerous aspect of cellular metabolism. It is also required for growth, optimum performance and modulation of immune system, partly due to its role as a co-factor of more than three hundred enzymes (Zago and Oteiza, 2001). Zinc plays an important role in the structure and function of biological membranes (Bettger and O'Dell, 1993), it has been shown to have an antioxidant potential and also exert critical physiological role in regulating the structure and function of cells (Powell, 2000; Sidhu *et al*., 2004).

Specifically, zinc is required for the adequate formation and function of the antioxidant enzyme copper-zinc superoxide dismutase (CuZnSOD), and various metallothioneins (Disilvestro, 2000). Since the complications of both AAT and HAT may be mediated, at least in part, through oxidative stress, which potentially affect the liver, kidney, spleen, and heart; zinc play a key role in the cellular antioxidative defense (Bonnefont-Rousselot, 2004).

Selenium (Se) is an essential component of antioxidant enzymes such as glutathione peroxidase (GPx), thioredoxin reductase (TrxR) and iodothyronine deiodinases (IDD); it is also a natural antioxidant (Tapiero *et al*., 2003) and immunostimulant (Beck *et al*., 2003; Broome *et al*., 2004; Kiremidjian-Schumacher *et al*., 1994). In recent years, Se research has attracted tremendous interest because of its important role in antioxidant selenoproteins for protection against oxidative stress initiated by excess reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Rayman, 2012). This helps to maintain membrane integrity, protects prostacyclin production and reduces the likelihood of propagation of further oxidative damage to biomolecules such as lipids, lipoproteins and DNA with the associated increased risk of conditions such as atherosclerosis and cancer (Néve, 1996).

1.1 STATEMENT OF RESEARCH PROBLEM

African trypanosomiasis still remains a major constraint to human and animal health, and increased livestock production in most countries of Tropical Africa as well as constituting a major threat to food security in several parts of sub-Saharan Africa (Bourn *et al*., 2005; FAO, 2002; Ijagbone *et al*., 2004; Osanya *et al*., 2008; Samdi *et al*., 2010a; WHO, 2012).

Trypanosomiasis occurs throughout the Tropical regions of Africa, the Middle East, Asia and Latin America. In Tropical Africa, the disease is found between latitude 14ºN and 29ºS covering 10 million km² and stretching across over 37 countries in the region (Mulumba, 2003; Seifert, 1996; WHO, 2002). The prevalence of the disease in Nigeria and most African countries appears to be on the increase (Halid, 2001; Odoya *et al*., 2003; Samdi *et al*., 2010a, WHO, 2012). It is reported that over 587, 273 km^2 (63.4%) of the Nigerian total land mass of 928,300 km^2 is affected by trypanosomiasis (Balalak *et al*., 2003; Usman *et al*., 2008; Yanan *et al*., 2004). The disease is responsible for about 3 million livestock and 55,000 human deaths annually in sub-Saharan Africa (Abenga *et al*., 2003; Abenga *et al*., 2010; Mulumba, 2003; Samdi *et al*., 2010a). According to Food and Agriculture Organization of the United Nations (FAO, 2002), it is one of the neglected diseases which has profoundly affected the settlement and economic development of major parts of sub-Saharan Africa. Nagana prohibits cattle rearing in large areas of Africa causing further malnutrition.

In Nigeria, tsetse flies which are the vector for trypanosomiasis still occupy the nation land mass extending from latitude 4°N to approximately 13°N infesting about 75-80% of the 928,300 Km^2 landmass. There is variation in the prevalence of animal trypanosomiasis, which increases with increase in tsetse population (Kristjanson *et al*., 1999), with over 14

million cattle, 22 million sheep and 33 million goats (NV20:2020) at risk of infection. Given the resurgence of both human and animal African trypanosomiasis, the epidemic potential, high fatality rate, and significant impact on socioeconomic development, many countries adopted measures for the control of the disease (WHO, 2005). The rural poor in Africa are the worst hit by the disease and without adequate treatment it is 100% fatal in patients (WHO, 2002).

Furthermore, since the prospect of vaccine development is still far (Nok, 2005), control of trypanosomiasis is principally achieved by means of either chemoprophylactic or chemotherapeutic agents (Antia *et al*., 2009). Most of these drugs for the control of both animal and human trypanosomiasis are chemically related (Bizimana *et al*., 2006) and have been in use for decades, in addition to been expensive (Kioy and Mattock, 2005; Moore, 2005). Hence, the repeated use of these trypanocides has led to the development of drug-resistant trypanosome populations. Also, most of the available trypanocides are beset with many undesirable toxic side effects (Deterding *et al*., 2005) that are serious and life-threatening (Hoet *et al*., 2007).

1.2 JUSTIFICATION OF THE RESEARCH

Several factors have been implicated in cellular injury in African trypanosomiasis and increasing evidence indicates that free radical-induced oxidative stress plays an important role in the pathogenesis of African trypanosomiasis (Akanji *et al*., 2009; Igbokwe, 1994; Umar *et al*., 2007). An antioxidant is any agent that limits the deleterious effects of free-radical stimulated oxidant reactions. Zinc, however, limits oxidant-induced damage in other ways through stabilization of membranes, which increases membrane resistance to oxidant damage and down-regulate radical production (Heather *et al*., 2014).

However, data are available on the use of different plants, foods and other natural products rich in antioxidants in the prevention or amelioration of free radicals-related diseases such as cancers (Kris-Etherton *et al*., 2002), neurodegenerative diseases (Di Matteo and Esposito, 2003) and trypanosomiasis (Akanji *et al*., 2009; Kingsley and Silvanus, 2011; Umar *et al*., 2010), but there is little scientific research on the use of zinc and selenium as antioxidant micronutrients and their effects on the pathophysiological damage due to trypanosome infection.

Therefore, this study was designed to evaluate the effect of zinc and selenium, as antioxidant micronutrients in the prevention or amelioration of free radicals generated during trypanosome infection.

1.3 Aim of the Study

The aim of this study was to evaluate the effect of zinc and selenium in *Trypanosoma brucei brucei* infected Wistar Albino rats.

1.4 Specific Objectives

i. To determine the level of parasitaemia of trypanosome-infected rats treated with zinc and selenium pre and post-infection.
ii. To determine the haematological parameters of trypanosome-infected rats treated with zinc and selenium pre and post-infection.
iii. To determine some biochemical parameters of trypanosome-infected rats treated with zinc and selenium pre and post-infection.
iv. To assay for the levels of some endogenous antioxidants enzymes (catalase, superoxide dismutase and glutathione peroxidase) and lipid peroxidation in the

tissues of trypanosome-infected rats treated with zinc and selenium pre and post-infection.

CHAPTER TWO

LITERATURE REVIEW

2.1 African Trypanosomiasis

Trypanosomes are extracellular protozoan parasites of the order Kinetoplastida. They cause diseases that are generally referred to as trypanosomiasis. Trypanosomes found in sub-Saharan Africa are generally transmitted by tsetse flies, a biting fly of the genus *Glossina*. These protozoa can infect all classes of vertebrates: fishes, amphibians, reptiles, birds and mammals (Connor and Van den Bossche, 2004). They are the causative agents of sleeping sickness in humans and *Nagana* in cattle in sub-Saharan Africa. The term 'Nagana' also refers to disease caused in ruminants by *Trypanosoma congolense* (Welburn *et al*., 2006).

2.2 Geographical Distribution of African Trypanosomiasis

Trypanosomes can be found wherever the tsetse fly exists. Tsetse flies are the vectors and they transmit the salivarian species which cause the most important forms of trypanosomiasis in Africa. The disease occurs in most regions of the Middle East, Asia, Latin America and Tropical Africa. In the Tropical Africa, trypanosomiasis is endemic in the regions between latitude 14^{o}N and 29^{o}S. These areas total about 10 million Km^2 (Losos, 1986; Seifert, 1996). The rural populations living in the regions where transmission occurs and which depends on agriculture, fishing, animal husbandry or hunting are the most exposed to the bite of the tsetse fly and therefore to the disease.

There are three subspecies of the African *Trypanosoma brucei*: *Trypanosoma brucei brucei, Trypanosoma brucei rhodesiense,* and *Trypanosoma brucei gambiense* (Hoare, 1972). *Trypanosoma b. brucei* that cause Nagana in cattle resides in the subgenus Trypanozoon. Horses, dogs, cats, camels and pigs are very susceptible to *Trypanosoma b.*

brucei infection. Infection of cattle, sheep, goats and sometimes pigs results in mild or chronic infection (Mulligan, 1970). Although this last observation is widely accepted, Moulton and Sollod, (1976) indicated evidences that this organism is widespread in East and West sub-Saharan Africa and that it can cause serious disease and high mortality in cattle, sheep, and goats.

Trypanosoma b. rhodesiense causes human sleeping sickness in the East of sub-Saharan Africa; this sub-species is also infective to cattle and wild animals which are a zoonotic reservoir of the disease (Welburn *et al*., 2006). *Trypanosoma b. gambiense* causes human sleeping sickness in West and Central sub-Saharan Africa and is only infective to humans (Gibson, 1986). There are 36 countries in sub-Saharan Africa in which sleeping sickness is a threat to 60 million people. In East Africa, *T. congolense* is considered to be the single most important cause of African animal trypanosomiasis (AAT). This trypanosome is also a major cause of the disease in cattle in West Africa. Sheep, goats, horses, and pigs may also be seriously affected. In domestic dogs, chronic infection often results in a carrier state (Mulligan, 1970).

Although *Trypanosoma vivax* is considered to be less pathogenic for cattle than *Trypanosoma congolense*, it is nevertheless the most important cause of AAT in West Africa (Gardiner and Wilson, 1987; Hoare, 1972). This trypanosome readily persists in areas free of tsetse flies (for example, in Central and South America and in the Caribbean), where it is transmitted mechanically by biting flies or contaminated needles, syringes, and surgical instruments (Mulligan, 1970).

2.3 The Trypanosomes

Trypanosomes are unicellular haemoflagellated protozoan parasites that cause trypanosomiasis in animals and man. These are obligate parasites that usually have two hosts: the vertebrate and the invertebrate (Seifert, 1996; Soulsby, 1986).

2.3.1 Classification of Trypanosomes

Trypanosomes belong to the phylum *Sarcomastigophora*, class *Zoomastogophora*, the order of *Kinetoplastidae*; The family of *Trypanosomatidae* and the genus of *Trypanosome* (Soulsby, 1986). The trypanosomes that infect mammals have been divided into two groups, Stercorarian and Salivarian, based on their sites of development in the vector. Transmission in the Stercorarian and the Salivarian trypanosomes occur through the fecal and saliva contamination of the wound caused by the bite of the vector respectively (Losos, 1986).

The Stercorarian is further divided into three subgenera: *Magatrypanum, Schizotrypanum* and *Herpetosoma*. With the exception of a few human cases, trypanosomes that belong to the subgenus *Herpetosoma* (*T. lewisi, T. musculi* and *T. microtis*) parasitize rodents. *Trypanosoma theileri* belong to the *Megatrypanum* subgenus, whereas *Trypanosoma cruzi* and *Trypanosoma rangeli* belong to *Schizotrypanum* subgenus.

The salivarian trypanosomes are divided into four subgenera: the subgenus *Pycnomonas, Nannomonas, Duttonella* and *Trypanozoon*. The subgenus *Pycnomonas* contains one species; *Trypanosoma suis,* which is known to infect pigs. *Trypanosoma suis* has short, stout monomorphic structure with a free flagellum. This species has received limited attention, perhaps because it is of little importance. The subgenus *Nannomonas* contains

two recognized species; *Trypanosoma congolense* and *Trypanosoma simiae* and a proposed new specie *Trypanosoma godfreyi* (McNamara *et al*., 1994). The trypanosomes in this group have medium-sized marginal kinetoplast with no free flagellum. They could be monomorphic or pleomorphic and with a moderately developed undulating membrane. All the three species are morphologically identical but differ with respect to host specificity and disease symptoms in the host. Furthermore, *Trypanosoma congolense* is made up of four genotypic groups: the Savannah, Kilifi, Tsavo and West African Riverine Forest. The subgenus *Trypanozoon* contains three species: *Trypanosoma equiperdum*, *Trypanosoma evansi* and *Trypanosoma brucei* which are morphologically indistinguishable, variations within the species exist on the basis of distribution, pathogenicity and host range (Hoare, 1972). The latter species is the only member of this subgenus that can undergo cyclic transmission by tsetse flies. Mechanical transmission of *Trypanosoma equiperdum* and *Trypanosoma evansi* by the tsetse vector is however possible. *Trypanosoma brucei* is further divided into three subspecies; *Trypanosoma brucei gambiense*, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei brucei*. *Trypanosoma vivax* is found in the subgenus *Duttonella* (Stevens and Brisse, 2004).

2.3.2 Morphology of the Family Trypanosomatidae

Members of the family are leaf-like in shape, with a single flagellum attached to the body by an undulating membrane. They are whip-like unicellular organisms, of 8-50µm in size, depending on the species. In Figure 2.1 is a simplified structure of a trypanosome (trypamastigote) (Uilenberg, 1998). *Trypanosoma congolense*, the smallest of the three livestock-infective trypanosomes, is between 8 and 20µm in length, *Trypanosoma vivax* is between 20 and 26µm while *Trypanosoma brucei* is between 23 and 30µm. A number of

cellular organelles can be clearly seen under an electron microscope. In the trypamastigote, the kinetoplast is situated near the posterior end, the flagellum can be seen arising from the parabasal body, the undulating membrane is seen along the length of the body and the nucleus is placed in the center. The kinetoplast is distinct and well-defined, and its size and position differs among species. It plays an important role in reproduction, metabolism and in the cyclic transmission of trypanosomes. The flagellum is used for movement through the blood plasma and tissue fluid (Uilenbeng, 1998). Members of the genus *Trypanosoma* are covered by a single cell membrane but the developmental stages are covered by an additional 10-15 mm thick surface coat which apparently protects the parasite against the resistance mechanisms of the host (Seifert, 1996).

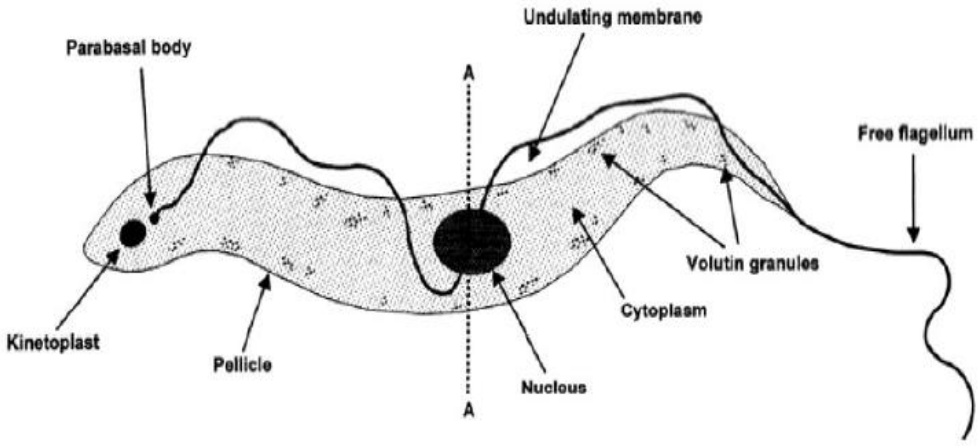


Figure 2.1: A simplified drawing of a trypanosome (Taken from Uilenbeng, 1998).

2.3.3 Reproduction

Trypanosomes reproduce asexually by binary fission, i.e., by the process of division of one parent cell to produce two daughter cells. They can also reproduce sexually where by

genetic exchange takes place between two of them. The division into two daughter cells follows a sequence of events, where the kinetoplast divides first. A second parabasal body develops, from which a second flagellum develops. The nucleus divides next, followed by the rest of the trypanosome body duplicating all the structures present in the cytoplasm. The body then divides into two daughter cells, beginning at the anterior end (Uilenbeng, 1998). The process is rapid, and may result in a vast population in the host within a short period of time.

2.3.4 Life Cycle of Trypanosomes

2.3.4.1 Overview of the Life Cycle

Typically trypanosomes require two hosts to complete their lifecycle which is made up of different developmental stages before it becomes infective (figure 2.2). The life cycle of this parasite involves transmission between mammalian hosts by an insect vector, which is the tsetse fly. Tsetse flies are bloodsucking insects; the metacyclic infective stages of the parasite reside in the salivary glands of the fly and are passed on when the fly feed on the blood of an infected mammalian host. The parasites then multiply rapidly in the bloodstream as ‘slender’ forms and, once a threshold density is reached, the cells begin to differentiate to ‘stumpy’ forms. The stumpy cells are cell cycle arrested and can only re-enter the cell cycle once they have been taken up in the fly blood meal and differentiated to the procyclic insect form. Figure 2.2 summarized the life cycle and the morphology of the parasites at each stage.

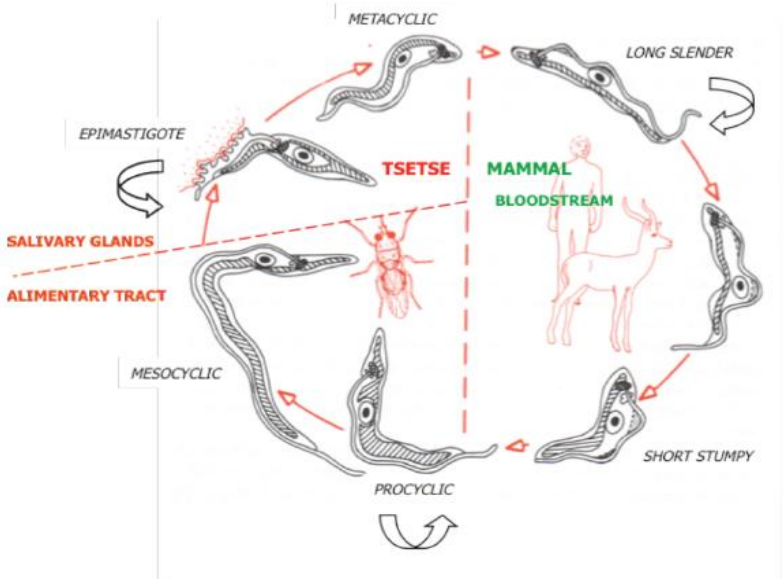


Figure 2.2: Summarized life cycle of the African trypanosome. The different cell morphologies of each stage of the life cycle are shown. This figure was made by Melanie Buhlmann using cell drawings by Keith Vickerman (Vickerman, 1985).

2.3.4.2 Life Cycle in the Mammalian Host.

The infective metatrypanosomes undergo development and multiplication at the site of infection where a swelling or chancre may be detected in the skin, and finally the mature blood trypanosomes (or trypanomastigotes) are released via lymph vessels and lymph nodes into the blood circulation **(**Uilenbeng, 1998**).** Trypanosomes feed by absorbing nutrients, through their outer membrane, from the body fluids of the host. The proteins, carbohydrates and fats are digested by enzyme systems within their protoplasm. Oxygen dissolved in the tissue fluids or blood plasma of their host is absorbed in a similar manner, to generate the energy necessary for the vital processes. Waste products are disposed of by a reverse process, through the outer membrane, into the body fluids of the host. They include carbon dioxide formed during respiration, as well as more complex metabolic products.

2.3.4.3 The Insect Stages of the Life Cycle

The tsetse fly insect vector of African trypanosomiasis is blood-feeding, and the parasites are transmitted to the vector in the blood meal. Stumpy-form cells are competent to undergo differentiation to the insect stage of the life cycle. Procyclic forms are characterized by a metabolism based on mitochondrial activity, the expression of the Procyclin coat, and the ability to evade the tsetse fly innate immune system and complete the insect stage of the life cycle over a course of several weeks (Aksoy *et al*., 2003). However, it is not only the capability of the trypanosome versus the immune system of the fly that determines if an infection can progress. Female flies are typically found to have higher infection rates, as are older flies, and a warmer climate also correlates with higher infection rates (Aksoy *et al*., 2003).

