PUTATIVE EFFECTS OF CYANOCOBALAMIN IN THE REGULATION OF INFLAMMATORY RESPONSES, OXIDATIVE STRESS AND PATHOPHYSIOLOGICAL EVENTS DURING SEVERE STAGE OF HUMAN AFRICAN TRYPANOSOMIASIS

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DECLARATION

This thesis is my original work and has not been presented in any other institution for a degree award or other qualification.

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DEDICATION

This work is dedicated to my mother Mrs. Jenipha Okombo Oula for her support and love for education even though she never acquired any form of education, and to my brother Mr. Tobias Oula for his unconditional support and wise counsel. Special dedication to my wife Mrs. Millicent Akoth, daughter Annastacia Shanaya and son Nyakinda Francis for their support as well as allowing me to take their precious time to do this work which was quite involving, for this I say thank you.

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ABBREVIATIONS / ACRONYMS

aaMϕ: alternatively activated macrophages

ALT: Alanine aminotransferase

APOA1: Apolipoprotein A1

APOL1: Apolipoprotein L1

AST: Aspartate aminotransferase

BBB: Blood brain barrier

caMϕ: Classically activated macrophages

CNS: Central nervous system

CSF: Cerebrospinal fluid

FeSODs: Iron superoxide dismutases

GSH: Reduced glutathione

H_2O_2: Hydrogen peroxide

HAT: Human African trypanosomiasis

Hb: Haemoglobin

Hcy: Homocysteine

HRP: Haptoglobin related protein

IFN: Interferon

IL: Interleukin

iNOS: Inducible nitric oxide synthase

LAMP: Loop mediated isothermal amplification

MDA: Malondialdehyde

MGE: Mobile genetic element

mHCT: mini-anion-exchange centrifugation technique

MPV: Mean platelets volume

MyD88: Myeloid differentiation primary response 88

NADPH: Nicotinamide adenine dinucleotide phosphate

NETs: Neutrophil extracellular traps

NK: Natural killer cells

NO: Nitric oxide

$O_2^{\cdot -}$: Superoxide radical anion

$ONOO^-$: Peroxynitrite

PAMPs: Pathogen associated molecular patterns

PCR: Polymerase Chain Reaction

PCV: Packed cell volume

PRR: Pattern recognition receptors

PTRE: Post-treatment reactive encephalopathy

RAPD: Random amplified polymorphism DNA

RBC: Rred blood cell

RDTs: Rapid diagnostic tests

RMCBS: Rapid Murine Coma Behavioral Score

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

SOD: Superoxide dismutase

SRA: Serum resistance antigen

T2D: Type 2 diabetes

TGF-β: Transforming growth factor- beta

TH: T- helper cells

TLF: Trypanosome lytic factors

TLR: Toll like receptor

TLTF: Trypanosome-released triggering factor

TNF: Tumors necrosis factor

VSG: Variant Surface Glycoprotein

WBC: White blood cell

WHO: World Health Organization

ABSTRACT

Human African Trypanosomiasis (HAT) is a neglected tropical disease caused by T. b. rhodesiense and T. b. gambiense, with tsetse fly (Glossina spp) being the known vector. T. b. rhodesiense causes acute form of HAT in Africa. The severe late stage of the disease is characterized by infiltration of the brain through the blood brain barrier (BBB) as well as overwhelming inflammation and oxidative stress. Treatment of HAT is reliant on the stage of the disease and melarsoprol remains the drug of choice for treatment of late-stage HAT despite the fact that it causes post-treatment reactive encephalopathy (PTRE). It therefore implies that, new treatment strategies that control the breach of the BBB as well as inflammatory reactions and oxidative stress may benefit those on treatment. This study utilized cyanocobalamin (vitamin B12), a well characterized anti-inflammatory and anti-oxidant molecule to determine its effects on T. b. rhodesiense-driven deleterious events. Therefore, the objective was to determine the effects of cyanocobalamin in the regulation of immune response during T. b. rhodesiense infection. Mice were randomly assigned into four groups each containing 8 mice; with group one being the control. Group two was infected with 5.0 x 104 KETRI 2537 T.b.rhodesiense; group three was supplemented with 8mg/kg of vitamin B12 for two weeks before infection. For group four, administration of vitamin B12 was started 4 days post infection (dpi). The general health of the mice was assessed using rapid murine coma and behavior scale (RMCBS), while parasiteamia was determined microscopically. At 42 dpi, the mice were sacrificed to obtain blood, tissues and organs for various analyses. The statistical analysis was done by GraphPad Prism software package. One-way ANOVA was used to compare the treatment groups with controls, Tukey's post-hoc test for internal comparisons and Log-rank (Mantel-Cox) test for survival analysis. The results were given as a mean ±SEM with the level of significance set at $P<0.05$. The results showed that vitamin B12 enhanced the survival rate of T.b.rhodesiense infected mice independent of parasitemia of antilog 8.7 organism/ml and prevented T.b.rhodesiense-induced disruption of the BBB. Notably, T.b.rhodesiense-induced hematological alteration leading to microcytic hypochromic anemia and leukocytosis was nullified in mice administered with vitamin B12. T.b.rhodesiens-induced dyslipidemia was reversed by vitamin B12. T.b.rhodesiense-induced elevation of the liver alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, urea, uric acid and creatinine for kidney damage markers were attenuated by vitamin B12. Vitamin B12 blocked T.b.rhodesiense-driven rise in pro-inflammatory cytokines (TNF-α and IFN-γ), nitric oxide (NO) and malonaldehyde (MDA). T.b.rhodesiense-induced depletion of glutathione (GSH) levels were attenuated in the presence of vitamin B12 in the brain, spleen and liver tissues; a clear indication of the anti-oxidant activity of vitamin B12. The histological analysis of the brain and liver confirmed T.b.rhodesiense-driven damage of these vital organs; which was ameliorated by vitamin B12. In conclusion, treatment with vitamin B12 potentially protects against various pathological events associated with severe late-stage HAT and presents a great opportunity for further scrutiny to develop an adjunct therapy for severe late-stage HAT.