In the search for blood meal, the tsetse fly might suck from an infected host, where it takes in trypanosomes from the host into the midgut. Once in the fly midgut, the trypanosomes are enclosed in a peritrophic matrix, which partitions the blood meal. During the following three days, the blood meal is digested by the fly and the trypanosomes progress to the posterior midgut until they eventually penetrate the peritrophic matrix (Ellis and Maudlin, 1985). After approximately seven days, the trypanosomes halt cellular division and become proventricular forms which are very elongated; these return to the gut lumen and then migrate to the salivary glands. Proventricular forms become epimastigotes, which are proliferative and divide in an asymmetric fashion (Sharma *et al*., 2008; Van Den Abbeele *et al*., 1999). These cells become attached to the lining of the salivary glands via an outgrowth of their flagella membrane. A further developmental step results in metacyclic forms, which are motile, proliferative and ready to be transmitted by a tsetse fly when it

feeds on a mammalian host (Hendricks *et al*., 2000; Vickerman *et al*., 1988). Metacyclics are competent for transmission to the mammal, and express the Variant Surface Glycoprotein (VSG) surface coat (Tetley *et al*., 1987), from metacyclic expression sites (Ginger *et al*., 2002). When a fly bites a mammalian host, the infective metacyclic cells that matured in either the proboscis or salivary glands are transferred with the saliva into the dermis of the skin of the mammal where they initially divide at the site of the bite before progressing further into the bloodstream (Aksoy *et al*., 2003). In mammalian host the metacyclics differentiate into different bloodstream forms, i.e., the slender, intermediate and then stumpy forms one and two. Interestingly, it has been suggested that tsetse fly saliva passed into the mammal bite results in an increased rate of trypanosome infection (Caljon *et al*., 2006). At this stage the life cycle of a trypanosome would be complete, and the stumpy forms can be ingested by a tsetse fly and undergo cyclical changes again (Hendricks *et al*., 2000).

2.3.5 Pathogenesis of Trypanosomes

The pathogenesis of trypanosomiasis generally starts at the site of the tsetse bite, when the tsetse fly injects infective metacyclic trypanosomes into the skin of the host, where a hard, painful, red nodule known as chancre appears in the skin (Uilenbeng, 1998). Chancre may be several centimeters in diameter (usually 2-3cm) and it arises 4-14 days after a bite (Apted, 1970). Morphologically, the chancre is a localized inflammatory site with oedema, infiltrations of macrophages and lymphocytes, and the multiplying parasites (Maudlin *et al*., 2004). The metatrypanosomes divide and multiply in the chancre and give rise to the typical blood forms which invade the lymphatics and lymph nodes, and then the blood

stream. They continue to multiply and cause various types of tissue damage and disorders (Donelson, 2003; Faye *et al*., 2005).

Trypanosomiasis, like other infectious diseases, starts with an increase of the body temperature, a hyperthermia. This is the result of the contact between the trypanosomes multiplying in the host and the defense system of the host. The surface proteins of the trypanosomes provoke the host in making specific antibodies against these proteins, and after a few days almost all of the trypanosomes in the blood are destroyed by these antibodies and the body temperature drops. However, a few parasites survive as they have been able to replace their surface proteins by different ones, against which the antibodies cannot act. These surviving trypanosomes are able to multiply, and cause a new peak of parasitaemia and hyperthermia, until the organism of the host makes specific antibodies against the new surface proteins. This seesawing process continues for a long time, as the trypanosome is able to make an almost unlimited number of antigenic variants, and the host responds to each of them, until either the antigenic repertoire of the trypanosome is finally exhausted, in which case self-cure of the host follows, or the ability of the host to react to all of the antigenic variants is overwhelmed, and the host dies (Uilenbeng, 1998). The clinical and pathological manifestations of animal trypanosomiasis are common to domestic animals, irrespective of the species of the trypanosomes involved (Maudlin *et al*., 2004). The severity of these pathological changes are influenced by several factors including the virulence of the parasite, the susceptibility of the host, the period of the infection during which samples are taken, among others. The prepatent period is generally between 1-3 weeks (Maudlin *et al*., 2004).

2.3.6 Transmission of Trypanosomes

Trypanosomes are primarily transmitted from host to host by insect vectors, cyclically or mechanically. Cyclic transmission refers to the developmental cycle of the parasite in the tsetse fly vector (see details in the life cycle in the insect section). Biting flies such as *Tabanus, Stomoxys* and *Lyperosia* also transmit trypanosomes, but they do so mechanically. Other methods for transmission of trypanosomes are venereal, experimental and vertical. Venereally transmitted parasites such as *T. equiperdum* are transferred during coitus. Experimentally, trypanosomes may be transferred by the use of needles and syringes containing trypanosomes. Vertical transmission (from mother to offspring) is also possible whereby the parasites can cross the placental membrane and infect the foetus (Katakura *et al*., 1997; Sekoni, 1994).

2.4 Trypanosomiasis

2.4.1 Signs and Symptoms

Generally, clinical signs and symptoms of Africa trypanosomiasis include intermittent fever, oedema, and emaciation, pale mucous membranes due to anaemia, enlarged lymph nodes, rough and dull hair coats, occasional diarrhoea and infertility. Infected animals usually abort and the ejaculates of infected animals have reduced live spermatozoa with reduced motility as a result of testicular damage (Sekoni *et al*., 1988). In endemic areas, clinical signs alone can be non-specific for diagnosis of the disease because other disease state can result in similar symptoms. The morbidity and mortality of infected animals is largely dependent on the breed and age of the animal, virulence and dose of the infected organism. Cattle, sheep and goats experimentally infected with *T. b. brucei* developed an

acute disease that lasted for few days. Chronic phase follows with the involvement of central nervous system and may terminate in death (Ikede and Losos, 1972; Moulton, 1986). The disease in dogs is acute with high parasitaemia, moderate to severe anaemia and central nervous system disorders which often lead to death (Adamu *et al*., 2009). *Trypanosoma b. brucei* infection in horses and camels is characterized by high fever, anaemia, anorexia, subcutaneous oedema, emaciation and often death (Ikede *et al*., 1977). Inco-ordination and spinal paralysis may occur in horses. *Trypanosoma b. brucei* can develop in many sites including the eye producing lacrimation, photophobia and keratoconjuctivitis (Adamu *et al*., 2009). High prevalence of *T. brucei* infections was reported in Nigeria (Omotainse *et al*., 2000). The Clinical signs manifested in pigs include intermittent fever and parasitaemia, anaemia, loss of weight and body condition, dehydration, anorexia, paresis and wobbling of hind legs, ascites, petechial hemorrhages and death (Allam *et al*., 2006; Otesile *et al*., 1992).

The West African form of the disease, caused by *Trypanosoma b. gambiense*, causes a chronic infection where the patient may survive for several months or years. The East African form of the disease, caused by *Trypanosoma b. rhodesiense*, is more acute and usually kills the patient within weeks or months. The initial period of the disease is characterized by fever and joint pain, during which stage the parasites are in the bloodstream and lymphatic system. Once the parasites have crossed the blood-brain barrier, sleep alterations occur, as do psychiatric and sensory imbalances (Lundkvist *et al*., 2004).

2.4.2 Diagnosis of Trypanosomiasis

In regions where the disease is known to occur, clinical symptoms and post-mortem lesions are important indications, especially in combination with the history of the disease and the region in which it occurs. However, symptoms and lesions of trypanosomiasis are never pathognomonic (which means specific for the disease), and suspicion has to be confirmed by other means (Uilenbeng, 1998). The disease may also run a subclinical course and a variety of diseases have similar clinical manifestations. Generally, for field diagnosis, microscopy-based techniques using direct observation of wet blood film, or concentration techniques such as the buffy coat technique (BCT) and the haematocrit centrifugation technique (HCT) (Ezebuiro *et al*., 2009) are the most common methods of parasites detection, and have historically been considered as the standard techniques. It has been demonstrated that microscopy examination has a very poor sensitivity compared to the molecular tools, highlighting that previous studies using these methods were likely to have significantly underestimated both animal and herd-level prevalence of the pathogens (Picozzi *et al*. 2002).

The diagnosis of trypanosomes has been improved, since the 1980s, by DNA-based techniques. DNA diagnosis is either based on hybridization profiles of parasite DNA with DNA probes or polymerase chain reaction (PCR) technology. A DNA-probe is a known DNA sequence which can be obtained by cloning or by PCR with labeled nucleotides (enzymes or isotopes). DNA probing entails exposing a denatured DNA sample fixed on nitro-cellulose to a labeled DNA-probe under specific salt and temperature conditions. If the complementary DNA sequence is present in the sample, the probes will bind to it and remain on the nitro-cellulose where they can be visualized (Desquesnes and Dàvila, 2002).

The molecular diagnostic tools, and in particular polymerase chain reaction (PCR), has vastly improved the detection of trypanosome infections over standard parasitological techniques, by lowering the parasitaemia limit by several orders of magnitude.

The polymerase chain reaction (PCR) is an *in vitro* technique which allows the amplification of a specific deoxyribonucleic acid (DNA) region that lies between two regions of a known DNA sequence. The technique exploits a thermostable enzyme, Taq polymerase, which synthesizes a new strand of DNA by copying an original DNA template. PCR was originally developed to amplify sequences of interest and to increase the sensitivity of detection using DNA-probes (Saiki *et al*., 1986). However, since the DNA-probe technique is laborious and time consuming (Desquesnes and Dàvila, 2002), in recent years the development of PCR alone has been mainly devoted to diagnosis without the need of probes. Furthermore, primer design targeting precise DNA sequences ensures high specificity of PCR, making it independent of morphological differences required for speciation by microscopy. The PCR-based diagnosis of trypanosome infections in livestock has now been used as the diagnostic tool of choice in a wide number of studies investigating the epidemiology of trypanosomiasis across Africa (Cox *et al*., 2005; Ng'ayo *et al*., 2005; Pinchbeck *et al*., 2008), using different protocols and methods (Cox *et al*., 2005; Picozzi *et al*., 2008). The field applications of PCR include estimating prevalence for monitoring of control programs.

The analytical sensitivity of concentration techniques, such as the HCT or the BCT ranges between detectable parasitaemia levels of 2.5×10^2 to 5×10^3 parasites /ml of blood depending on the trypanosome species (Masake *et al*., 2002), whereas PCR can detect the presence of the parasite DNA equivalent to one trypanosome in 10ml of host blood (Eisler

et al., 1997).The analytical detection limit of trypanosomes especially *Trypanosoma brucei* specific PCR has been shown to be as low as 1/10 of the genetic material of a single trypanosome per PCR (Moser *et al*., 1989). The internal transcribed spacer (ITS)-PCR has been shown to detect trypanosomal DNA at detection limit equivalent to less than one parasite/ml of host blood (Cox *et al*., 2005). However, due to the cost and level of laboratory equipment involved, PCR is not suitable for diagnostic testing of individual animals for treatments at the local level (Bronsvoort *et al*., 2010).

2.4.3 Control for Trypanosomiasis

In the past, bush clearing to decrease tsetse natural habitat, elimination of game animals to decrease the natural reservoirs and aerial spraying of insecticides to kill arthropod vectors were the major control measures employed in the control of African trypanosomiasis. These measures however, were faced with a lot of problems, for instance, bush clearing was destructive to our natural environment whereas, game elimination and aerial spraying were detrimental to the ecosystem (Watson, 2004).

However, since vaccine for trypanosomiasis is yet to be developed, the control of trypanosomiasis in Africa principally involves three approaches: Vector control, management of the disease by use of drugs and the rearing of trypanotolerant breed of livestock in areas infested with tsetse flies (Barret, 1997; Vale *et al*., 1985).

2.4.3.1 Suppressing the Vector or Vector Control

Several approaches to fly control have been used with varying degrees of success. Many methods widely used for tsetse control in the past have ceased to be used in the last 10-20 years, either because they were ineffective, or because they have become environmentally

unacceptable (Uilenberg, 1998). Efforts aimed at controlling trypanosomiasis in sub-Saharan Africa are focused on controlling the tsetse fly vector. A tsetse population control method is accomplished by aerial application of ultra-low volume insecticides, traps designed to catch the flies and the sterile insect technique (SIT) (Torr *et al*., 2006).

The widespread use of insecticides to control tsetse populations in infested areas has seen some slight development of vector resistance to insecticides (Aksoy *et al*., 2003). Concerns over the effect of insecticides on the environment have been the main consideration raised. Thus, the use of insecticides in controlling trypanosomiasis is not seen as a permanent solution.

Tsetse traps (such as biconical, Challier-Laveissière, Nitse, Vavoua, Ngu, Lancien, pyramidal and bipyramidal) have been effective in controlling insect numbers, especially when used in addition to insecticides. However, traps require effort to maintain, as they are susceptible to damage, and therefore require long-term community involvement (Kabayo, 2002). Recently, efforts to improve the use of tsetse traps have included treated traps that lure tsetse with odour baits in order to increase the number of flies caught, as well as treating cattle and other livestock with insecticide (Omolo *et al*., 2009).

One of the newly introduced methods of tsetse control is the use of the sterile insect technique (SIT), where male flies are reared in large numbers, sterilized by irradiation and then released in infested areas. The effectiveness of this technique relies on the fact that the sterile males would mate with wild females resulting in no offspring. Because female flies rarely mate more than once in their lifetime, it is believed that the release of sterile males over a period of time would result in reduction and perhaps in the complete elimination of

tsetse flies, Unlike the other methods of tsetse control, this approach does not have effect on non- target organisms (Kuzoe and Schofield, 2004). SIT is effective in combating tsetse flies when it is integrated with other methods. For example, complete eradication of *G. austeni* was achieved in 1999 in the Unguja Island of Zanzibar. The success of the campaign relied on the integration of three techniques: reducing tsetse fly population by capturing them on insecticide impregnated screens and treating cattle with pour- on formulation of pyrethoids from 1988 to 1993, followed by a release of 8.5 million sterile males from 1994 to 1997 (Vreysen *et al*., 2001). This allowed for improved livestock production, which improved the socio-economic status of the communities in this region (Kabayo, 2002).

Research efforts are well underway to find ways to employ transgenesis to render vector tsetse flies insusceptible to trypanosome infection and replace natural vector populations with refractory ones (Aksoy *et al*., 2001). Earlier control methods, no longer extensively applied, included ground spraying of persistent insecticides such as dichlorodiphenyltrichloroethane (DDT), the clearing of brush, and extermination of native mammals that provide tsetse fly blood meals and act as trypanosome reservoirs.

2.4.3.2 Use of Trypanotolerant Livestock

Trypanotolerance refers to resistance of host animals to trypanosomiasis, which appears to be related to their ability to control parasitaemia in the bloodstream and resist anaemia. It has long been recognized that certain breeds of West African cattle are considerably more resistant to African trypanosomiasis than others. This is especially true of the West African short-horned cattle (Muturu, Baoule, Laguna, Samba, and Dahomey) and the long-horned

cattle (N'Dama). These breeds of cattle were found to be more tolerant to trypanosome infection and have higher calving rates than trypanosusceptible breeds (FAO, 2000; Lalmanach *et al*., 2002). These tolerant breeds, known as trypanotolerant cattle, once infected by trypanosomes, are able to limit the pathogenicity of the parasites and maintain a better state of health than other breeds (Stephen, 1966). The breeds that are more susceptible to disease following trypanosome infection are termed trypanosusceptible. Trypanosusceptible breeds are not as able to cope with infection as the trypanotolerant breeds, and frequently succumb to the disease as a result of high levels of parasitaemia and anaemia (Murray *et al*., 2004). The disadvantage of farming with the trypanotolerant breeds, such as the N'Dama, is that these are usually smaller, and in terms of milk production, less productive than the larger, trypanosusceptible breeds, such as the Zebu (Murray *et al*., 2004). The genetic factors resulting in trypanotolerance have up to now been largely unknown, however, research is currently underway to investigate the phenomenon by the use of whole transcriptome analysis of both trypanotolerant and trypanosusceptible cattle (Berthier *et al*., 2008). Because of the limitations of other methods of control, the use of trypanotolerant breeds is accepted as a means of keeping livestock in tsetse- infested areas.

2.4.3.3 Chemotherapy (Conventional Drugs Currently in Use)

The application of anti-trypanosomal drugs has been the most widely practiced means of controlling trypanosomiasis in domestic livestock since the early 1950s, either as curative or prophylactic drugs. A programme to eradicate tsetse flies from the sub-Saharan Africa is highly ambitious. It will be complex, take many years and possibly cost some US$ 20 billion (Budd, 1999). Thus, control of trypanosomiasis will depend in the foreseeable

future on the use of the existing trypanocidal drugs. The challenge, therefore, remains to make optimal use of the three relatively old compounds until new methods of treatment emerge, possibly through unanticipated cross-reactivity with new broad-spectrum anti-protozoa compounds such as those currently being developed for the treatment of malaria and cryptosporidiosis (Holmes *et al*., 2004). There are only four drugs currently available for the treatment of human African trypanosomiasis (HAT), of which three were developed more than 50 years ago (Fairlamb, 2003). These drugs include pentamidine and suramin for treatment of early-stage infection as well as melarsoprol (an arsenical) and eflonithine for late-stage treatment of HAT (Fairlamb, 2003). Similarly, only a few drugs are available for the treatment of trypanosomiasis in cattle, namely isometamidium chloride and homidium, which possess both prophylactic and therapeutic effects; as well as ethidium bromide and diminazene aceturate (berenil) has only therapeutic properties (Delespaux *et al*., 2008; Geerts *et al*., 2001). The efficacy and/or toxicity levels of all the drugs used to treat HAT are not satisfactory (Fairlamb, 2003; Gutteridge, 1985), whilst the main problems faced with the drugs used to treat animal trypanosomiasis are cost, protracted treatment protocols and drug resistance (Kamuanga, 2003; Legros *et al*., 2002,).

2.5 Nutrition and Trypanosomiasis

Nutrition has been recognized as an important factor influencing the host- parasite relationship and the ability to withstand the impact of the disease caused by trypanosome infection (Holmes *et al*., 2000; Igbokwe, 1995). It has been suggested that animals of low nutrition status could become less able to withstand the effects of infection (Holmes *et al*., 2000) and it is also shown that nutritional deprivation could result in more severe infection, even among trypanotolerant breeds (Pathak, 2009; Reynolds and Ekwurukwe, 1988).

Ameh, (1984) showed that the effect of *T. brucei* infection was more severe in young pig on the lower energy diet.

In an investigation, decrease in zinc levels coincided with the onset of *T. brucei gambiense* in peripheral blood of rabbit (Mwangia *et al*., 1995). Zinc-deficient animals showed three times the number of trypanosomes as that of the complete and pair-fed mice (Lee *et al*., 1983). A report suggested that cattle zinc profile could be responsible for either susceptibility or resistance to trypanosome infection (Traoré-Leroux *et al*., 1985). Egbe-Nwiyi *et al*., (2003, 2004) reported that rats infected with *T. brucei or T. congolense* and supplemented with oral Magnesium chloride and Zinc chloride withstood the effects of the infection better than infected unsupplemented animals. Patients with trypanosomiasis infection have demonstrated reduced selenium level with higher mortality rate when compared with the uninfected control (De Souza *et al*., 2002). In contrast, parasite load was not different for selenium fed and selenium deficient *Trypanosoma*- infected mice (De Sousa *et al*., 2002). Also, a report has it that selenium deficient and control mice post inoculation after a while ensured parasite clearance (Ongele *et al*., 2002). Therefore, trace mineral nutrition may be very important in the management of trypanosomiasis patients, also an understanding of the role of trace mineral nutrition in the pathogenesis of trypanosomiasis would help in designing nutritional protocols that can support control programs and afford practical husbandry strategies to counter the effect of the disease in areas where the disease is endemic. Zinc and Selenium supplementation will help to quench free radicals that may continue to cause pathological changes in extracellular fluid after the trypanosomes have been eliminated by trypanocidal drugs.

2.6 Zinc (Zn)

Zinc is one of the most important trace mineral elements in the Earth's crust. It is found in the air, soil, and water and is present in all foods. In its pure elemental (or metallic) form, zinc is a bluish-white, shiny metal. Powdered zinc is explosive and may burst into flames if stored in damp places. The mineral zinc is present in every part of the body and has a wide range of functions. It helps with the healing of wounds and is a vital component of many enzyme reactions. Zinc is vital for the healthy working of many of the body's systems. It is particularly important for healthy skin and is essential for a healthy immune system and resistance to infection.

2.6.1 Chemistry of Zinc

Zinc is a metallic chemical element; it has the symbol Zn and atomic number 30. It is the first element of group 12 of the periodic table; it is a moderately reactive metal and a strong reducing agent (CRC, 2006), with oxidation state of +2. It is the 24th most abundant element in the Earth's crust and has five stable isotopes. The chemistry of Zn is dominated by the +2 oxidation state. When compounds in this oxidation state are formed the outer shell electrons are lost, which yields a bare zinc ion (Ritchie, 2004). Zinc chemistry is similar to the chemistry of the late first-row transition metals nickel and copper, though it has a filled d- shell, so its compounds are diamagnetic and mostly colorless (Greenwood and Earnshaw, 1997). Elemental zinc is a lustrous, blue-white to grey metal that is virtually insoluble in water. It has a melting point of 419.5°C and boiling point of 908°C (ATSDR, 1995). Pure zinc is usually produced by an electrolytic process in which zinc oxide is leached from the roasted or calcined ore with sulfuric acid to form zinc sulfate solution

which is electrolyzed in cells to deposit zinc on cathodes (Lewis, 1998). Zinc can also combine with other elements, such as chlorine, oxygen, and sulfur, to form zinc compounds, such as zinc chloride, zinc oxide, zinc sulfate, and zinc sulfide. Most zinc ore found naturally in the environment is in the form of zinc sulfide.