CHAPTER ONE

INTRODUCTION

1.1: Background information

Human African Trypanosomiasis (HAT) commonly referred to as "sleeping sickness" is one of the neglected tropical diseases caused by extracellular flagellated protozoan blood parasites *Trypanosoma brucei gambiense* (*T. b. gambiense*) and *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) subspecies. Both forms are domiciled in the tropical regions of Africa where the tsetse fly of the Glossina species is the known vector involved in sleeping sickness (Jamonneau *et al*., 2012; Gaillot *et al*., 2017). The *T. b. gambiense* is found in West and Central Africa, where it causes the chronic form of HAT, that can last for several months to years before causing death in the mammalian host, while *T. b. rhodesiense* is common in Eastern and Southern Africa and is responsible for the acute form of HAT and can cause mammalian death within weeks to few months of infection (Kato *et al*., 2015; Buscher *et al*., 2017).

In both *T. b. gambiense* and *T. b. rhodesiense* forms of HAT, the mammalians are the main reservoirs and human infections are incidental and characterized by a quick and critical development of the ailment. Failure or delayed treatment of sleeping sickness may lead to the increased morbidity and mortalities among the mammalian hosts (Franco *et al*., 2014). Millions of people residing in rural areas with high tsetse fly infestation are still threatened by HAT in about 36 countries in sub-Saharan Africa, putting approximately 55 million people living in these regions at risk. The reported incidences of chronic form of HAT has dropped by 98% (from 27 862 to 565) between 1999 and 2020, with majority of these incidences reported in the

Democratic Republic of Congo, while the incidences of the acute form of HAT fell by 84% (from 619 to 98) during the same period (WHO, 2021). With regards to the main contributor to the global parasitic disease burden, large board of evidence has placed HAT in the third position after malaria and schistosomiasis (Cattand *et al*., 2001; Franco *et al*., 2014). Therefore, HAT still remains a disease of great public health concern in sub-Saharan Africa.

Naturally, the lifecycle of *T. b. gambiense* and *T. b. rhodesiense* starts when the hematophagous tsetse fly (Glossina spp) inoculates the metacyclic form of trypomastigotes into the vertebrate skin when taking a blood meal (Aksoy *et al*., 2014). The metacyclic forms of parasites then differentiate through binary fission into a long-slender form of trypomastigotes that are proliferative and able to establish infections as they travel to the lymph nodes through afferent lymphatic vessels before they are systemically disseminated in the bloodstream from which they actively colonize multiple tissues of the vertebrate host (Alfituri *et al*., 2019; CDC, 2016).

HAT is known to progress in two stages: The heamolymphatic stage (early-stage HAT), is usually characterized by the growth and multiplication of the trypanosomes in blood, subcutaneous tissues and lymph leading to high parasitaemia levels. The meningo-encephalytic stage (severe late-stage HAT) is manifested when the trypanosomes cross the blood brain barrier (BBB) and establish in the central nervous system (CNS). The severe late-stage HAT is associated with the development of neuro-inflammatory reactions, encephalitis, massive infiltration of effector inflammatory cells and inflammation of the parenchymal vessels (Brun and Blum, 2012; Jamonneau *et al*., 2012; Rodgers *et al*., 2017).