2.6.2 Food Source of Zinc

Zinc is present in a wide variety of foods, particularly in association with protein foods. Shellfish, beef and other red meats are rich sources of zinc. Nuts and legumes are relatively good plant sources. Lean red meat, whole-grain cereals, pulses, and legumes provide the highest concentrations of zinc, concentrations in such foods are generally in the range of 25–50mg/kg (380–760mmol/kg) raw weight. Processed cereals with low extraction rates, polished rice, and chicken, pork or meat with high fat content have moderate zinc content, typically between 10 and 25mg/kg (150–380 mmol/kg). Fish, roots and tubers, green leafy vegetables, and fruits are only modest sources of zinc, having concentrations <10mg/kg (<150mmol/kg). Saturated fats and oils, sugar, and alcohol have very low zinc contents. Supplements contain several forms of zinc, including zinc gluconate, zinc sulfate, and zinc acetate. The percentage of elemental zinc varies by form. For example, approximately 23% of zinc sulfate consists of elemental zinc; thus, 220 mg of zinc sulfate contains 50 mg of elemental zinc (Trumbo *et al*., 2001).

2.6.3 Biological Function of Zinc

Zinc is present in all body tissues and fluids. The total body zinc content has been estimated to be 30mmol (2g). Skeletal muscle accounts for approximately 60% of the total body content and bone mass, with a zinc concentration of 1.5–3mmol/g (100–200mg/g),

for approximately 30%. The concentration of zinc in lean body mass is approximately 0.46mmol/g (30mg/g). Plasma zinc has a rapid turnover rate and it represents only about 0.l% of total body zinc content. This level appears to be under close homeostatic control. High concentrations of zinc are found in the choroid of the eye (4.2mmol/g or 274mg/g) and in prostatic fluids (4.6–7.7mmol/l or 300–500mg/l) (Hambidge *et al*., 2000). Zinc is an essential component for the function of more than 300 enzymes, including alkaline phosphatase, alcohol dehydrogenase, Cu, Zn-superoxide dismutase, carboxypeptidase, aminolevulinic acid dehydratase (ALAD), carbonic anhydrase, deoxyribonucleic acid (DNA) polymerases (DNA polymerase alpha, DNA polymerase III), and reverse transcriptase (Vallee and Falchuk, 1993; Sandstead, 1994), that are participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. Zinc stabilizes the molecular structure of cellular components and membranes and in this way contributes to the maintenance of cell and organ integrity (Heather *et al*., 2014). Furthermore, zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression. The zinc coordinates with cysteine and histidine residues of certain peptides and produces a tertiary structure which has an affinity for unique segments of DNA in promoter gene regions (Prasad, 1993). Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all life forms. Zinc plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity (Shankar and Prasad, 1998).

2.6.4 Zinc Deficiency

Zinc deficiency has been recognized by a number of experts as an important public health issue, especially in developing countries. There have been reports on the prevalence and clinical consequences of zinc deficiency on growth delay, diarrhoea, pneumonia, disturbed neuropsychological performance and abnormalities of fetal development (Debjit *et al*., 2010). In people suffering from marginal zinc deficiency, clinical signs are depressed immunity, impaired taste and smell, unset of night blindness, impairment of memory and decreased spermatogenesis in males (Hambidge, 1989; Wang and Busbey, 2005; Zalewski, 1996). In more severe cases, zinc deficiency is characterized by severely depressed immune function, frequent infections, bullous pustular dermatitis, diarrhoea, delayed sexual maturation, impotence, and hypogonadism in males, alopecia, and mental disturbances (King and Cousins, 2005; Van Wouwe, 1995; Wang and Busbey, 2005). Similar effects of mild and severe zinc deficiency arise in zinc-deficient laboratory animals (Clegg *et al*., 1989). However, many of these symptoms are non-specific and often associated with other health conditions; therefore, a medical examination is necessary to ascertain whether a zinc deficiency is present.

2.6.5 Antioxidant Properties of Zinc

Zinc is one of the antioxidants trace mineral element of significant importance. Antioxidants are nutrients that block some of the damages caused by free radicals, which are by products that results when the body transform food into energy. Multiple studies in humans suggest that zinc may have a protective effect against free radical generation and oxidative stress. It acts as an antioxidant and is basically involved in some of the

biochemical decisive reactions, which includes protein synthesis, enzymatic function and carbohydrate metabolism. It has other properties that could contribute to its role in lymphocyte function. It is an antioxidant, protecting cells from the damaging effects of oxygen radicals generated during immune activation (Shankar and Prasad, 1998). Zinc also regulates the expression in lymphocytes of metallothionein and metallothionein-like proteins with antioxidant activity (Heather *et al*., 2014; Klatenberg *et al*., 2010). Membrane zinc concentrations are strongly influenced by dietary zinc deficiency and supplementation. Zinc concentrations in cell membranes appear to be important in preserving their integrity through poorly defined mechanisms involving binding to thiolate groups (King, 2011). It is noteworthy that zinc release from thiolate bonds can prevent lipid peroxidation (Kroncke *et al*., 1994). In addition, nitric oxide induces zinc release from metallothionein, the primary zinc binding and transport protein in the body, which may limit free radical membrane damage during inflammation.

2.6.6 Recommended Dietary Allowance (RDA)

For some essential vitamins and minerals, the human body normally has the capacity to either store excess quantities or excrete them through urine. The human body unfortunately has no storage mechanism for zinc. The RDA for zinc is around 11mg per day for men and 8mg per day for women (12mg per day for breastfeeding women). Idea dosage of 50mg per day coming from supplements, considering absorption rates around 25% is recommended (Maret and Sandstead, 2006).

2.6.7 Toxicity

Zinc toxicity can occur in both acute and chronic forms. Acute adverse effects of high zinc intake include nausea, vomiting, loss of appetite, abdominal cramps, diarrhoea, and headaches (Trumbo *et al*., 2001). One case report cited severe nausea and vomiting within 30 minutes of ingesting 4 g of zinc gluconate (570 mg elemental zinc) (Lewis and Kokan, 1998). Taking too much zinc into the body through food, water, or dietary supplements can also affect health. The levels of zinc that produce adverse health effects are much higher than the Recommended Dietary Allowances (RDAs) for zinc of 11 mg/day for men and 8 mg/day for women. If large doses of zinc (10–15 times higher than the RDA) are taken by mouth even for a short time, stomach cramps, nausea, and vomiting may occur.

2.7. Selenium (Se)

Selenium is an essential trace mineral of fundamental importance for animals and humans. As a constituent of more than two dozen selenoproteins, selenium has structural and enzymic functions, playing critical roles in reproduction, thyroid hormone metabolism, DNA synthesis, and protection from oxidative damage and infection (Sunde, 2012).

Most selenium is in the form of selenomethionine in animal and human tissues, where it can be incorporated nonspecifically with the amino acid methionine in body proteins. Skeletal muscle is the major site of selenium storage, accounting for approximately 28% to 46% of the total selenium pool (Terry and Diamond, 2012). Both selenocysteine and selenite are reduced to generate hydrogen selenide, which in turn is converted to selenophosphate for selenoprotein biosynthesis (Davis, 2012).

2.7.1 Chemistry of Selenium

Selenium is a non-metal chemical element with symbol Se and atomic number 34; it has the oxidation state of -2, +2, +4, and +6 and atomic mass of 78.96. It rarely occurs in its elemental state in nature, or as pure ore compounds. It is chemically related to sulphur and tellurium. Selenium exists in two forms: inorganic (selenate and selenite) and organic (selenomethionine and selenocysteine) (Sunde, 2006). Both forms can be good dietary sources of selenium (Terry and Diamond, 2012).

2.7.2 Food Source of Selenium

Foods are the major source of Se and, in general, seafood, cereals and meat products contain relatively high levels of Se, while low levels are found in milk, vegetables and fruit (Sunde, 2012; Tinggi, 1999). The levels of Se in foods can vary widely between geographical regions depending on soil Se levels, and these wide variations in soil Se level are reflected in the wide variations found in the Se status of human populations around the world (Reilly, 2006). Soils contain inorganic selenites and selenates that plants accumulate and convert to organic forms, mostly selenocysteine and selenomethionine and their methylated derivatives.

Selenium in foods and biological materials can exist in both organic and inorganic chemical forms (Dumont *et al*., 2006). In turn, the chemical form of the Se can affect its bioavailability from diet. In general, the organic forms, such as selenomethionine, are more bioavailable than the inorganic selenites or selenates. Selenium is also available in multivitamin/multimineral supplements and as a stand-alone supplement, often in the forms of selenomethionine or of selenium-enriched yeast (grown in a high-selenium medium) or

as sodium selenite or sodium selenate (Sunde, 2006, 2010). The human body absorbs more than 90% of selenomethionine but only about 50% of selenium from selenite.

2.7.3 Biological Function of Selenium

Ironically, until approximately 40 years ago, selenium was known only as a poison. It is now known that selenium is essential for the normal function of many systems of the body and selenium deficiency can have adverse consequences on these systems. Since its discovery as an important component of antioxidant enzymes, such as glutathione peroxidase (GPx), thioredoxin reductase (TrxR) and iodothyronine deiodinases (IDD), there has been an increased interest in the study of other Se-containing proteins (selenoproteins) or enzymes (selenoenzymes) (Tapiero *et al*., 2003). It has now been recognised that all these enzymes are selenium-dependent, generally with selenocysteine at the active site (Sunde, 1997). Here selenium functions as a redox centre, for instance when the selenoenzyme, thioredoxin reductase, reduces nucleotides in DNA synthesis and helps control the intracellular redox state (Allan *et al*., 1999), or when the selenium-dependent iodothyronine deiodinases produce active thyroid hormone from inactive precursor (Sunde, 1997). The best-known example of this redox function is the reduction of hydrogen peroxide and damaging lipid and phospholipid hydroperoxides to harmless products (water and alcohols) by the family of selenium-dependent glutathione peroxidases (Allan *et al*., 1999; Broome *et al*., 2004). This function helps to maintain membrane integrity (Diplock, 1994; Néve, 1996), protects prostacyclin production (Néve, 1996), and reduces the likelihood of propagation of further oxidative damage to biomolecules such as lipids, lipoproteins, and DNA with the associated increased risk of conditions such as atherosclerosis and cancer (Diplock, 1994; Néve, 1996).

There are at least 30 selenoproteins that have been identified in mammals, and it has been estimated that humans have about 25 selenoproteins (Kryukov *et al*., 2003). The functional roles of some of these selenoproteins are still not fully understood, even though they have been conserved throughout evolution because of their unique physiochemical properties (Mix *et al*., 2007). Because of their antioxidant activity, there has been a tremendous interest in the study of Se and its compounds in cancer chemoprevention, heart disease and in proper functioning of immune system.

2.7.4 Deficiency of Selenium

Selenium deficiency produces biochemical changes that might predispose people who experience additional stresses to develop certain illnesses (Food and Nutrition Board Staff, Panel on Dietary Antioxidants and Institute of Medicine Staff, 2000). For example, selenium deficiency in combination with a second stress (possibly a viral and protozoa infection) leads to Keshan disease, a cardiomyopathy that occurred in parts of China prior to a government-sponsored selenium supplementation program that began in the 1970s (Chen, 2012). Selenium deficiency is also associated with male infertility and might play a role in Kashin-Beck disease, a type of osteoarthritis that occurs in certain low-selenium areas of China, Tibet, and Siberia (Jirong *et al*., 2012; Rayman, 2012; Sunde, 2006, 2010). Selenium deficiency could exacerbate iodine deficiency, potentially increasing the risk of cretinism in infants (Sunde, 2006, 2010).

2.7.5 Antioxidant Properties of Selenium

Selenium behaves both as an antioxidant and anti- inflammatory agent. This is because selenium in its antioxidant role, notably as glutathione peroxidase (GPx), can reduce hydrogen peroxide, lipid and phospholipid hydroperoxides, thereby dampening the

propagation of free radicals and reactive oxygen species; reduce hydroperoxide intermediates in the cyclo-oxygenase and lipoxygenase pathways diminishing the production of inflammatory prostaglandins and leukotrienes; and modulate the respiratory burst, by removal of hydrogen peroxide and reduction of superoxide production (Spallholz *et al*., 1990).

Selenoproteins help prevent the oxidative modification of lipids, reducing inflammation and preventing platelets from aggregating (Rayman, 2012). There is evidence for a protective effect of selenium in pancreatitis, a disorder associated with a high level of oxidative stress. At Manchester Royal Infirmary, administration of selenium (600g per day) along with other antioxidants to patients with chronic and recurrent pancreatitis significantly reduced pain and frequency of attacks. Treatment has been revolutionised by obviating the need for surgery for pancreatic pain (McCloy, 1998).

2.7.6 Recommended Dietary Allowance of Selenium

The standard of recommended intake levels of selenium is under debate. The UK reference nutrient intake (RNI) of 75 µg per day for men and 60 µg per day for women has been determined as the intake believed to be necessary to maximize the activity of the antioxidant selenoenzyme GPx in plasma (MacPherson, 1997). The American recommended dietary allowance (RDA), set at 55 µg per day for both men and women, is based on the investigations of the selenium intake required to achieve plateau concentrations of plasma GPx (Institute of Medicine, the National Academics with Health Canada, 2000). WHO/FAO/IAEA (1996) expert group recommended an intake level of only 40 µg per day for men and 30 µg per day for women, assuming only two-thirds of the

full expression of GPx activity is required. However, as Rayman (2012) points out, if levels of GPx activity saturation are determined using platelets rather than plasma, then the intake levels needed should be approximately 80-100g per day.

2.7.7 Toxicity

Although selenium is an essential trace element, it is toxic if taken in excess. Exceeding the Tolerable Upper level of 400 micrograms per day can lead to selenosis. Chronically high intakes of the organic and inorganic forms of selenium have similar effects. Early indicators of excess intake are a garlic odor in the breath, gastrointestinal disorders and a metallic taste in the mouth. The most common clinical signs of chronically high selenium intakes, or selenosis, are hair and nail loss or brittleness. Other symptoms include lesions of the skin and nervous system, nausea, diarrhoea, skin rashes, mottled teeth, fatigue, irritability, and nervous system abnormalities. Acute selenium toxicity can cause severe gastrointestinal and neurological symptoms, acute respiratory distress syndrome, myocardial infarction, hair loss, muscle tenderness, tremors, lightheadedness, facial flushing, kidney failure, cardiac failure, and, in rare cases, death (Food and Nutrition Board Staff, Panel on Dietary Antioxidants and Institute of Medicine Staff, 2000; Sunde, 2006).

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Chemicals/Reagents

All assays kits for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline Phosphatase (ALP), bilirubin were purchased from Randox laboratories Ltd® (Northern Ireland, UK), Ardmore, Co. Antrum UK. All chemicals used were of analytical grade. Hydrogen Peroxide, Sodium chloride, Sodium hydroxide, Sodium carbonate, Sodium bicarbonate, Acetic acid, Potassium dichromate, Potassium phosphate, Potassium iodide, Picric acid, Hydrochloric acid, Dinitrophenyl hydrazine, Magnesium chloride, Copper sulphate, Sodium citrate, Dipotassium phosphate, Trichoroacetic acid, Adrenalin, Thiobarbituric acid were all purchased from Sigma-Aldrich Company limited®. Zinc gluconate (Good'N Natural, Bohemia USA) and Selenium (Sigma-Aldrich®).

3.1.2 Experimental Animals

A total of fifty four (54) wistar albino rats of both sexes, weighing between 150 – 250g, were used for the experiment. The rats were purchased from the animal house of Trypanosomiasis Research Department (TRD), Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria. The animals were kept in well-aerated laboratory cages and allowed to adjust to the laboratory environment for a period of two weeks before commencement of the experiment. They were fed, with standard feed (Vital Feeds, Jos, Nigeria) and water was provided *ad libitum.*

3.1.3 Trypanosome Isolates

Strain of *Trypanosoma brucei brucei* was obtained from the stabilates that was cryopreserved in Vector and Parasitology Studies Department, Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria.

3.2 METHODOLOGY

3.2.1 Induction of Parasitaemia

The parasite was maintained by serial passage in a donor rat. The infected blood from the donor rat (at peak parasitaemia) was collected and diluted with phosphate buffered saline (PBS). The number of parasites in the diluted blood was determined (Herbert and Lumsden, 1976) and 0.1mL of blood containing approximately 1 x 10^3 trypanosomes was inoculated intraperitoneally into each rat in the infected groups.

3.2.2 Experimental Design

A total of fifty four (54) rats were used. The rats were randomized into nine (9) groups, with six (6) rats per group. Groups I and II served as controls, while group III served as infected untreated. Groups IV, V and VI represented the pre-treated infected rats that were administered with daily dose of 50mg per kilogram body weight of zinc gluconate (Ambali *et al*., 2011), 10mg per kilogram body weight of selenium (Rayman, 2012) and combination of zinc gluconate and selenium respectively for seven (7) days. Rats in groups VII, VIII and IX represented the post-infected treated groups that were administered with daily dose of 50mg per kilogram body weight of zinc gluconate (Ambali *et al*., 2011), 10mg per kilogram body weight of selenium (Rayman, 2012) and combination of zinc gluconate and selenium immediately parasite was sighted in the blood for seven (7) days.

Thereafter the infected untreated, pre and post-infected treated groups were monitored for parasitaemia every 24 hours.

Group I: Normal rats fed with normal chow and distilled water *ad libitum*

Group II: Normal rats treated with zinc gluconate + selenium

Group III: *Trypanosoma brucei brucei* infected untreated rats

Pre-treated infected groups

Group IV: *Trypanosoma brucei brucei* infected rats + zinc gluconate

Group V: *Trypanosoma brucei brucei* infected rats + selenium

Group VI: *Trypanosoma brucei brucei* infected rats + zinc gluconate + selenium

Post- infected treated groups

Group VII: *Trypanosoma brucei brucei* infected rats + zinc gluconate

Group VIII: *Trypanosoma brucei brucei* infected rats + selenium

Group IX: *Trypanosoma brucei brucei* infected rats + zinc gluconate + selenium

The samples were reconstituted in distilled water, and administered intraperitoneally, daily for 7 days. Blood samples were collected from the tail vein of rats daily to monitor the development of parasitaemia, and every 5 days for the determination of packed cell volume and haemoglobin concentration.

3.2.3 Determination of Parasitaemia

Blood sample was collected from the tail vein of rats, pre-sterilized with methylated spirit every day to monitor the development of parasitaemia. The number of parasites was determined microscopically at x 40 magnification using the “Rapid Matching” method

described by Herbert and Lumsden (1976) to estimate the number of parasite per field. The method involves microscopic counting of parasites per field in blood appropriately diluted with buffered phosphate saline (pH 7.2). Logarithm values of these counts were obtained by matching with the Table of Herbert and Lumsden (1976) and converted to antilog to provide absolute number of trypanosomes per ml of blood.

3.3 EXPERIMENTAL SAMPLE COLLECTION

3.3.1 Collection and Preparation of Sera Samples and Organ Extract

At the end of the experiment, the animals were decapitated under chloroform anaesthesia and bled via cardiac puncture. Blood samples were collected in plain bottles for biochemical parameters and Ethylene diamine tetraacetic acid (EDTA) coated tubes for haematological parameters. Blood samples collected in plain tubes were allowed to clot and the supernatant (serum) separated by centrifugation using Denley BS400 centrifuge (England) at 3000 rpm for 10 minutes. The serum was removed and dispensed into properly labelled sterile vials and stored at -20^0C until further analysis.

3.3.2 Collection and Preparation of Organs

Immediately after the blood sample was collected, the liver, kidney, spleen, heart and lungs were quickly dissected out and trimmed, rinsed with saline to eliminate blood contamination, then blotted with filter paper and weighed to calculate the relative weight and kept in the refrigerator. The liver and kidney were crushed using pestle and mortar. Five (5) milliliter of phosphate buffered saline (pH 7.4) was used for homogenization. A portion of the organ was taken and buffer was added and then homogenized. It was then centrifuged at 4000 rpm for 15 minutes. Thereafter, the supernatant was collected using

Pasteur pipette for further analysis. The percentage change in organ weight of each of the animals was calculated as follows;

Percentage change in organ weight = $\frac{\text{organ weight}}{\text{animal weight}} \times 1($ ×

3.4. DETERMINATION OF HAEMATOLOGICAL PARAMETERS

Haematological parameters were determined by standard methods. Blood samples were collected through the tail vein. Packed Cell Volume and haemoglobin concentrations were determined on days 0, 1, 6, and 11

3.4.1 Packed Cell Volume (PCV)

The PCV is the volume of red blood cells (RBC) expressed as a fraction of the total volume of the blood was determined by the microhaematocrit method (Cheesbrough, 2000).

Principle: The red blood cells are heavier than plasma with specific gravity of 1090 and 1030 respectively. When blood is placed in a capillary tube and centrifuged, they settle and pack because of the centrifugal force acting on them. The volume occupied by the cells is measured and its ratio with volume of the whole blood is calculated.

Procedure: Blood from EDTA bottle was allowed by capillary action to flow through the capillary tube and one end of the tube was sealed with plasticine. It was then centrifuged at a speed of 3000 rpm for 10 minutes. The PCV was estimated using a microheamatocrit reader and expressed as percentage of the erythrocytes the blood contain.

3.4.2 Determination of Haemoglobin Concentration (Hb)

Hemoglobin concentration was determined by the Cyanomethemoglobin method (Jain, 1986).

Principle: Blood is mixed with dilute hydrochloric acid. This process hemolyzes the red cells, disrupting the integrity of the red cells' membrane and causing the release of hemoglobin, which, in turn, is converted to a brownish-colored solution of acid hematin. The acid hematin solution is then compared with a color standard.

Procedure: Sample solutions and standard solutions were prepared as follows:

Random sample: Drabkin reagent (5000µl) was mixed with 20µl of distilled water
Standard: Drabkin reagent (5000µl) was mixed with 20µl of sample solution of hemoglobin

Target sample: 5000µl of Drabkin reagent + 20µl of blood

The concentration of hemoglobin was marked with Drabkin's method, with the use of a spectrophotometer, at 540 nm. Once Drabkin reagent was mixed with the blood, the solution was incubated at room temperature for the duration of 5 minutes and absorbance was measured. The spectrophotometer was set to zero using distilled water.

The concentration of hemoglobin was calculated according to the following formula:

$$\text{Hb (g/dl)} = \frac{A_T}{A_S} \times C_S$$

Where:

A_T- absorbance of tested sample

A_S- absorbance of standard

C_S- concentration of standard in g/dl

3.4.3 Determination of White Blood and Red Blood Cells Count

Improved Neubeur haemocytometer (counting chamber) was used to count both white cells and red cells under the light microscope (Dacie and Lewis, 1995).

Principle: Blood is diluted with a fluid containing glacial acetic acid that lyses the red cells but not the white cells and a dye (gentian violet) which stains the nucleus of the white cells to facilitate counting.

Procedure: The white blood cell and red cell pipettes were used to draw blood and fill to 0.5 marks of both WBC and RBC pipettes. WBC diluting fluid was drawn to 11 and 101 marks respectively. The fluid and the blood were then mixed gently and transferred into the counting chamber. It was allowed to settle for 2 minutes and the chamber placed on the stage of the microscope for counting. The white blood count was done using the x 10 objective while the red blood cell counts were done using the x 40 objective.

Calculation:

$$\frac{\text{Number of Cells Counted} \times \text{Dilution Factor} \times \text{Depth Factor}}{\text{Number of Square Millimeters Counted}} = \text{WBC per mm}^3$$

3.4.4 Differential White Blood Cell (WBC) Count

Principle: The white blood cell differential count determines the number of each type of white blood cell present in the blood. A stained smear is examined in order to determine the percentage of each type of leukocyte present.

Procedure: A drop of blood from each rat was placed in the center line of a slide about 1-2cm from one end. The spreader was used to spread out the blood quickly along the line of contact of the spreader with the slide to make a blood film. The blood films were fixed with acetone free methyl alcohol for 1 minute in order to prevent hemolysis before washing with water. The blood films were then stained using Leishmans stain (powdered Leishmans stain 0.15g, methyl alcohol 133ml) by pouring few drops (about 8) on the slide and waited for 2 minutes. Thereafter, buffered water was added double the amount of drops, then mixed by rocking and waited for 10 minutes. The stain was then flooded off with distilled water and allowed to dry. The dry and stained films were then examined by counting the number of WBC in each of 5 to 6 low power fields under oil immersion objective. A total of 100 cells were counted and recorded.

3.4.5 Mean Cell Volume: The mean cell volume (MCV) was calculated as follows:

$$MCV = 10 \times \frac{\text{Hamatocrit}}{\text{Red Blood Cell Count}}$$

3.4.6 Mean Corpuscular Haemoglobin: Mean Corpuscular Haemoglobin (MCH) was calculated as follows:

$$MCH = 10 \times \frac{\text{Haemoglobin}}{\text{Red Blood Cell Count}}$$

3.4.7 Mean Corpuscular Haemoglobin Concentration (MCHC): It was calculated as follows:

$$MCHC = \frac{\text{Haemoglobin}}{\text{Hamatocrit}} \times 100$$

3.5 DETERMINATION OF BIOCHEMICAL PARAMETERS

3.5.1 Determination of Serum Aminotransferases

Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed by the colorimetric method of Reitman and Frankel (1957) using Randox assay kits.

3.5.1.1 Serum Alanine Aminotransferase (ALT)

Principle

$$\alpha\text{-oxoglutarate} + \text{L-alanine} \xrightarrow{\text{ALT}} \text{L-glutamate} + \text{pyruvate}$$

Alanine Aminotrasferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine at 37^{O}C and 540 nm.

Reagent Composition: R1. Buffer: Phosphate buffer (100mmol/l, pH 7.4), L-alanine (200mmol/l), α-oxoglutarate (2mmol/l). R2. 2, 4-dinitrophenylhydrazine: (2mmol/l).

Procedure: Exactly, 0.5ml of reagent 1 which is made up of phosphate buffer, L-alanine and α-Oxoglutarate was added into two clean test tubes, one containing 0.1ml of serum and the other 0.1ml distilled water (blank). The content in each test tube was mixed, incubated for 30 minutes at 37^{O}C. Reagent 2 (0.5ml) which is made up of 2, 4-dinitrophenylhydrazine was added to each of the test tubes, mixed and allowed to stand for 20 minutes at 25^{O}C. Then 0.5ml of 0.4N sodium hydroxide solution was added to each of the test tubes, the content in each test tube was mixed and absorbance was read against the blank at 540 nm

after 5 minutes. The ALT concentration (U/I) was determined from the standard calibration Table provided in the manual of Randox Lab. Ltd, UK Reagent Kit.

3.5.1.2 Serum Aspartate Aminotransferase (AST)

Principle

$$\alpha\text{-oxoglutarate} + \text{L-aspartate} \xrightarrow{\text{AST}} \text{L-glutamate} + \text{Oxaloacetate}$$

AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine at 37^{O}C and 540 nm.

Reagent Composition: R1: Buffer: Phosphate buffer (100mmol/l, pH 7.4), L-aspartate (100mmol/l), α-oxoglutarate (2mmol/l). R2: 2, 4-dinitrophenylhydrazine (2mmol/l).

Procedure: Exactly, 0.5ml of reagent 1 (R1) which is made up of phosphate buffer, L-aspartate and α-Oxoglutarate was added into two clean test tubes labelled as test sample and reagent blank, containing 0.1ml of serum and 0.1ml of distilled water respectively, the content in each test tubes was mixed and incubated for 30 minutes at 37^{O}C. Reagent 2 (0.5ml) which is made up of 2, 4-dinitrophenylhydrazine was added to each of the test tubes, the content of each of the test tubes was mixed and allowed to stand for exactly 20 minutes at 25^{O}C. To each of the test tubes, 0.5ml of sodium hydroxide solution were added, mixed and after 5 minutes absorbance was read against the blank at 540 nm. The AST concentration (U/l) was determined from the standard calibration Table provided in the manual of Randox Lab. Ltd, UK Reagent Kit.

3.5.2 Alkaline Phosphatase (ALP)

The serum level of alkaline phosphatase was quantified by optimized standard method described by Haussament (1977) using Randox assay kits.

Assay principle

P-nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ phosphate + p-nitrophenol

Alkaline phosphatase is measured by monitoring the concentration of phosphate hydrazone formed with 2, 4-dinitrophenylhydrazine at $37^{O}C$ and 405 nm.

Reagent Composition: Rla: Diethanolamine buffer (1mol/l, pH 9.8), $MgCl_2$ (0.5mmol/l), Rlb: Substrate- p-nitrophenylphosphate (10 mmol/l).

Reagent Preparation: One vial of substrate Rlb was reconstituted with 20ml of Buffer Rla.

Procedure: Exactly 1ml of reagent 1 containing diethanolamine buffer, magnesium chloride and substrate (P-nitrophenylphosphate), was added into a clean test tube containing 0.02ml of serum. This was mixed, initial absorbance was read and timer was started simultaneously, absorbance at 405nm was read again after 1, 2 and 3 minutes.

Calculation:

The ALP activity was calculated using the following formulae:

$U/l = 2760 \times \Delta A$ 405 nm/ min.

ΔA 405 nm/ min= change in absorbance at 405 nm per minute

3.5.3 Total Protein (TP)

Total protein was determined colorimetrically according to the method described by Fine (1935) using Randox assay kits.

Principle: The protein assay is based on the reaction of protein with alkaline copper tartarate solution and Folin reagent. Two steps lead to colour development. First is the reaction between protein and copper at alkaline pH, and subsequently, the reduction of Folin reagent by the copper-treated protein. Colour formation is due to amino acids that reduce the Folin reagent, yielding reduced species that imparts a characteristic blue colour. Bovine serum albumin is used as standard.

Reagent Composition: R1: Biuret reagent. Sodium hydroxide (100mmol/l), Na-K-tartrate: (16mmol/l), Potassium iodide (15mmol/l), Copper Sulphate (6mmol/l). R2: Blank Reagent. Sodium hydroxide (100mmol/l), Na-K-tartrate: (16mmol/l). CAL. Standard: Protein (58.48g/l).

Reagent Preparation: R1: Biuret reagent. The contents of bottle R1 was diluted with 400ml of double distilled water, rinsing the bottle thoroughly. R2: Blank reagent. The contents of bottle R2 was diluted with 400ml of double distilled water, rinsing the bottle thoroughly.

Procedure: Exactly 1ml of biuret reagent was added to three (3) clean test tubes labelled as sample, standard and blank containing 0.02ml of serum, 0.02ml of standard and 0.02ml

of distilled water respectively, the content in each of the test tube was mixed, incubated for 10 minutes at room temperature and absorbance was read at 540nm. The total protein concentration was calculated using the formula below

$$\text{Total Protein Concentration (g/dl)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times \text{Standard Concentration}$$

ΔA sample = Change in absorbance for sample

ΔA standard = Change in absorbance for standard

3.5.4 Albumin (ALB)

The serum albumin was determined by the method of Doumas *et al.,* (1971) using Randox assay kit.

Principle: The measurement of serum albumin is based on its quantitative binding to the indicator 3, 3, ', 5, 5' – tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The absorbance at 630 nm of the blue green colour of albumin-BCG-complex is directly proportional to the concentration of albumin in the sample.

Reagent Composition: R 1**:** BCG concentrate, Succinate buffer (75mmol/l): pH 4.2, Bromocresol green (0.15 mmol). CAL. Standard **(**46.75g/l**).**

Reagent Preparation: R1. BCG Concentrate**.** The content of bottle R1 was diluted with 87 ml of distilled water.

Procedure: Exactly 1ml of Bromocresol green reagent (R1) was added to three clean test tubes labelled test sample, standard and reagent blank containing 0.02ml of serum sample, 0.02ml of standard and 0.02ml distilled water respectively. The mixtures in each of the test

tubes were incubated for 10min under room temperature. The absorbance at 630 nm of the serum sample (A sample) and of the standard (A standard) was read against the reagent blank.

Calculation:

The albumin concentration in the sample was calculated from the following formula in g/l:

$$\text{Albumin concentration} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times \text{Concentration of the standard}$$

Where:

ΔA sample = Change in absorbance for sample

ΔA standard = Change in absorbance for standard

3.5.5 Determination of Serum Total Bilirubin (TB) Concentration

The serum TB concentration was estimated by acid diazo method as described by Doumas *et al*., (1971), using assay Kits (Randox laboratories limited UK).

Principle: Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction which diazotized sulphanillic acid.

Procedure: Exactly, 200ml of reagent 1 (sulphanillic acid) was dispensed each into two different test tubes labeled ‘sample blank’ and ‘sample’ followed by the addition of 1 drop (50µl) of reagent 2 (nitrite) and 1000µl of reagent 3 (caffeine). Test serum (200µl) was then dispensed into each of the test tubes and the mixtures incubated in a water bath for 10 minutes at 25^{O}C. This was followed by the addition of 100µl of reagent 4 (tartrate) with

the mixture incubated again at $25^{O}C$ for 10 minutes. The absorbance at 578nm of the sample (A_{TB}) was then read against the sample blank.

The total bilirubin concentration was then calculated as follows:

Total bilirubin (mg/dl) = $10.8 \times A_{TB}$ (578nm)

Where: A_{TB}- Absorbance of Total Bilirubin

3.5.6 Determination of Serum Direct/Indirect Bilirubin (DB/IB) Concentration

The serum DB concentration was determined using the Randox Kit (Randox laboratories limited UK) based on the method of Doumas *et al*., (1971).

Principle: Direct/indirect bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex**.**

Procedure: Exactly, 200ml of reagent 1 (sulphanilic acid) was dispensed each into two different test tubes labeled 'sample blank' and 'sample' followed by the addition of 1 drop (50µl) of reagent 2 (nitrite) and 2000µl of 0.9 % NaCl. Test serum (200µl) was dispensed into each of the test tubes and the mixtures incubated in a water bath for 10 minutes at $25^{O}C$. The absorbance at 546 nm of the sample (A_{DB}) was then read against the sample blank.

The direct bilirubin concentration was then calculated as follows:

Indirect bilirubin (mg/dl) = $14.4 \times A_{DB}$ (546nm)

Where: A_{DB}- Absorbance of Direct Bilirubin

3.5.7 Serum Zinc

Serum Zinc was determined using atomic absorption spectrophotometry by the method described by Fernandez and Kalm (1991).

Principle: Zinc binds to 5-Br-PAPS [(2-5-Bromo-2-Pyridylazo)-5-(N-propyl-N-sulfo-propylamino) phenol], forming a reddish-violet chelate. The absorbance of this reddish-violet chelate is measured at 560nm, and is directly proportional to the amount of zinc in the sample.

Reagent Composition: R1: Colour reagent A [Carbonate- Bicarbonate Buffer pH 9.8 (0.2 mol/L), 5-Br-PAPS (0.07 mmol/L), Sodium Citrate (170 mmol/L) and Dimethylglyoxime (4 mmol/L)]. R2: Colour reagent B [Salicylaldoxime (29 mmol/L)]. R3: Deproteinizing Reagent [Trichloroacetic Acid (7%)]. CAL. Standard (200µg/dL).

Reagent Preparation: Color Reagent A and Color Reagent B was mixed (20ml of Color Reagent A was mixed with 5ml of Color Reagent B) and then stored at room temperature.

Procedure: Exactly 0.5ml of deproteinizing reagent (R3) was added to three clean test tubes labelled serum sample, standard and reagent blank containing 0.5ml of serum sample, 0.5ml of standard and 0.5ml distilled water respectively. The mixtures in each of the test tubes were centrifuged for 10 minutes at 3000 rpm. Thereafter, 0.5ml of the supernatant was removed from each of the test tubes after centrifugation. Then 2.5ml of the working solution (R1 and R2) was added into each test tube. The mixtures were then incubated for

10 minutes at room temperature. The absorbance at 560 nm of the serum sample (A sample) and standard (A standard) was read against the reagent blank.

Calculation:

The zinc concentration corresponds to the measured absorbance of the sample (A_S) was determined using the formula:

$$\frac{\text{A sample}}{\text{A standard}} \times \text{Standard concentration} = (\mu\text{mol/l})$$

Where: A- Absorbance

3.5.8 Determination of Serum Creatinine Concentration

Colorimetric method was used to determined serum creatinine concentration according to Bartels and Bohmer (1973) using Randox assay kits.

Principle: Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is proportional to the creatinine concentration.

Reagent Composition: CAL. Standard (2.06mg/dl), Rla: Picric Acid (35mmol/l), Rlb: Sodium Hydroxide (0.32mol/l).

Reagent Preparation: An equal volume of solutions Rla + Rlb was mixed.

Procedure: Exactly 1.0ml of working reagent containing picric acid and sodium hydroxide was added into two clean test tubes labelled sample test and standard, containing 0.1ml of

sample and 0.1ml of standard solution. The content in each test tube was mixed and after 30 seconds, the absorbance A_1 of the standard and sample was read at 490 nm. Exactly 2 minutes later, absorbance A_2 of the standard and sample were also taken.

Calculation:

The Concentration of creatinine in serum was calculated as follows:

$$\text{Concentration of Creatinine (mg/dl)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times \text{Standard concentration (mg/dl)}$$

$A_2 - A_1 = \Delta A$ sample or ΔA standard

Where: ΔA- Change in Absorbance of sample or standard

3.5.9 Determination of Serum Urea Concentration

Principle: The serum urea concentration was determined using urea Randox assay kit. Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia formed is then measured photometrically by Berthelot's reaction (Fawcett and Scout, 1960).

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{hypochlorite} + \text{phenol} \longrightarrow \text{indophenols (blue compound).}$$

Reagent Composition: R1: EDTA (116mmol/l), Sodium nitroprusside (6mmol/l), Urease (1mmol/l). R2: Phenol (diluted) (120mmol/l). R3: Sodium hypochlorite (diluted) (27mmol/l), NaOH (0.14 N). CAL. Standard: (80.65 mg/dl).

Reagent Preparation: R1. Sodium nitroprusside and urease (Solution Rl). The contents of vial Rla was transferred into bottle Rlb and mixed gently. The content of bottle R2 (phenol) was diluted with 660ml of distilled water. Then contents of bottle R3 (Sodium hypochlorite) was diluted with 750ml of distilled water.

Procedure: Exactly 100µl of reagent 1 containing sodium nitropusside and urease was added into three clean test tubes labelled as test sample, standard and reagent blank containing 10µl each of sample, standard reagent and distilled water respectively. The content in each of the test tube was mixed and incubated at 37^{o}C for 10 minutes, then 2.5ml of reagent 2 containing diluted phenol and reagent 3 containing diluted sodium hypochlorite and sodium hydroxide were added to each of the three test tubes after which content of each test tube was mixed immediately and incubated for 15 minutes. The absorbance at 500 nm of the test sample (A sample) and standard (A standard) were read against blank.

Calculation:

The serum urea concentration was calculated as follows:

$$\text{Urea Concentration (mg/dl)} = \frac{\Delta A\text{sample}}{\Delta A\text{standard}} \times \text{Concentration of Standard}$$

ΔA sample = Change in absorbance for sample or standard

3.6 DETERMINATION OF ANTIOXIDANTS PROPERTIES AND LIPID PEROXIDATION

3.6.1 Thiobarbituric Acid Reactive Substances (TBARS)

Lipid peroxidation of low density lipoprotein (LDL) as evidenced by the level of malondialdehyde (MDA) concentration was assessed by Thiobarbituric acid reactive substances (TBARS) formation (Ohkawa *et al.,* 1979).

Principle: Malondialdehyde (MDA) is a product of lipid peroxidation and is used as an indicator of tissue damage. The MDA form a 1:2 adduct with thiobarbituric acid (TBA) and produces a pink coloured product which has an absorption maximum at 532 nm.

Reagents Preparation: Thiobarbituric acid (100mg of TBA in 30ml distilled water and 30ml of acetic acid)

Trichloroacetic (TCA) 10%: 10g of TCA was dissolved in distilled water and made up to 100ml with distilled water. Normal saline solution (0.9%): 0.9g of NaCl was dissolved in distilled water and made up to 100ml.

Procedure: Normal saline (0.2ml) was pipetted into test tubes labelled as, sample test and sample blank, then 0.2ml of tissue homogenate was also pipetted into the sample test tube and 0.5ml of TCA solution was added to both the sample test and sample blank tubes followed by addition of 0.1ml of TBA solution and 0.2ml of distilled water was added into sample blank tube in place of tissue homogenate. The mixture in each test tube was heated for 60 minutes in a water bath at 95^{o}C. After cooling to room temperature on ice bath, 3ml of n-butanol was added to the content in each test tube and then mixed vigorously, the

butanol phase was mixed by centrifugation at 1000 X g for 5 minutes and absorbance at 532 nm of the sample test was read against the absorbance of the sample blank.

Calculations:

Concentration of TBARS is expressed in terms of Malondialdehyde (MDA) in mmol/g of tissue.

Molar extinction of MDA $1.56 \times 10^5 cm^{-1}m^{-1}$

MDA concentration = Absorbance / $1.56 \times 10^5 cm^{-1}m^{-1}$

TBARS = (Volume of homogenate $\times$ MDA Concentrate) / weight of tissue

3.6.2 Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) activity was determined by method described by Fridovich (1989).

Principle: The ability of superoxide dismutase (SOD) to inhibit auto oxidation of adrenaline at pH10.2 forms the basis of this assay.

Reagents: Carbonate buffer (0.05M): 14.3g of Na_2CO_3 and 4.2g of $NaHCO_3$ was dissolved in distilled water and made up to 100ml mark in a volumetric flask. The buffer was adjusted to pH 10.2.

Adrenaline (0.3mM): 0.01g of adrenaline was dissolved in 17ml of distilled water. The solution was always prepared fresh.

Procedure: Tissue homogenate of 0.1ml was diluted in 0.9ml of distilled water to make 1:10 dilution of microsome. An aliquant mixture of 0.20ml of the diluted microsome was added to 2.5ml of 0.05M Carbonate buffer. The reaction was started with the addition of 0.3ml of 0.3mM Adrenaline. The reference mixture contains 2.5ml of 0.05M Carbonate buffer, 0.3ml of 0.3mM Adrenaline and 0.20ml distilled water. Absorbance was measured over 30s up to 150s at 480 nm.

Calculations:

Increase in absorbance per minute $= (A_5 - A_1)/ 2.5$

$$\% \text{ inhibition} = 100 - \frac{\text{Increase in absorbance for substrate}}{\text{Increase in absorbance of blank}} \times 100$$

1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adenochrome in 1 minute.

SOD activity = % inhibition / (50 $\times$ weight of tissue).

3.6.3 Catalase (CAT) Activity

Catalase activity was determined using the method described by Sinha (1972).

Principle: The method is based on the reduction of dichromate in acetic acid to chromic acetate, when heated in the presence of hydrogen peroxide with the formation of perchromic acid as unstable intermediate. Chromic acetate so produced is measured colorimetrically at 570 nm. This is because dichromate has no absorbance at 570 nm and does not interfere with the determination of catalase.

Reagents: 0.01M Phosphate buffer (pH 7.0): 5% potassium heptaoxochromate (V1), $K_2Cr_2O_7$: 5g of $K_2Cr_2O_7$ was dissolved in little quantity of distilled water and made up to 100ml.

0.2M H_2O_2: 0.6 ml was dissolved in little quantity of distilled water and made up to 100ml. It was stored at 4^oC.

Dichromate / Acetic acid solution: 5% potassium heptaoxochromate (V1), $K_2Cr_2O_7$, was mixed with glacial acetic acid in ratio 1:3, and was stored in brown bottle at room temperature.

Procedure: Exactly 1ml of phosphate buffer, 0.2ml of tissue homogenate and 0.4ml of H_2O_2 were added into two separate test tubes each, 1ml of potassium dichromate/glacial acetic acid was added immediately to one of the test tubes followed by the addition of H_2O_2 (T0), after which 10 minutes later 1ml of potassium dichromate was also added to the second test tube (T1). The mixture in the test tubes was heated in water bath at 80^0C for 10 minutes and absorbance was read at 570 nm.

Calculations:

The quantity of H_2O_2 was calculated as follows:

$C_T/C_S = A_T/A_S$

Where:

C_T = Concentration of the test

C_S = Standard concentration = 0.2M

A_T = Absorbance of test (T0 – T1)

A_S = Absorbance of blank (T0)

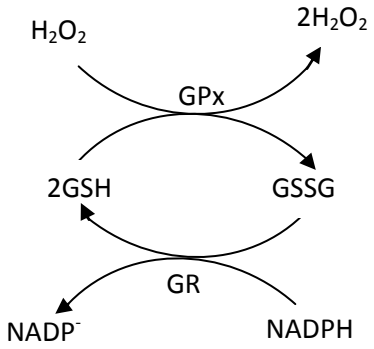
$$\text{Catalase activity} = \frac{C_T \times \text{total volume of homogenate}}{10 \text{ minutes} \times \text{volume of homogenate used} \times \text{weight of tissue}}$$

Catalase activity was expressed in moles of H_2O_2/min/g of tissue.

3.6.4 Glutathione Peroxidase (GPx)

Glutathione peroxidase (GPx) was determined by the method of Ellman (1959).

Principle: Glutathione Peroxidase catalyzes the reduction of hydrogen peroxide (H_2O_2), oxidizing reduced glutathione (GSH) to form oxidized glutathione (GSSG). GSSG is then reduced by glutathione reductase (GR) and β-nicotinamide adenine dinucleotide phosphate (NADPH) forming $NADP^+$ (resulting in decreased absorbance at 340 nm) and recycling the GSH. Glutathione peroxidase is limiting hence, the decrease in absorbance at 340 nm is directly proportional to the glutathione peroxidase concentration.



Reagents Preparation: NADPH Reagent: (β-nicotinamide adenine dinucleotide phosphate and GSH reduced) was reconstituted with the entire contents of one NADPH diluent

(glutathione reductase in buffer with stabilizer and 4mM NaN_3) and was labelled as working NADPH.

H_2O_2 Reagent: Dilute 3% hydrogen peroxide (0.02ml of 3% H_2O_2 to 2ml assay buffer and mark as dilution 1 and further diluted (0.3ml of 3% H_2O_2 diluted to 10ml with assay buffer) and mark as working H_2O_2.

Procedure: All reagents were brought to room temperature and samples (tissue homogenate) were placed on ice. Microplate was removed from the plastic bag and 50µL of tissue homogenate was added to the wells and followed by addition of 50µL working NADPH solution into each well. Thereafter, 50µL of working H_2O_2 (0.3ml of 3% H_2O_2 diluted to 10ml with assay buffer) solution was also added to each well and allowed to stand for 1 minute. Sample blank tube was prepared by replacing the sample with 50µL of distilled water. The mixtures in both tubes were transferred to cuvettes and absorbance monitored at 340 nm for 5 minutes at recording interval of every 30 seconds against sample blank. The GPx activity was calculated from the net rate.

Calculation:

GPx Concentration was calculated using the NADPH Absorption Coefficient:

Glutathione Peroxidase activity, expressed as mU/mL, was calculated as follows:

$$[GPx] = \frac{2(mRate_s - mRate_b).\ V_{Rxm}\ .\ df}{6.22\ V_s}$$

Where:

$mRate_s = 1000 \times \Delta A_{340}$/min of sample

$mRate_b = 1000 \times \Delta A_{340}$/min of blank

6.22 = Absorption Coefficient at 1cm path length.

V_{Rxm} = Volume of Reaction Mixture (150µL)

V_s = Volume of Sample (50µL)

2 = Correction for 2 moles GSH oxidized to 1 mole GSSG per mole NADPH oxidized.

df = Sample dilution factor

3.7 STATISTICAL ANALYSIS

Results were expressed as mean ± standard deviation (SD). The data obtained were analyzed using analysis of variance (ANOVA) (SPSS program, version 20 SPSS Inc., Chicago, IL, USA for windows Computer software Package). The difference between the experimental groups were compared using the Duncan Multiple Range Test. Values of P less than 0.05 ($P<0.05$) were taken as significant.

CHAPTER FOUR

RESULTS

4.1 Parasitaemia

Effect of pre and post infection administration of zinc and selenium on parasitaemia on *Trypanosoma brucei brucei* infection in wistar albino rats is presented in Figure 4.1. Parasitaemia was detected in the infected untreated control and post-infected treated groups on day 3 while in the pre-treated infected groups parasitaemia was detected on day 4 post infection (PI). The results indicate that the rats that were administered with zinc and selenium pre and post-infection shows significant ($P<0.05$) reduction in mean parasitaemia level throughout the administration period, compared to the trypanosomiasis infected untreated group. The pre-treated infected groups recorded significantly ($P<0.05$) lower parasitaemia compared to the post-infected treated groups.

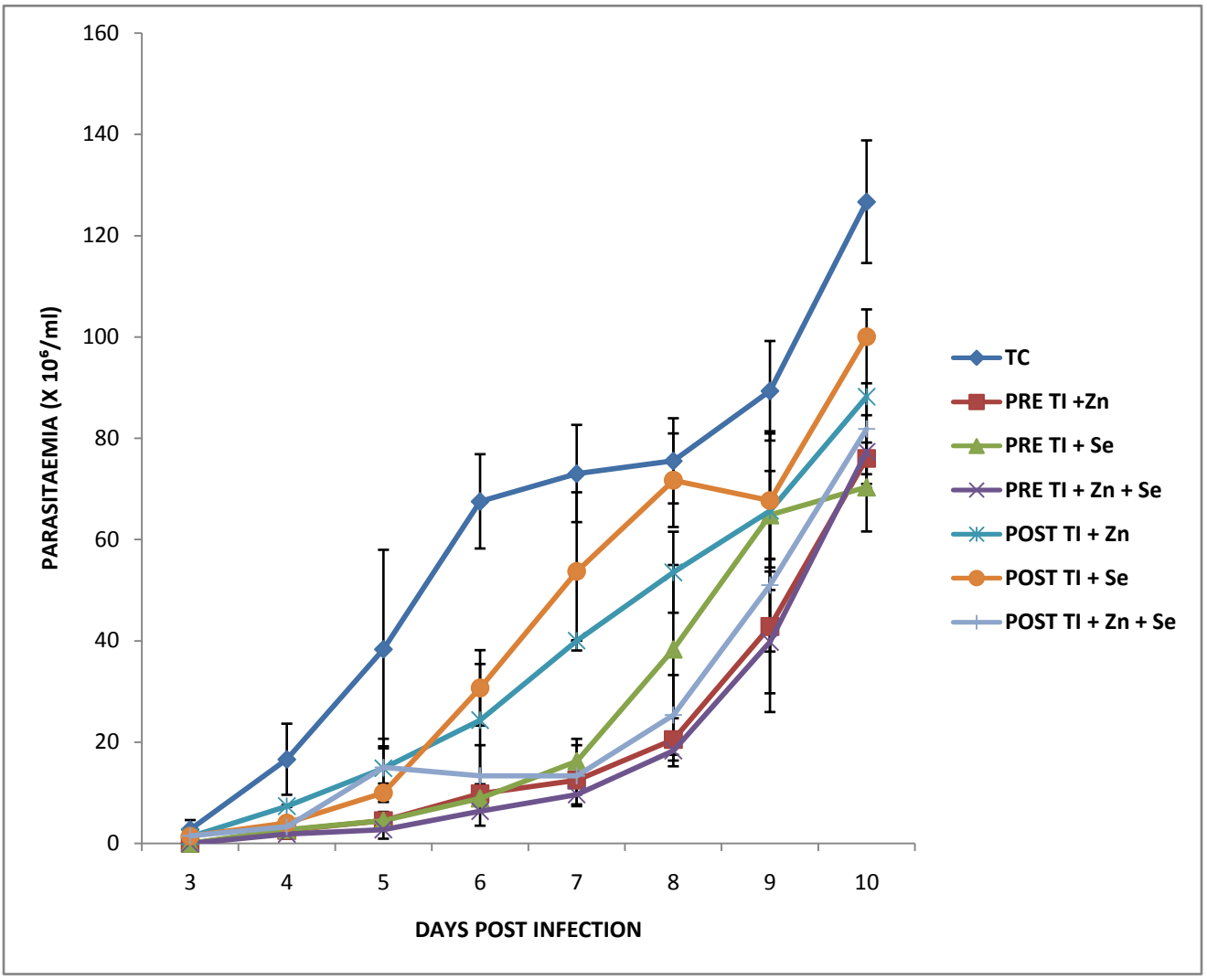


Figure 4.1: Effects of Pre and Post Infection Administration of Zinc and Selenium on Parasitaemia Level in *Trypanosoma brucei brucei* Infected Wistar Albino Rats.

TC: Trypanosomiasis Control, PRE TI + Zn: Pre-Treated Infected + Zinc, PRE TI + Se: Pre-Treated Infected + Selenium, PRE TI + Zn + Se: Pre-Treated Infected + Zinc + Selenium, POST TI + Zn: Post-Treated Infected + Zinc, POST TI + Se: Post-Treated Infected + Selenium, POST TI + Zn + Se: Post-Treated Infected +Zinc + Selenium

4.2. Effect of Pre and Post Infection Administration of Zinc and Selenium on Heamatological Parameters in *Trypanosoma brucei brucei* Infected Wistar Albino Rats.

4.2.1 Mean Packed Cell Volume and Haemoglobin Concentration

Effect of zinc and selenium administration on mean packed cell volume (PCV) and haemoglobin concentration (Hb) in normal and *Trypanosoma brucei brucei* infected wistar albino rats are shown in Figures 4.2 and 4.3 respectively. The result revealed that infection of rats with *T. b. brucei* caused significantly ($P<0.05$) lower values of PCV and Hb concentration in the infected untreated group compared to the normal and combined Zn +Se control groups.

Significantly ($P<0.05$) lower values of PCV and Hb were recorded in the post-infected treated group administered with Se and combined Zn +Se compared to the normal and combined Zn + Se control groups.

Significantly ($P<0.05$) higher values of PCV and Hb concentration in the pre and post infected treated groups was recorded with the exception of post-infected treated with Se and combined Zn + Se when compared to the infected untreated group. On the other hand, there were significantly ($P<0.05$) higher values of PCV and Hb of all the pre and post-infected treated groups compared to the infected untreated group on day 6 and 11 PI.

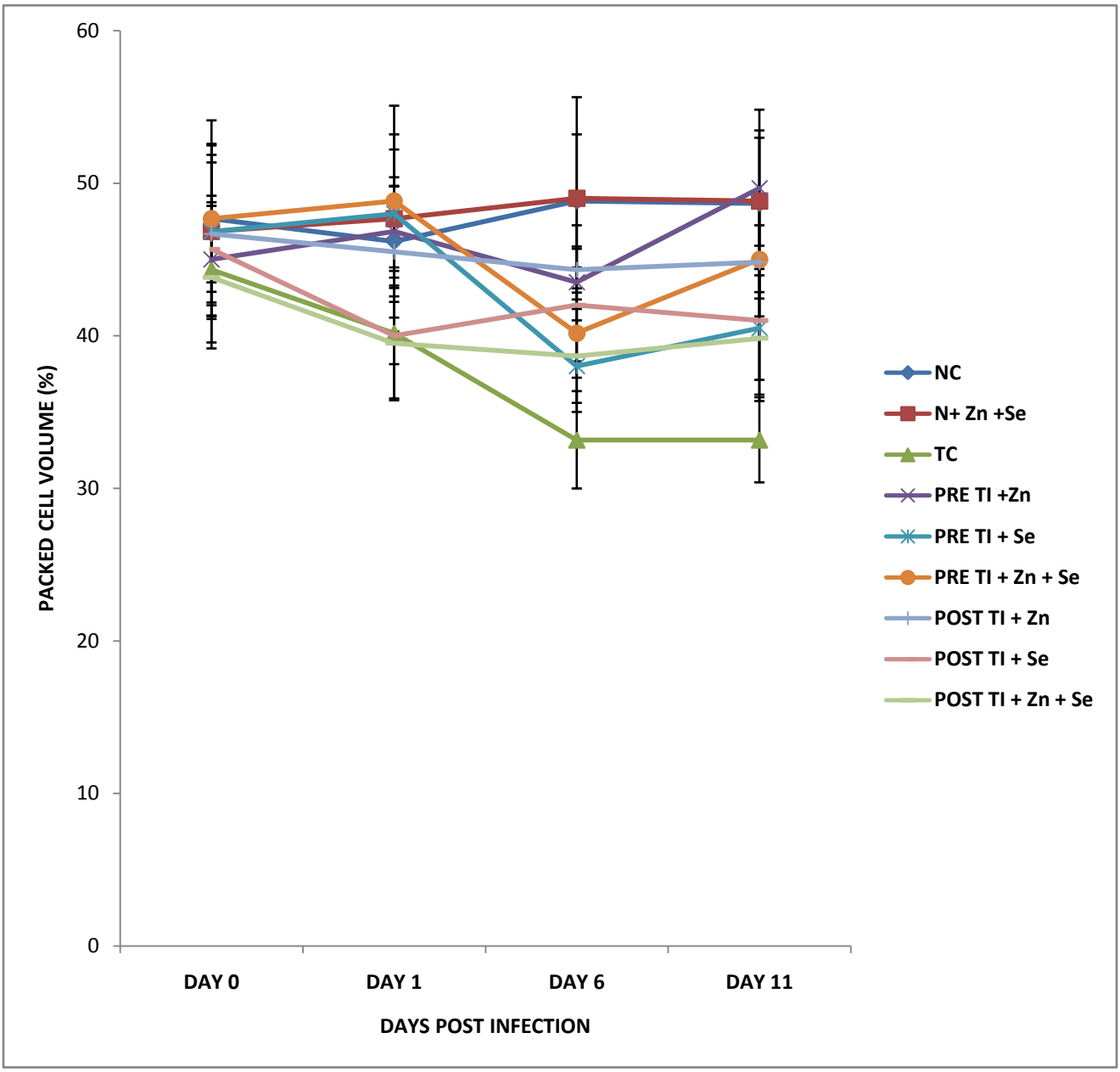


Figure 4.2: Effect of Pre and Post Infection administration of Zinc and Selenium on Packed Cell Volume in *Trypanosoma brucei brucei* Infected Wistar Albino Rats

NC: Normal Control rats, N + Zn +Se: Normal rats + Zinc + Selenium, TC: Trypanosomiasis Control, PRE TI + Zn: Pre-Treated Infected + Zinc, PRE TI + Se: Pre-Treated Infected + Selenium, PRE TI + Zn + Se: Pre-Treated Infected + Zinc + Selenium, POST TI + Zn: Post-Treated Infected + Zinc, POST TI + Se: Post-Treated Infected + Selenium, POST TI + Zn + Se: Post-Treated Infected +Zinc + Selenium

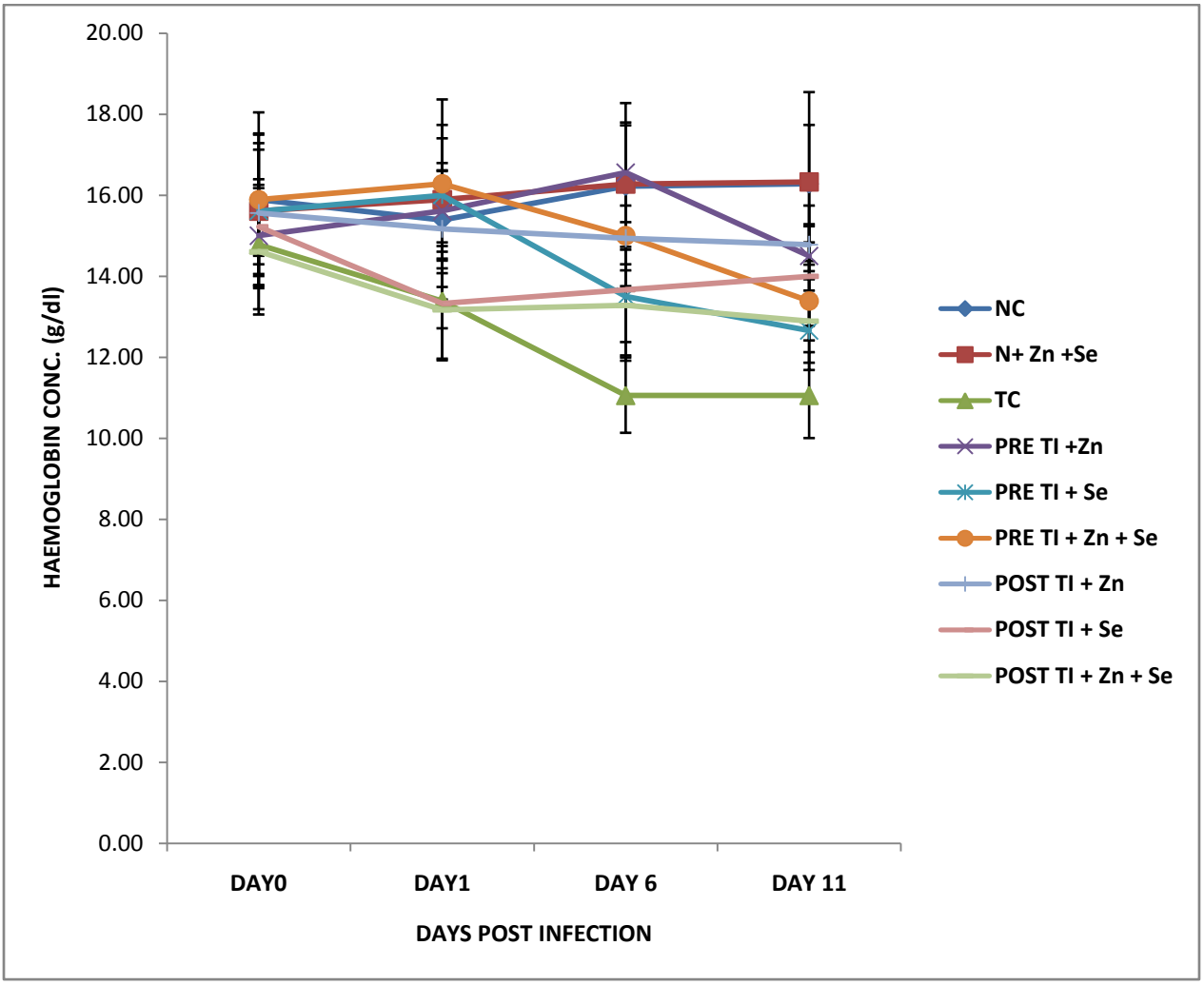


Figure 4.3: Effect of Pre and Post Infection Administration of Zinc and Selenium on Haemoglobin Concentration in *Trypanosoma brucei brucei* Infected Wistar Albino Rats

NC: Normal Control rats, N + Zn +Se: Normal rats + Zinc + Selenium, TC: Trypanosomiasis Control, PRE TI + Zn: Pre-Treated Infected + Zinc, PRE TI + Se: Pre-Treated Infected + Selenium, PRE TI + Zn + Se: Pre-Treated Infected + Zinc + Selenium, POST TI + Zn: Post-Treated Infected + Zinc, POST TI + Se: Post-Treated Infected + Selenium, POST TI + Zn + Se: Post-Treated Infected +Zinc + Selenium

4.2.2: Effect of Pre and Post Infection Administration of Zinc and Selenium on Other Haematological Parameters.

Effect of zinc and selenium administration on Red Blood Cell (RBC) count, total white blood cell (WBC) count, mean cell volume (MCH), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and total differential white blood cell (WBC) count (lymphocyte, neutrophil and eosinophil) of normal and *T. brucei brucei* infected rats is presented on Table 4.1. The result show significantly ($P<0.05$) lower values of RBC, WBC and lymphocyte in the infected untreated control group with the exception of neutrophil and eosinophil which was significantly ($P<0.05$) higher compared to the normal and combined Zn + Se control groups.

The rats that were administered with zinc and selenium pre and post infection with *T. b. brucei*, showed significantly ($P<0.05$) lower values of RBC and lymphocyte, and was significantly ($P<0.05$) higher in neutrophil, compared to the normal and combined Zn + Se control groups.

However, in all the pre and post infected treated groups, there were significantly ($P<0.05$) higher values of RBC, WBC and lymphocyte except for post infected treated with Se, Zn as well as a significantly ($P<0.05$) lower value of neutrophil and eosinophil compared to the infected untreated control group.

Table 4.1: Effects of Pre and Post Infection Administration of Zinc and Selenium on Other Haematological Parameters

Group	RBC Count ($X 10^6$/L)	MCV(fl)	MCH (Pg)	MCHC (g/dl)	WBC Count ($x10^9$)/L	Lymphocyte (%)	Neutrophil (%)	Eosinophil (%)
NC	9.17 ± 0.79^{d}	60.20 ± 0.64^{ab}	20.07 ± 0.20^{ab}	33.23 ± 0.01^{a}	14.88 ± 2.30^{b}	91.80 ± 4.28^{d}	10.09 ± 2.90^{a}	5.24 ± 0.15^{a}
N+ Zn +Se	9.15 ± 1.10^{d}	60.13 ± 0.28^{ab}	20.04 ± 0.10^{ab}	33.23 ± 0.01^{a}	14.82 ± 3.32^{b}	90.00 ± 3.52^{d}	11.02 ± 0.49^{a}	5.13 ± 0.13^{a}
TC	5.55 ± 0.52^{a}	55.75 ± 0.63^{a}	17.92 ± 0.21^{a}	33.33 ± 1.00^{a}	8.07 ± 1.55^{a}	68.97 ± 5.76^{a}	26.00 ± 2.94^{c}	10.99 ± 1.22^{b}
PRE TI +Zn	7.05 ± 0.55^{b}	62.28 ± 2.46^{ab}	20.76 ± 3.16^{ab}	33.63 ± 0.01^{a}	15.43 ± 1.08^{b}	80.33 ± 2.37^{c}	17.71 ± 3.42^{b}	7.50 ± 0.52^{a}
PRE TI + Se	6.77 ± 0.45^{b}	56.35 ± 5.84^{a}	18.79 ± 1.94^{a}	33.27 ± 0.01^{a}	15.12 ± 2.40^{b}	78.00 ± 3.55^{b}	18.00 ± 5.40^{b}	6.31 ± 0.88^{a}
PRE TI+Zn+Se	7.38 ± 0.23^{b}	54.39 ± 3.24^{a}	18.13 ± 1.08^{a}	33.33 ± 0.03^{a}	15.07 ± 3.59^{b}	83.50 ± 7.45^{c}	16.67 ± 0.54^{b}	6.57 ± 1.02^{a}
POST TI + Zn	6.40 ± 0.51^{b}	67.90 ± 14.54^{b}	22.64 ± 4.84^{b}	33.34 ± 0.01^{a}	14.98 ± 2.54^{b}	77.58 ± 6.81^{b}	19.86 ± 1.25^{ab}	6.80 ± 0.49^{a}
POST TI + Se	6.33 ± 0.48^{b}	66.86 ± 4.91^{b}	22.29 ± 3.30^{b}	33.04 ± 0.02^{a}	14.85 ± 2.21^{b}	74.38 ± 8.8^{b}	20.33 ± 4.12^{ab}	5.63 ± 0.38^{a}
POST TI+Zn+Se	8.75 ± 1.27^{c}	60.42 ± 0.17^{ab}	20.14 ± 0.06^{ab}	33.23 ± 0.01^{a}	15.00 ± 1.10^{b}	82.75 ± 5.09^{c}	18.67 ± 4.36^{b}	5.45 ± 0.55^{a}

Values are means ± SD of six replicate determinations. Values with different superscript down the column are significantly different ($P<0.05$).

NC: Normal Control rats, N + Zn +Se: Normal rats + Zinc + Selenium, TC: Trypanosomiasis Control, PRE TI + Zn: Pre-Treated Infected + Zinc, PRE TI + Se: Pre-Treated Infected + Selenium, PRE TI + Zn + Se: Pre-Treated Infected + Zinc + Selenium, POST TI + Zn: Post-Treated Infected + Zinc, POST TI + Se: Post-Treated Infected + Selenium, POST TI + Zn + Se: Post-Treated Infected +Zinc + Selenium. RBC: Red blood cell, WBC: White blood cell, MCV: Mean cell volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration.

4.3: Effect of Pre and Post Infection Administration of Zinc and Selenium on Some Biochemical Parameters in *Trypanosoma brucei brucei* Infected Wistar Albino Rats.

4.3.1. Effects on Liver Marker Enzymes

Table 4.2 presents the effects of pre and post infection administration of zinc and selenium on liver marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in normal and *T. b. brucei* infected rats. The result shows that there were no significant ($P>0.05$) difference in these maker enzymes in the animals that were administered combined Zn + Se without infection compared to the normal control.

The result revealed that there were significantly ($P<0.05$) higher values in the serum activities of AST, ALT and ALP in *Trypanosoma brucei brucei* infected untreated control when compared to the normal and Zn + Se control groups.

The result also show significantly ($P<0.05$) higher values in the serum activities of AST, ALT and ALP in the pre-treated infected groups with the exception of the pre-treated infected group that were administered with Zn which were not significantly ($P>0.05$) higher in AST and ALT when compared to the normal and combined Zn + Se control groups.

The pre-treated infected administered with Zn, Se, shows significantly ($P<0.05$) higher values in the serum activities of AST, ALT and ALP with the exception of the group that received Zn + Se combined which were not significantly ($P>0.05$) higher in AST value compared to the normal and combined Zn + Se control groups.

The pre and post infected treated groups showed significantly ($P<0.05$) lower values in serum activities of AST, ALT and ALP compared to the infected untreated control group (Table 4.2).

Table 4.2: Effects of Pre and Post Infection Administration of Zinc and Selenium on Liver Marker Enzymes in *Trypanosoma brucei brucei* Infected Wistar Albino Rats.

Group	AST (U/I)	ALT (U/I)	ALP (U/I)
NC	33.50 ± 4.85^{a}	33.30 ± 1.75^{a}	120.17 ± 3.67^{a}
N+ Zn +Se	35.00 ± 3.58^{a}	34.67 ± 2.16^{a}	123.83 ± 4.14^{a}
TC	51.33 ± 3.01^{d}	52.83 ± 4.11^{d}	258.67 ± 12.43^{e}
PRE TI +Zn	35.5 ± 0.83^{ab}	36.17 ± 0.79^{ab}	204.83 ± 1.91^{c}
PRE TI + Se	44.50 ± 4.22^{c}	35.12 ± 2.89^{ab}	217.33 ± 6.19^{cd}
PRE TI + Zn + Se	38.00 ± 3.85^{b}	31.50 ± 1.83^{a}	168.50 ± 4.42^{b}
POST TI + Zn	41.20 ± 5.58^{bc}	45.33 ± 2.47^{c}	213.00 ± 2.29^{cd}
POST TI + Se	42.16 ± 6.01^{bc}	46.33 ± 3.03^{c}	231.17 ± 3.56^{d}
POST TI + Zn + Se	36.83 ± 2.53^{ab}	40.83 ± 1.76^{b}	200.50 ± 2.32^{c}

Values are means ± SD of six replicate determinations. Values with different superscript down the column are significantly different ($P<0.05$).

NC: Normal Control rats, N + Zn +Se: Normal rats + Zinc + Selenium, TC: Trypanosomiasis Control, PRE TI + Zn: Pre-Treated Infected + Zinc, PRE TI + Se: Pre-Treated Infected + Selenium, PRE TI + Zn + Se: Pre-Treated Infected + Zinc + Selenium, POST TI + Zn: Post-Treated Infected + Zinc, POST TI + Se: Post-Treated Infected + Selenium, POST TI + Zn + Se: Post-Treated Infected +Zinc + Selenium. AST: Aspartate amino transferase, ALT: Alanine amino transferase, ALP: Alkaline phosphatase

4.3.2. Effects on Liver Function Parameters and Serum Zinc

Effect of pre and post- infection administration of zinc and selenium on serum zinc level and liver function indices of total protein (TP), albumin (ALB) concentrations and the bilirubin's: total bilirubin (TB), direct bilirubin (DB) and indirect bilirubin (IB) in the serum of normal and *T. brucei brucei* infected wistar albino rats is presented in Table 4.3. The rats that were infected untreated showed significantly ($P<0.05$) lower values of TP, ALB and higher values in the levels of TB and IB compared to the normal and combined Zn +Se control groups.

There were significantly ($P<0.05$) lower value in TP by Zn +Se combined in the pre-treated infected group compared to the normal and combined Zn +Se control groups. There was also a significantly ($P<0.05$) lower value in ALB by Zn, Se and combined Zn +Se compared with the normal and combined Zn +Se control groups.

The result also shows significantly ($P<0.05$) higher level of serum Zn in the pre-treated infected group administered with zinc, no significant ($P>0.05$) difference in Zn level by Se administered group and not significantly ($P<0.05$) higher in serum Zn level by the combined Zn +Se treated group compared to the normal and combined Zn +Se control groups.

On the other hand, rats that were infected with *T. brucei brucei* post administration of Zn, Se and combined Zn +Se shows no significant ($P>0.05$) difference in TP and significantly ($P<0.05$) lower in ALB when compared to the normal and combined Zn +Se control groups. Also the post-infected treated rats were not significantly ($P<0.05$) higher in TB,

serum Zn level and no significant (P>0.05) difference in DB, IB, when compared to the normal and combined Zn +Se control groups.

All pre and post-infected treated groups shows increase (P<0.05) in TP and significantly (P<0.05) higher in ALB compared to the infected untreated group. There were significantly (P<0.05) lower values of TB, IB and no significant (P>0.05) difference in DB when compared to the infected untreated group. When the pre and post-infected treated groups were compared to the trypanosomiasis infected untreated control group, there were significantly (P<0.05) higher level of serum Zn in the pre-treated infected Zn administered group, no significant (P<0.05) difference in Zn level in the pre-treated infected Se administered group, and not significantly (P<0.05) higher in Zn level in the pre-treated infected Zn +Se administered group, post-infected treated with Zn, post-infected treated with Se and post-infected treated with combined Zn +Se compared to the infected untreated group (Table 4.3).

Table 4.3: Effects of Pre and Post Infection Administration of Zinc and Selenium on Liver Function Parameters and Serum Zinc in *Trypanosoma brucei brucei* Infected Wistar Albino Rats.

Group	TP (g/dl)	ALB (g/dl)	TB (mg/dl)	DB (mg/dl)	IB (mg/dl)	Zinc (µmol/l)
NC	44.17±1.47^{b}	42.67±1.75^{e}	10.88±1.12^{a}	6.52±0.48^{a}	4.36±1.33^{a}	0.49±0.30^{a}
N+ Zn +Se	42.33±1.50^{b}	40.00±2.28^{e}	10.13±1.39^{a}	7.10±0.42^{a}	3.03±1.27^{a}	1.00±0.37^{ab}
TC	37.83±3.54^{a}	31.83±2.48^{a}	14.15±1.29^{c}	7.08±0.60^{a}	7.07±1.30^{b}	0.32±0.69^{a}
PRE TI +Zn	40.17±0.52^{ab}	37.50±1.64^{cd}	11.62±1.28^{ab}	6.90±0.85^{a}	4.72±1.02^{a}	1.32±0.65^{b}
PRE TI + Se	39.67±2.92^{ab}	36.50±2.26^{b}	11.85±1.02^{ab}	7.00±0.84^{a}	4.85±1.43^{a}	0.42±0.29^{a}
PRE TI + Zn + Se	44.47±2.56^{b}	39.83±0.47^{d}	11.01±0.65^{a}	6.83±0.45^{a}	4.18±0.41^{a}	0.95±0.22^{ab}
POST TI + Zn	42.67±1.75^{b}	34.67±2.26^{b}	12.88±1.07^{b}	7.63±0.38^{a}	5.25±1.48^{a}	0.91±0.50^{ab}
POST TI + Se	38.83±4.00^{ab}	32.00±1.67^{ab}	12.20±1.84^{ab}	7.02±0.58^{a}	5.18±0.95^{a}	0.48±0.30^{a}
POST TI +Zn +Se	43.03±3.67^{b}	36.83±2.32^{c}	11.78±0.98^{ab}	6.00±0.31^{a}	5.78±1.31^{a}	0.85±0.30^{ab}

Values are means ± SD of six replicate determinations. Values with different superscript down the column are significantly different ($P<0.05$).

NC: Normal Control rats, N + Zn +Se: Normal rats + Zinc + Selenium, TC: Trypanosomiasis Control, PRE TI + Zn: Pre-Treated Infected + Zinc, PRE TI + Se: Pre-Treated Infected + Selenium, PRE TI + Zn + Se: Pre-Treated Infected + Zinc + Selenium, POST TI + Zn: Post-Treated Infected + Zinc, POST TI + Se: Post-Treated Infected + Selenium, POST TI + Zn + Se: Post-Treated Infected +Zinc + Selenium. TP: Total protein, ALB: Albumin, TB: Total bilirubin, DB: Direct bilirubin, IB: Indirect bilirubin

4.4: Effects of Pre and Post Infection Administration of Zinc and Selenium on Lipid Peroxidation and Endogenous Antioxidant Enzymes in *Trypanosoma brucei brucei* Infected Wistar Albino Rats.

4.4.1. Effects on Liver Lipid Peroxidation

Table 4.4, shows lipid peroxidation as evidenced by the level of malondialdehyde (MDA) in the liver of normal and trypanosomiasis infected rats administered with zinc and selenium for seven (7) days. Rats that were infected with *T. brucei brucei* untreated shows significantly ($P<0.05$) higher level of MDA compared to the normal and Zn +Se control groups.

On the other hand, animals that were treated, pre and post infection were significantly ($P<0.05$) higher in MDA level with the exception of the pre-infected treated and post-infected treated groups that were administered combined Zn +Se compared to the normal and combined Zn +Se control groups.

There were significantly ($P<0.05$) lower value in MDA level of all the pre and post infected treated, when compared to the infected untreated control group.

4.4.2. Effects on Liver Antioxidants

The effect of daily interperitoneal administration of zinc and selenium on liver endogenous antioxidant enzymes in normal and *Trypanosoma brucei brucei* infected wistar albino rats is presented in Table 4.4. The result shows that, normal rats that were administered combined Zn +Se without infection had no significant ($P>0.05$) difference in liver antioxidant enzymes (SOD, CAT, and GPx) level compared to the normal control. There

were significantly ($P<0.05$) lower values of liver antioxidant enzymes in the infected untreated group compared to the normal and combined Zn +Se control groups.

Rats that were treated, pre and post infection shows significantly ($P<0.05$) lower value in SOD, compared to the normal and combined Zn +Se control groups. All the pre and post *Trypanosoma b. brucei* infected treated rat shows significantly ($P<0.05$) higher level in liver antioxidant enzymes compared to the infected untreated group.

Table 4.4: Effect of Pre and Post Infection Administration of Zinc and Selenium on Lipid Peroxidation and Endogenous Antioxidant Enzymes in *Trypanosoma brucei brucei* Infected Wistar Albino Rats in the Liver.

Group	MDA(mmol/g of tissue)	SOD(mmol/min/g of tissue)	CAT (moles of H_2O_2/min/g of tissue)	GPx (mU/mL)
NC	1.35 ± 0.33^{a}	2.18 ± 0.19^{e}	51.00 ± 1.26^{b}	50.00 ± 2.23^{bc}
N+ Zn +Se	1.36 ± 0.18^{a}	2.13 ± 0.16^{e}	52.17 ± 2.14^{b}	48.83 ± 1.94^{bc}
TC	1.95 ± 0.26^{c}	1.28 ± 0.46^{a}	42.33 ± 1.63^{a}	41.00 ± 4.38^{a}
PRE TI +Zn	1.65 ± 0.28^{b}	1.92 ± 0.15^{d}	52.00 ± 1.03^{bc}	48.33 ± 1.86^{bc}
PRE TI + Se	1.67 ± 0.29^{b}	1.87 ± 0.20^{bc}	52.00 ± 1.74^{bc}	47.59 ± 2.59^{bc}
PRE TI + Zn +Se	1.43 ± 0.38^{a}	2.00 ± 0.05^{cd}	54.67 ± 2.80^{c}	50.50 ± 1.23^{c}
POST TI + Zn	1.70 ± 0.33^{b}	1.77 ± 0.18^{c}	52.83 ± 3.06^{bc}	48.67 ± 2.73^{bc}
POST TI + Se	1.77 ± 0.23^{b}	1.53 ± 0.31^{b}	52.50 ± 3.08^{bc}	46.17 ± 3.06^{b}
POST TI + Zn + Se	1.44 ± 0.32^{a}	1.90 ± 0.21^{bcd}	51.17 ± 2.79^{bc}	48.00 ± 1.53^{bc}

Values are means ± SD of six replicate determinations. Values with different superscript down the column are significantly different ($P<0.05$).

NC: Normal Control rats, N + Zn +Se: Normal rats + Zinc + Selenium, TC: Trypanosomiasis Control, PRE TI + Zn: Pre-Treated Infected + Zinc, PRE TI + Se: Pre-Treated Infected + Selenium, PRE TI + Zn + Se: Pre-Treated Infected + Zinc + Selenium, POST TI + Zn: Post-Treated Infected + Zinc, POST TI + Se: Post-Treated Infected + Selenium, POST TI + Zn + Se: Post-Treated Infected +Zinc + Selenium. MDA: malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: glutathione peroxidase

4.4.3. Effects on the Kidney Lipid Peroxidation

Result of the study on the kidney lipid peroxidation as evidenced by the level of malondialdehyde (MDA) is presented in Table 4.5. Rats that were infected with *Trypanosoma b. brucei* untreated shows significantly ($P<0.05$) higher level of MDA compared to the normal and combined Zn +Se control groups.

There were also a significantly ($P<0.05$) higher level of MDA in all the pre and post infected treated compared to the normal and combined Zn +Se control groups.

On the other hand, all the pre and post infected treated groups shows significantly ($P<0.05$) lower value of MDA compared to the infected untreated control group.

4.4.4. Effects on the Kidney Antioxidants

Table 4.5 also presents the effect of pre and post-infection administration of zinc and selenium on kidney antioxidants enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities in normal and *T. b. brucei* infected albino rats. The results indicates that, there were significantly ($P<0.05$) lower values of kidney antioxidant enzymes (SOD, CAT and GPx) in the infected untreated compared to the normal and combined Zn +Se control groups.

The result also shows that rats that were treated pre and post infection, shows a significant ($P<0.05$) difference in SOD, no significant ($P>0.05$) difference in CAT and GPx activities, with the exception of the pre-treated infected group that were administered with Se where there were significantly ($P<0.05$) higher value of GPx compared to the normal and combined Zn +Se control groups.

Furthermore, the pre and post infected with *T. b. brucei* and treated shows significantly ($P<0.05$) higher level of SOD, CAT and GPx with the exception of post-treated infected administered with Zn, Se which were not significantly ($P>0.05$) higher compared to the infected untreated control group.

Table 4.5: Effect of Pre and Post Infection Administration of Zinc and Selenium on Lipid Peroxidation and Endogenous Antioxidant Enzymes in *Trypanosoma brucei brucei* Infected Wistar Albino Rats of the Kidney.

Group	MDA (mmol/g of tissue)	SOD(mmol/min/g of tissue)	CAT (moles of H_2O_2/min/g of tissue)	GP_X (mU/mL)
NC	1.40 ± 0.13^{a}	2.37 ± 0.30^{d}	47.50 ± 1.33^{b}	45.17 ± 1.17^{b}
N+ Zn +Se	1.30 ± 0.14^{a}	2.22 ± 0.21^{d}	48.00 ± 1.22^{b}	45.67 ± 2.80^{b}
TC	1.78 ± 0.23^{d}	1.63 ± 0.27^{a}	33.67 ± 3.93^{a}	38.50 ± 1.38^{a}
PRE TI +Zn	1.58 ± 0.17^{bc}	2.25 ± 0.24^{b}	46.83 ± 1.14^{b}	46.17 ± 1.94^{b}
PRE TI + Se	1.63 ± 0.25^{bc}	1.95 ± 0.24^{ab}	46.17 ± 3.06^{b}	48.83 ± 1.60^{c}
PRE TI + Zn +Se	1.45 ± 0.22^{b}	2.98 ± 0.27^{b}	47.60 ± 0.22^{b}	46.00 ± 2.37^{b}
POST TI + Zn	1.68 ± 0.19^{bc}	1.80 ± 0.28^{ab}	45.00 ± 3.22^{b}	45.15 ± 3.11^{b}
POST TI + Se	1.70 ± 0.23^{c}	1.75 ± 0.19^{ab}	44.25 ± 1.82^{b}	46.12 ± 2.81^{b}
POST TI +Zn+Se	1.46 ± 0.24^{b}	2.91 ± 0.32^{c}	46.08 ± 2.00^{b}	45.08 ± 3.28^{b}

Values are means ± SD of six replicate determinations. Values with different superscript down the column are significantly different ($P<0.05$).

NC: Normal Control rats, N + Zn +Se: Normal rats + Zinc + Selenium, TC: Trypanosomiasis Control, PRE TI + Zn: Pre-Treated Infected + Zinc, PRE TI + Se: Pre-Treated Infected + Selenium, PRE TI + Zn + Se: Pre-Treated Infected + Zinc + Selenium, POST TI + Zn: Post-Treated Infected + Zinc, POST TI + Se: Post-Treated Infected + Selenium, POST TI + Zn + Se: Post-Treated Infected +Zinc + Selenium. MDA: malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GPx: glutathione peroxidase

4.5. Effects of Pre and Post Infection Administration of Zinc and Selenium on Kidney Function Parameters in *Trypanosoma brucei brucei* Infected Wistar Albino Rats.

Figure 4.4 shows the mean creatinine and urea concentrations in the serum of pre and post-infection administration of zinc and selenium in normal and trypanosomiasis infected rats. The result indicates that, there were significantly ($P<0.05$) higher level in concentrations of creatinine and urea in *T. b. brucei* infected untreated compared to the normal and combined Zn +Se control groups.

In the pre-treated infected groups, there were significantly ($P<0.05$) higher levels of creatinine and urea concentrations compared to the normal and combined Zn +Se control groups.

There were also significantly ($P<0.05$) higher levels of creatinine and urea concentrations in the post-infected treated groups, with the exception of the group that were administered with combined Zn +Se where there was no significant ($P<0.05$) difference in creatinine level compared to the normal and combined Zn +Se control groups.

Significant ($P<0.05$) reduction in creatinine and urea concentrations in the pre and post infected treated groups compared to the infected untreated group was also recorded, with the exception of creatinine value of the post-infected treated group that were administered with Se which were not significantly ($P>0.05$) lower.

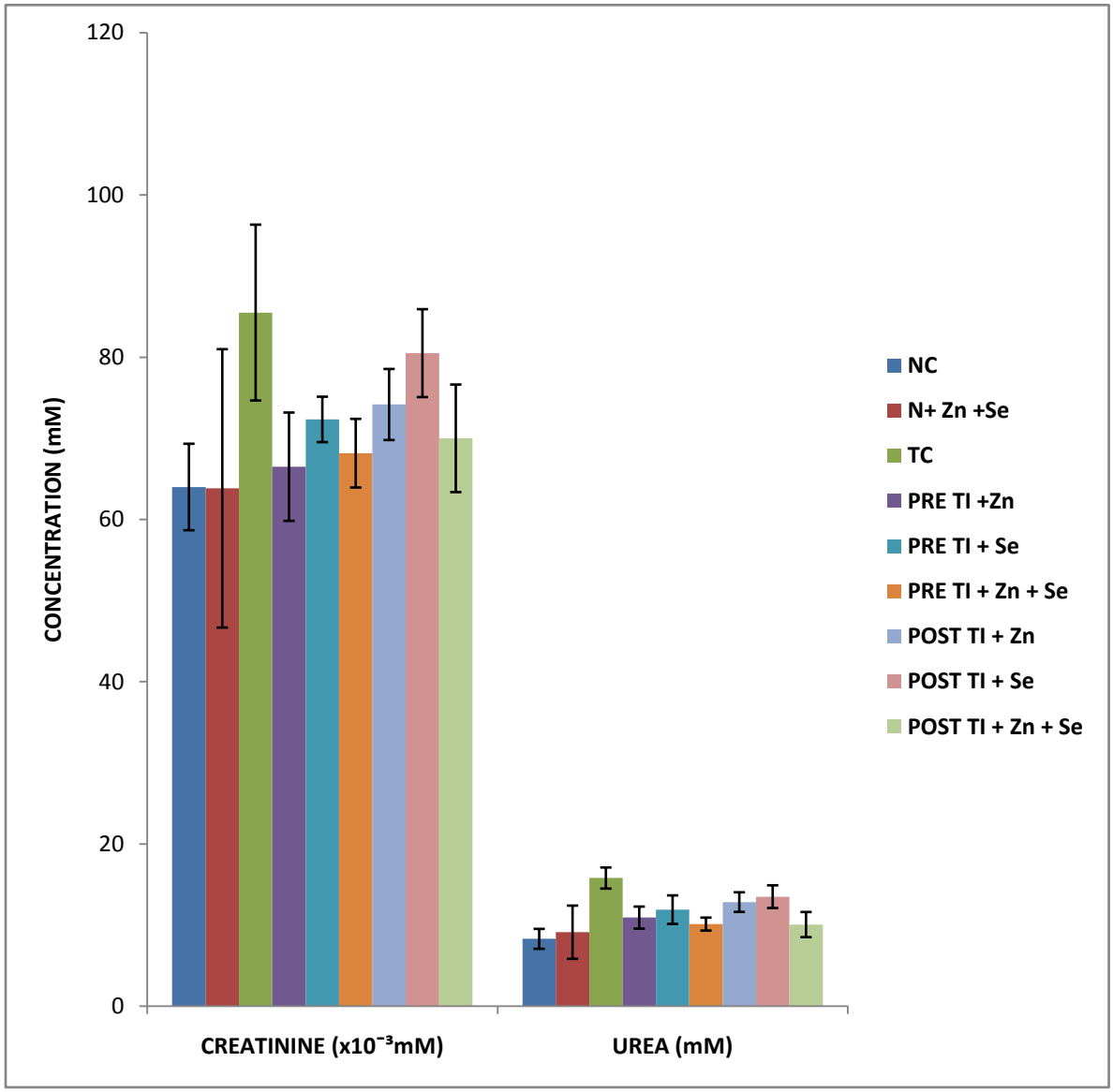


Figure 4.4: Effects of Pre and Post Infection Administration of Zinc and Selenium on Serum Creatinine and Urea in *Trypanosoma brucei brucei* Infected Wistar Albino Rats.

NC: Normal Control rats, N + Zn +Se: Normal rats + Zinc + Selenium, TC: Trypanosomiasis Control, PRE TI + Zn: Pre-Treated Infected + Zinc, PRE TI + Se: Pre-Treated Infected + Selenium, PRE TI + Zn + Se: Pre-Treated Infected + Zinc + Selenium, POST TI + Zn: Post-Treated Infected + Zinc, POST TI + Se: Post-Treated Infected + Selenium, POST TI + Zn + Se: Post-Treated Infected +Zinc + Selenium

4.6. Effect of Pre and Post infection Administration of Zinc and Selenium on Relative Organ Weight in *Trypanosoma brucei brucei* Infected Wistar Albino Rats.

Studies on the effect of pre and post infection administration of zinc and selenium in normal and *T. b. brucei* infected rats on mean relative organ weights such as kidney, liver, lungs, spleen and heart is presented in table 4.6. Result shows that infection with *T. b. brucei* untreated shows significantly ($P<0.05$) higher values of relative organ weights compared to the normal and combined Zn +Se control groups. The result also showed that there were significantly ($P<0.05$) lower values of relative organ weight in the pre and post infected treated rats when compared to the infected untreated group.

Table 4.6: Effect of Pre and Post Infection Administration of Zinc and Selenium on Relative Organ Weight in *Trypanosoma brucei brucei* Infection in Wistar Albino Rats.

Group	Kidney (% x 10^{-1})	Liver (%)	Lungs (%)	Spleen (%)	Heart (%)
NC	5.5±0.80[a]	2.71±0.49[a]	0.46±0.06[a]	0.38±0.84[a]	0.14±0.18[a]
N+ Zn +Se	4.9±0.87[a]	2.78±0.38[a]	0.45±0.15[a]	0.39±0.10[a]	0.19±0.04[a]
TC	7.3±0.15[b]	4.09±0.52[c]	1.08±0.04[c]	1.57±0.30[c]	1.06±0.26[d]
PRE TI +Zn	5.3±0.16[a]	3.07±0.40[b]	0.44±0.08[a]	0.96±0.19[b]	0.28±0.46[b]
PRE TI + Se	5.2±0.84[a]	3.03±0.50[b]	0.44±0.05[a]	0.90±0.30[b]	0.35±0.50[bc]
PRE TI + Zn + Se	4.2±0.91[a]	2.57±0.28[a]	0.46±0.11[a]	0.94±0.23[b]	0.33±0.10[bc]
POST TI + Zn	4.9±0.12[a]	2.81±0.35[a]	0.69±0.22[b]	0.98±0.17[b]	0.44±0.21[c]
POST TI + Se	5.3±0.66[a]	3.02±0.13[b]	0.70±0.29[b]	0.99±0.30[b]	0.41±0.12[c]
POST TI +Zn+Se	5.0±0.19[a]	2.90±0.52[a]	0.68±0.15[b]	0.91±0.31[b]	0.36±0.03[bc]

Values are means ± SD of six replicate determinations. Values with different superscript down the column are significantly different ($P<0.05$).

NC: Normal Control rats, N + Zn +Se: Normal rats + Zinc + Selenium, C: Trypanosomiasis Control, PRE TI + Zn: Pre-Treated Infected + Zinc, PRE TI + Se: Pre-Treated Infected + Selenium, PRE TI + Zn + Se: Pre-Treated Infected + Zinc + Selenium, POST TI + Zn: Post-Treated Infected + Zinc, POST TI + Se: Post-Treated Infected + Selenium, POST TI + Zn + Se: Post-Treated Infected +Zinc + Selenium

CHAPTER 5

5.0 DISCUSSION

When rats are injected with trypanosomes, trypanosomiasis is inflicted and thus provides an animal model of experimentally infected trypanosomiasis. The parasite *Trypanosoma brucei brucei* produced a very severe acute infection in the infected rats. The parasitaemia in infected untreated rats continued to rise steadily. Administration of zinc and selenium was able to significantly keep the parasitaemia of the pre and post infected treated rats lower than that of the infected untreated control. In the pre-treated infected groups, parasitaemia was detected on day 4 post infection; which could be attributed to the immune boosting function of the antioxidant micronutrients. The combination of zinc and selenium appear to suppress the early appearance of parasitaemia than the administration of zinc, and/or selenium singly.

Anemia is a constant feature of trypanosome infection, whose severity is correlated to the level of parasitaemia (Coustou *et al*., 2012), which is caused by; amongst other factors, oxidative damage to erythrocyte membrane components. Anemia, indicated by a significant drop in PCV of *T. b. brucei* infected untreated rats (Figure 4.2) is in agreement with earlier reports (Allam *et al*., 2011; Oduola *et al*., 2010; Faremi and Ekanem, 2011) in trypanosome infected animals. Anaemia in trypanosome infection has also been reported by several workers (Eghianruwa and Anika, 2011; Toma *et al*., 2008). Despite being considered a significant pathological feature of the disease, the etiology of this anaemia is complex and not completely elucidated. However many factors have been implicated in the pathogenesis of the anaemia; these factors may be attributed to the direct release of hemolytic factors into the animals blood by dead trypanosomes causing destruction of erythrocytes and

hence, reduction in PCV (Atawodi *et al*., 2011; Olukunle *et al*., 2011), existence of erythrophagocytosis in the spleen. It has also been reported that anaemia may be caused by erythrocyte injury caused by lashing action of trypanosome flagella, undulating pyrexia, platelet aggregation, toxins and metabolites from trypanosomes, lipid peroxidation and malnutrition (Mbaya *et al*., 2012). Severity of anaemia usually reflects the intensity and duration of parasitaemia, this corroborates with several reports by Ogunsanmi and Taiwo, (2001), Umar *et al*., (2007), and Saleh *et al*., (2009) who attributed acute anaemia in trypanosomiasis to rapidly increase in parasitaemia.

The administration of zinc and selenium showed a significantly higher level in all the hematological parameters (the mean values of RBC count, HB concentration, WBC, MCV, and MCH) in the pre and post-infected treated rats (Table 4.1). Results obtained here are in agreement with that obtained by Faremi and Ekanem (2011); who recorded a significant decrease in the erythrocytic count and blood indices in *T. brucei* infected rats. Eghianruwa and Anika (2011) and Ekanem and Yusuf (2008) had also reported increase in hematological parameters (PCV, HB and RBC). The observed increase in HB, WBC and RBC concentrations are probably as a result of reduced severity of the infection as a result of increased host action in the presence of zinc and selenium, as these will contribute to the development of phagocytes and antibodies against the recognizable antigens of parasite origin. The decreased level of RBC observed, suggested the occurrence of a hemolytic crisis in this period of the infection. *T. brucei* infection like other trypanosome infections precipitated increased red blood cell destruction which resulted in anemia and tissue damage (Takeet and Fagbemi, 2009).

WBC is involved in the defense mechanism and health. WBC was used as an index for immune function. Administration of zinc and selenium caused significantly ($P<0.05$) higher level in total WBC count of the pre and post-infected treated rats compared to the *T. brucei brucei* infected untreated and the normal control groups (Table 4.1)

The increase in the mean values of neutrophil and eosinophil in the infected untreated group observed in this study disagrees with the findings by Anosa and Isoun (1980), but agrees with Chaudhary and Iqbal (2000), who observed an increase in the levels of neutrophils in camels infected with *T. evansi*.

In the present study, the lymphocyte count lowered significantly in the infected untreated when compared to the pre and post-infected treated rats (Table 4.1). The persistent decrease in lymphocyte level agrees with observation by Chaudhary and Iqbal (2000), who reported a decrease in lymphocyte in camels, infected with *Trypanosoma evansi* but disagrees with the findings by Allam *et al*., (2011), who reported persistent increase in the mean lymphocyte count in gilts infected with *Trypanosoma brucei*.

Trypanosoma brucei infection, like other trypanosome infections may precipitate increased biochemical changes in the host in response to invading parasites and these changes in part could be responsible for infection-induced tissue damage. The biochemical indices monitored in the liver and kidneys are useful 'markers' for assessment of tissue damage. Analysis of serum enzymes have been shown to be a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue and are early warning signs for certain diseased conditions. There are many enzymes, found in the serum that did not originally originate from the serum. During tissue damage, some of

these enzymes from the liver cytosol find their way into the blood stream probably by leakage or changes in the permeability of liver membranes (Moss and Henderson, 1996).

Generally, hepatic injury is often associated with alterations in the serum and liver levels of some enzymes notably Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phospatase (ALP) (Arhoghro *et al*., 2014). The increase in serum alkaline phosphatase activity may indicate hepatic damage probably by the altered cell membrane permeability leading to the leakage of the enzymes from the tissues to the serum (Appidi *et al.*, 2009 ; Akanji and Yakubu, 2000). Alanine and aspartate aminotransferase are considered to be sensitive indicators of hepatocellular damage and within limit can provide a quantitative evaluation of the degree of damage to the liver (Al-Habori *et al.*, 2002). Hepatic damage can affect the metabolic processes in the body due to the role of liver in general metabolism. Hepatic cells appear to participate in a variety of enzymatic metabolic activities. Enzymes are necessary for normal cellular metabolism including that of the liver (Rajamanickam and Muthuswamy, 2008). Infection with *T. brucei brucei* damaged the hepatic cells leading to a significant increase in serum levels of AST, ALT, and ALP respectively (Table 4.2). The significantly ($P<0.05$) higher levels of serum AST, ALT, and ALP activities were observed in infected untreated rats compared to the pre and post-infected treated and control groups. These results are in agreement with previous studies where ALT was elevated in *Trypanosoma evansi* infected (Sazmand *et al*., 2011) and *T. brucei brucei* infected (Yusuf *et al*., 2012) animals. Several other studies have also reported elevated serum AST, ALT, and ALP (Abd El-Baky and Salem, 2011; Allam *et al*., 2011; Oluwatosin *et al*., 2013 ; Umar *et al*., 2007). Since these enzymes are the major liver marker enzymes; the elevation of these enzymes is usually an indication of liver damage,

haemolytic conditions or partly to cellular damage caused by lysis or destruction of the trypanosomes (Yusuf *et al*., 2012). The significant decrease in the activities of the liver marker enzymes in all the pre and post-infected treated rats could be attributed to the reduction in hepatic granuloma size and fibrosis as well as absence of necrotic hepatic tissue in the pre and post-infected treated rats. Apparently it appears that the membrane damage seems to be the prime culprit for the marked increase in the serum marker enzymes, AST, ALT, and ALP. The pre-treated infected rats were better at reducing hepatic damage than the post-infected treated rats. Serum albumin and total protein are some of the markers of liver dysfunction while albumin transports bilirubin and other substances in blood (Vasudevan and Sreekumari, 2007).

Serum levels of total protein (TP), total bilirubin (TB), indirect bilirubin (IB) and direct bilirubin (DB) are indices used to assess liver function as well as disease progression (Saad *et al.*, 2006). Serum levels of total protein and albumin were reduced significantly ($P<0.05$) in the infected untreated as compared to normal control groups (Table 4.3). However administration of zinc and selenium to the pre and post-infected rats resulted in elevation of total protein and albumin levels when compared to the infected untreated rats. The increased synthesis of protein occurs at the expense of muscle protein catabolism and loss in body weight.

The gradual decrease in the mean values of serum total proteins, observed in the infected untreated rats during this study, agrees with previous findings (Biryomumaisho *et al*., 2003; Katunguka-Rwakishaya, 1996), but contradicts observations made in sheep infected with *T. brucei* by Taiwo *et al*., (2003), who observed no change in levels of total plasma proteins

from pre-infected values at the initial stage of the infection, but in the later stage the levels increased significantly above pre-infection levels.

Albumin is synthesized in the liver; therefore decrease in albumin concentration may be attributed to the damage in the liver where there could be less synthesis of albumin. The result obtained here agrees with that of Ogunsanmi *et al*., (1994) who studied the serum biochemical changes in West African dwarf sheep experimentally infected with *T. brucei*. They found that the serum albumin values were markedly decreased. The findings suggest that there might be a hepatic and/or renal malfunction. Similar observations were noticed by Arora and Pathak, (1995) and Yusuf *et al*., (2012). The cause of the decrease in albumin is difficult to elucidate. Albumin is a negative acute phase protein during trypanosomiasis (Karori *et al*., 2008). Its decrease could result from reduced synthesis in the liver as part of the acute phase response, loss through the kidney and intestine or increased utilization by the trypanosomes as a nutrient, since they require it for optimal survival (Coopens *et al*., 1987).

Bilirubin is the main bile pigment that is formed from the breakdown of heme in the red blood cells. In addition, it is transported to the liver where it is secreted by the liver into the bile. As such interference with the normal liver functions affects its rate of conjugation or excretion. Thus a high level of bilirubin is used as an index for liver function and bile excretion status (Usha *et al*., 2008). The present results showed significantly ($P<0.05$) higher level of total bilirubin in *T. brucei* infected untreated rats. The high level of total bilirubin in infected untreated rats in this experiment supports earlier observations in several trypanosome-infected animals (Adeyemi *et al*., 2012; Boniface *et al*., 2011; Ezeokonkwo *et al*., 2012). The bilirubin formed from breakdown of red blood cells in the

reticulo endothelial cells are transported in plasma bound to albumin (Vasudevan, and Sreekumari, 2007), so the increase in bilirubin is suggestive of haemolytic anemia which may be due to the activity of proliferating parasites. It could also be associated to the inability of the liver to conjugate bilirubin (Adeyemi *et al*., 2012). The liver detoxifies harmful substances, secretes bile into the intestine, synthesizes and stores up important material, hence, it is common in clinical practice to screen for liver disease, monitor the progression of a known disease and monitor the effect of potentially hepatotoxic drugs (Kapoor, 2011).

It is well recognized that zinc is an essential trace element, which influences cellular growth and affect the development and integrity of the immune system (Saker, 2006; Gonçalves-Neto *et al*., 2011). An effect of the mineral was observed in the current study. However the best results were observed in the pre-treated infected groups. Oral zinc supplementation leads to an effective host immune response by up-modulating it, thus contributing to a reduction of blood parasites and the harmful pathogenic effects in experimental Chagas' disease (Brazão *et al*., 2008). The same group found that zinc supplementation could be an efficient therapeutic strategy in the control of *T. evansi* in rats due to increased IFN-gamma levels (Brazão *et al*., 2008). Similar results were observed in this study; however, our administration was intraperitoneal rather than oral. Another study using zinc sulphate ($ZnSO_4$) supplementation (20mg/kg/day) during pregnancy, demonstrated a reduction in placental parasitism by *T. cruzi* which may account for reduced impairment in the fetuses (Gonçalves-Neto *et al*., 2011). We believe that the treatment with zinc used in this experiment directly assisted the immune response to *T. b. brucei*, as in *T. cruzi* infection (Brazão *et al*., 2008; Gonçalves-Neto *et al*., 2011).

Creatinine and urea are indices for assessment of kidney function. The kidney helps in maintaining homeostasis of the body by reabsorbing important material and excreting waste products. Creatinine is a waste product formed in muscle by creatinine metabolism. Creatinine is synthesized in the liver, passes into the circulation and is taken up almost entirely by skeletal muscle. Its retention in the blood is evidence of kidney impairment.

Urea is the main end product of protein catabolism. Amino acid deamination takes place in the liver, which is also the site of urea cycle, where ammonia is converted into urea and excreted through urine. It represents 90% of the total urinary nitrogen excretion. Urea varies directly with protein intake and inversely with the rate of excretion. Renal diseases which diminish the glomerular filtration lead to urea retention. Infection with *T. brucei brucei* caused nephrotoxicity as indicated by significantly ($P<0.05$) higher levels in serum urea and creatinine concentration (Figure 4.4). These results were in agreement with earlier findings (Allam *et al*., 2011; Ezeokonkwo *et al*., 2012; Umar *et al*., 2010). But administration of zinc and selenium significantly ($P<0.05$) lowered urea and creatinine levels in the pre and post-infected treated rats. The significant decrease in urea and creatinine levels especially in the pre-treated infected groups indicated the ability of the micronutrients to provide some degree of protection to the kidneys during the course of the disease.

Oxidative stress describes an inbalance between the pro-oxidant load and the antioxidant defense system in the body in favor of the former, leading to a potential damage (Sies *et al*., 2005). In the state of oxidative stress, there is a characteristic depression of the free radical defense mechanism including alterations in the activities of antioxidant enzymes, essential polyunsaturated fatty acids, vitamins and minerals elements are compromised,

exposing the body's own antioxidant defense system to damage by reactive oxygen species (ROS) (Omoregie and Osagie, 2011). Malondialdehyde (MDA) is the major oxidation product of the peroxidation of polyunsaturated fatty acids and MDA formed is quantified by the reaction with TBARS, thus making an increased MDA level, an important index to lipid peroxidation. Biological system protects itself against the damaging effects of free radicals and activated species by the actions of free radical scavengers and chain terminator enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) system (Kurata *et al*., 1993). Catalase on the other hand, is an enzymatic antioxidant widely distributed in all animal tissues including the red blood cell and liver. Catalase decomposes hydrogen peroxide (H_2O_2) and helps protect the tissues from highly reactive hydroxyl radicals. SOD, another antioxidant enzyme, removes superoxide radical by converting it to H_2O_2 (Krishnaraju *et al*., 2009). Glutathione protects the cellular system against the toxic effects of lipid peroxidation.

In the present study, results shown in (Table 4.4) and (Table 4.5) indicated that MDA levels were significantly ($P<0.05$) higher in liver and kidney tissue homogenates of infected untreated rats compared to the pre and post-infected treated and normal control groups. The decreased MDA levels in liver and kidney tissue homogenates following administration of zinc and selenium confirms their antioxidant properties (DiSilvestro, 2000). The micronutrients may have also prevented or delayed the on-set of oxidative stress which arises as a result of imbalance between radical-generating and radical-scavenging activity. Ogunsanmi and Taiwo (2001) have earlier demonstrated that oxidative stress plays an important etiologic role in the pathogenesis of trypanosomiasis. Similarly, it

has also been shown that infections with *T. brucei* group of parasites may alter the host's antioxidant defense against free radicals (Igbokwe *et al*., 1996; Omer *et al*., 2007).

Increase in MDA concentrations have been related to the amount of stress and are well correlated with lipid membrane damage and deterioration of membrane integrity. Increased generation of MDA in plasma, tissues and erythrocytes was also reported in murine models and humans infected with *Trypanosoma cruzi* (Malvezi *et al*., 2004). Aleksandro *et al*., (2009) reported that plasma MDA levels were significantly higher in *Trypanosoma evansi* infected than in the uninfected group, and in animals with acute (day 21) and chronic (day 49) trypanosomiasis, MDA levels were found to be proportional to the time of infection.

Intraperitonial administration of zinc and selenium in the pre and post-infected rats resulted in increased levels of GPx in liver and kidney tissue homogenate (Table 4.4) and (Table 4.5), when compared with the infected untreated rats. Decreased level of GPx observed in the liver and kidney of infected untreated rats is an indication of an increased utilization due to oxidative stress. This may be because their antioxidant defense system, which included GPx, was mobilized to fight the presence of the parasites. This is in agreement with previous studies that showed a decrease in erythrocytic and hepatic glutathione concentrations in rats infected with *T. brucei* (Ameh, 1984). Significant decrease in glutathione peroxidase concentrations in the liver and kidney tissue homogenate of infected untreated rats compared to the pre and post-infected treated groups might be attributed not only to the oxidation of GSH to GSSH by activated oxygen produced as a result of trypanosome infection (Igbokwe *et al*., 1996; Ogunsanmi and Taiwo, 2001), but also to high increase in the glutathione peroxidase activity (Omer *et al*., 2007) since the reaction catalyzed by this enzyme consumes GSH. Similarly it has been reported that depletion of

endogenous glutathione antioxidant may be a significant factor in the pathogenesis of *T. congolense* infection but upon administration of exogenous vitamin C to infected animals prevented these disease-induced decreases in glutathione and ascorbic acid (Umar *et al*., 2010).

Catalase (CAT) is a peroxisomal maker enzyme found in blood, bone marrow, mucous membrane, kidney and liver. It functions assumed to be destruction of hydrogen peroxide. The result showed increase in liver and kidney CAT activities in all the pre and post infection treated compared to the infected untreated (Table 4.4) and (Table 4.5). Oluwatosin *et al*., (2013) noticed that the reduction in catalase activity could be attributed to its utilization in scavenging the free radicals overload which is generated during trypanosome infection.

Superoxide dismutase (SOD) is also an endogenous antioxidant enzyme that protects blood cells from oxidative stress and damage. SOD activities decreased in liver and kidney in the infected untreated control compared to the pre and post-infected treated groups. A decrease in SOD activity recorded in this study could have resulted from increased removal of superoxide anions. Omer *et al*., (2007), reported decreased of SOD activity in *T. evansi* infected rats. However, others reported increased SOD activity (Ogunsanmi and Taiwo, 2001; Yusuf *et al*., 2012). Ataley *et al*., (2000) also reported that under condition of oxidative stress, activities of antioxidant enzymes such as SOD, CAT and glutathione peroxidase increases.

The results on organ/body weight ratio showed that *Trypanosoma brucei brucei* infection had caused significant ($P<0.05$) inflammation of liver, kidney, lungs and spleen weight

(Table 4.6) in the infected untreated rats compared with the pre and post-infected treated and normal control rats. The enlargement of the organs (heart, liver and spleen) otherwise known as cardiomegaly, hepatomegaly and splenomegaly respectively as observed in the study, is presumably due to membrane damage caused by the large amount of free radicals and other oxidative species being generated and the concomitant reduction in systemic antioxidant reserves. This agrees with the findings of Morrison *et al*., (1978) who reported hepatomegaly and splenomegaly in trypanosomiasis. Umar *et al*., (2007) and Yusuf *et al*., (2012) had also reported hepatomegaly in trypanosomal infection. The increase in size of liver and spleen is caused by the activation of the immune system during trypanosome infection.

CHAPTER 6

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6. 1 Summary

i) Results of the present study shows that, the pre-treated infected groups, administered with zinc and selenium had significant ($P<0.05$) effect on the rats especially the pre-treated infected group that was administered combined Zn + Se than the post-infected treated which could be attributed to the immune boosting function of the micronutrients.

ii) Infection with *T. b. brucei* in the midst of daily administration of zinc and selenium caused significantly ($P<0.05$) higher values of RBC, PCV, HB, MCV, MCH and WBC count. However, there was a significant increase in neutrophil, eosinophil and significant decrease in lymphocyte counts in infected untreated group compared to the pre and post-infected treated and normal control rats.

iii) The administration of zinc and selenium caused significantly ($P<0.05$) higher activities of endogenous antioxidant enzymes (CAT, SOD, and GPx) in the liver and kidneys of the pre and post infected treated rats.

iv) The mineral elements also possessed some hepatocurative/protective effect as evident by the significant ($P<0.05$) decrease in serum liver enzymes (ALP, AST, ALT) and albumin in the pre and post-infected treated rats.

6.2 Conclusion

This study shows that administration of zinc and selenium prevented depletion of endogenous antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) and also reduced the rate of development of anaemia and degenerative changes in the liver and kidney of rats. Therefore, understanding the role of these micronutrients in the pathogenesis of trypanosomiasis may help in designing nutritional support and control programs as a strategy to combating the effect of the disease in areas where it is endemic.

6.3 Recommendations

i) Investigate the effect of antioxidant deficiency in chronic type of infections which may probably clarify the extent to which antioxidant deficiency adds to the severity of the disease.

ii) Examination of other organs such as the brain, where trypanocides hardly pass through the blood-brain barrier and lymph nodes.

iii) Other species of the parasite may be used for comparative study.

iv) Study should also be carried out with mixed infection.

v) Investigation should be carried out using zinc and selenium plus established trypanocides to evaluate the usefulness of such combination in the treatment of infection.

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APPENDICES

Appendices 1: Effects of Pre and Post Infection Administration of Zinc and Selenium on Parasitaemia Level in *Trypanosoma brucei brucei* Infected Wistar Albino Rats.

GROUP (N=6)	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
NC	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
N+Zn+Se	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
TC	2.73 ± 1.84^{c}	16.58 ± 7.02^{d}	38.33 ± 19.60^{d}	67.50 ± 9.31^{d}	73.00 ± 9.60^{e}	75.50 ± 8.41^{e}	89.33 ± 9.83^{d}	126.66 ± 12.1^{g}
PRE TI+Zn	0.00 ± 0.00^{a}	2.57 ± 1.17^{ab}	4.50 ± 1.39^{ab}	9.83 ± 1.81^{b}	12.50 ± 2.89^{b}	20.50 ± 4.18^{b}	42.83 ± 13.23^{b}	76.00 ± 0.00^{c}
PRE TI+Se	0.00 ± 0.00^{a}	2.73 ± 1.84^{ab}	4.52 ± 1.66^{ab}	8.92 ± 1.39^{b}	16.25 ± 4.36^{b}	38.33 ± 16.60^{c}	64.83 ± 8.66^{c}	70.33 ± 8.78^{b}
PRE TI + Zn + Se	0.00 ± 0.00^{a}	1.87 ± 0.71^{ab}	2.73 ± 1.84^{ab}	6.35 ± 2.89^{ab}	9.67 ± 2.00^{b}	18.33 ± 3.14^{b}	39.77 ± 13.87^{b}	77.33 ± 7.17^{c}
POST TI + Zn	1.33 ± 0.19^{b}	7.35 ± 0.19^{c}	14.83 ± 4.30^{c}	24.33 ± 11.06^{c}	40.00 ± 0.00^{c}	53.50 ± 8.00^{d}	65.67 ± 15.67^{c}	88.17 ± 17.23^{e}
POST TI + Se	1.42 ± 0.33^{b}	4.00 ± 0.00^{b}	$9.96\pm1.83b^{c}$	30.67 ± 7.45^{c}	53.67 ± 15.6^{d}	71.67 ± 9.24^{e}	67.67 ± 13.22^{c}	100.00 ± 0.00^{f}
POST TI + Zn + Se	1.50 ± 0.52^{b}	3.30 ± 1.48^{b}	15.00 ± 5.62^{c}	13.33 ± 6.02^{b}	13.33 ± 6.02^{b}	25.33 ± 7.87^{b}	51.00 ± 13.16^{b}	81.83 ± 8.98^{d}

Values are means ± SD. Values with different superscript down the column are significantly different ($P<0.05$)

NC: Normal Control rats, N + Zn +Se: Normal rats + Zinc + Selenium,TC: Trypanosomiasis Control, PRE TI + Zn: Pre-Treated Infected + zinc, PRE TI + Se: Pre-Treated Infected + Selenium, PRE TI + Zn + Se: Pre-Treated Infected + Zinc + Selenium, POST TI + Zn: Post-Treated Infected + Zinc, POST TI + Se: Post-Treated Infected + Selenium, POST TI + Zn + Se: Post-Treated Infected +Zinc + Selenium

Appendices 2: Effect of Pre and Post Infection administration of Zinc and Selenium on Packed Cell Volume in *Trypanosoma brucei brucei* Infected Wistar Albino Rats.

Group (n=6)	Day 0	Day 1	Day 6	Day 11
NC	47.67 ± 4.18^{a}	46.17 ± 3.60^{b}	48.67 ± 4.30^{c}	48.83 ± 4.36^{c}
N+ Zn +Se	46.83 ± 7.28^{a}	47.67 ± 7.40^{b}	48.83 ± 5.98^{c}	49.00 ± 6.63^{c}
TC	44.33 ± 3.01^{a}	40.17 ± 2.04^{a}	33.17 ± 2.79^{a}	33.17 ± 3.19^{a}
PRE TI +Zn	45.00 ± 3.74^{a}	46.83 ± 3.55^{b}	49.67 ± 3.78^{c}	43.50 ± 3.73^{c}
PRE TI + Se	46.83 ± 5.74^{a}	48.00 ± 4.20^{b}	40.50 ± 4.37^{b}	38.00 ± 3.00^{b}
PRE TI + Zn + Se	47.67 ± 4.80^{a}	48.83 ± 4.36^{b}	45.00 ± 3.74^{bc}	40.17 ± 2.93^{bc}
POST TI + Zn	46.67 ± 4.68^{a}	45.50 ± 4.32^{b}	44.83 ± 2.40^{c}	44.33 ± 1.51^{bc}
POST TI + Se	45.67 ± 3.50^{a}	40.00 ± 4.24^{a}	41.00 ± 3.90^{bc}	42.00 ± 3.69^{b}
POST TI + Zn +Se	43.83 ± 4.67^{a}	39.50 ± 3.62^{a}	39.83 ± 4.12^{b}	38.67 ± 3.08^{b}

Values are means ± SD. Values with different superscript down the column are significantly different ($P<0.05$)

NC: Normal Control rats, N + Zn +Se: Normal rats + Zinc + Selenium, TC: Trypanosomiasis Control, PRE TI + Zn: Pre-Treated Infected + Zinc, PRE TI + Se: Pre-Treated Infected + Selenium, PRE TI + Zn + Se: Pre-Treated Infected + Zinc + Selenium, POST TI + Zn: Post-Treated Infected + Zinc, POST TI + Se: Post-Treated Infected + Selenium, POST TI + Zn + Se: Post-Treated Infected +Zinc + Selenium

Appendices 3: Effect of Pre and Post Infection administration of Zinc and Selenium on Haemoglobin Concentration in *Trypanosoma brucei brucei* Infected Wistar Albino Rats.

Group (n=6)	Day 0	Day 1	Day 6	Day 11
NC	15.89 ± 1.39^{a}	15.39 ± 1.20^{b}	16.22 ± 1.50^{c}	16.28 ± 1.45^{c}
N+ Zn +Se	15.61 ± 2.43^{a}	15.89 ± 2.47^{b}	16.28 ± 1.99^{c}	16.33 ± 2.21^{c}
TC	14.78 ± 1.01^{a}	13.39 ± 0.68^{a}	11.06 ± 0.93^{a}	11.06 ± 1.06^{a}
PRE TI +Zn	15.00 ± 1.25^{a}	15.61 ± 1.18^{b}	16.56 ± 1.23^{c}	14.50 ± 1.24^{c}
PRE TI + Se	15.61 ± 1.91^{a}	16.00 ± 1.40^{b}	13.50 ± 1.46^{b}	12.66 ± 0.98^{b}
PRE TI + Zn + Se	15.89 ± 1.60^{a}	16.28 ± 1.45^{b}	15.00 ± 1.25^{bc}	13.39 ± 0.98^{bc}
POST TI + Zn	15.56 ± 1.56^{a}	15.17 ± 1.44^{b}	14.94 ± 0.80^{bc}	14.78 ± 0.50^{c}
POST TI + Se	15.22 ± 1.17^{a}	13.33 ± 1.41^{a}	13.67 ± 1.30^{b}	14.00 ± 1.23^{bc}
POST TI + Zn+Se	14.61 ± 1.56^{a}	13.17 ± 1.21^{a}	13.28 ± 1.37^{b}	12.89 ± 1.03^{b}

Values are means ± SD. Values with different superscript down the column are significantly different ($P<0.05$)

NC: Normal Control rats, N + Zn +Se: Normal rats + Zinc + Selenium, TC: Trypanosomiasis Control, PRE TI + Zn: Pre-Treated Infected + Zinc, PRE TI + Se: Pre-Treated Infected + Selenium, PRE TI + Zn + Se: Pre-Treated Infected + Zinc + Selenium, POST TI + Zn: Post-Treated Infected + Zinc, POST TI + Se: Post-Treated Infected + Selenium, POST TI + Zn + Se: Post-Treated Infected +Zinc + Selenium

Appendices 4: Effects of Pre and Post Infection Administration of Zinc and Selenium on Serum Creatinine and Urea in *Trypanosoma brucei brucei* Infected Wistar Albino Rats.

Group (n=6)	Creatinine ($x10^{-3}$ mM)	Urea (mM)
NC	64.00 ± 5.33^{a}	8.30 ± 1.23^{a}
N+ Zn +Se	63.83 ± 17.16^{a}	9.12 ± 3.28^{a}
TC	85.50 ± 10.84^{d}	15.80 ± 1.30^{e}
PRE TI +Zn	66.50 ± 4.23^{b}	10.92 ± 1.36^{c}
PRE TI + Se	72.33 ± 2.80^{bc}	11.90 ± 1.76^{bcd}
PRE TI + Zn + Se	68.17 ± 4.23^{b}	10.12 ± 0.8^{b}
POST TI + Zn	74.83 ± 4.38^{c}	12.83 ± 1.22^{cd}
POST TI + Se	80.50 ± 5.42^{cd}	13.50 ± 1.40^{d}
POST TI + Zn + Se	70.00 ± 6.63^{abc}	10.06 ± 1.55^{bcd}

Values are means ± SD. Values with different superscript down the column are significantly different ($P<0.05$)

NC: Normal Control rats, N + Zn +Se: Normal rats + Zinc + Selenium, TC: Trypanosomiasis Control, PRE TI + Zn: Pre-Treated Infected + Zinc, PRE TI + Se: Pre-Treated Infected + Selenium, PRE TI + Zn + Se: Pre-Treated Infected + Zinc + Selenium, POST TI + Zn: Post-Treated Infected + Zinc, POST TI + Se: Post-Treated Infected + Selenium, POST TI + Zn + Se: Post-Treated Infected +Zinc + Selenium

Appendices 5: Experimental Design.