A number of factors such as the host immune response, parasite specific subspecies and the stage of the disease have been reported to influence the signs and symptoms during trypanosomiasis

(MacLean *et al*., 2010; Odiit *et al*., 1997). Nevertheless, both *T. b. gambiense* and *T. b. rhodesiense* forms of HAT may cause impairment of consciousness, incontinence, seizures and even death if left untreated or when the treatments are delayed (Jamonneau *et al*., 2012; Urech *et al*., 2011). Studies have reported a dermal reaction due to *T.b. rhodesiense* inoculation by the tse-tse fly bite resulting to the development of a chancre of about 3-4 cm in diameter that occur within 2-3 days after the bite, however this phenomenon is very rare in *T.b. gambiense* form of HAT (MacLean *et al*., 2010; Küpfer *et al*., 2011; Kato *et al*., 2015).

The interaction between the trypanosomes and the host immune cell defines their destiny in the mammalian host. The parasites can either be cleared by host antibodies or escape the host immune cells by mounting resistance to serum apolipoprotein L1 (APOL1) (Frenkel *et al*., 2016). Indeed, the parasites evade the host immune system via the variant surface glycoprotein (VSG); a surface antigen found on the cell membrane of parasites that enables them to dodge specific immune responses through a phenomenon of antigenic variation (Tachado and Schofield, 1994; Okomo *et al*., 1995). The trypanosomal VSG can also induce the secretion of autoantibodies and pro-inflammatory cytokines, in particular tumour necrosis factor (TNF-a) (Okomo *et al*., 1995; Magez *et al*., 2002).

Tissue damage associated with oxidative stress has been reported in the pathophysiology of HAT (Igbokwe, 1994; Ogunsanmi and Taiwo, 2001; Umar *et al*., 2007). An oxidant stress occurs when there is an imbalance between radical-generating and radical-scavenging activity. Therefore, this imbalance may cause an increase in the formation of oxidation products (Saleh *et al*., 2009). It has been shown that infections by the *T. brucei* may alter the host's antioxidant defense against free radicals (Igbokwe *et al*., 1996; Omer *et al*., 2007; Umar *et al*., 2007). Peroxides and oxygen radicals are cellular toxins which are very aggressive and can destroy both

connective tissues and biological membranes. They can also oxidize the sulphydryl groups and inactivate enzymes as well as cause peroxidative damage of nucleic acids (Ogunsanmi and Taiwo, 2001). Lipids in particular the polyunsaturated fatty acids are known to be very sensitive to oxidation, thus the term lipid peroxidation of which, malondialdehyde (MDA) is the most abundant (Igbokwe *et al*., 1996). The accumulation of MDA in tissues or biological fluids is an indication of the extent to which free radical are generated, oxidative stress and tissue damage (Gutteridge, 1995). Some studies have suggested that lipid peroxidation is involved in cell destruction during HAT (Igbokwe, 1994). The products of lipid peroxidation are normally scavenged by antioxidants such as reduced glutathione (GSH), the most essential cellular antioxidants that play a major role in protecting mammalian cells against oxidative stress caused by ROS (Shan *et al*., 1990). The decline in the concentration of serum and liver GSH was associated with oxidative haemolysis in *T. brucei* infected rats (Ogunsanmi and Taiwo, 2001).

About five different drugs are currently used in the treatment HAT and they include: pentamidine and suramin for the treatment of early-stage HAT, and melarsoprol, eflornithine and nifurtimox for the late stage ailment. These drugs are donated by the manufacturers in collaboration with non-governmental organisation (Graf *et al*, 2013). Some studies have reported that drug therapy for the early-stage HAT is effective and less toxic compared to the ones used in the late-stage therapy (Büscher *et al*., 2017; Kennedy, 2013). The pentamidine drug administered through intramuscular or intravenous is somehow effective for both stages of *T. b. rhodesiense* and the late stage of *T. b. gambiense* though it has been associated with side effects such as hypotension, abnormalities of glucose metabolism, renal dysfunction, and gastro-intestinal symptoms (Atouguia *et al*., 2000). On the other hand, suramin therapy is used in the early-stage of *T. b. rhodesiense* and administered through the intravenous route. Even though it is somehow

effective therapy, it has been associated with side-effects such as mild renal dysfunction, peripheral neuropathy, anaphylactic reactions, and bone marrow toxicity leading to peripheral blood abnormalities (Kennedy, 2008; Kennedy, 2004). Intravenous melarsoprol remains the only treatment presently available for the treatment of the late-stage *T. b. rhodesiense*. Though very effective, melarsoprol is painful to administer and is very toxic, with a fatality rate estimated to be 5–9% as a result of a severe post-treatment reactive encephalopathy (PTRE) which occur in 5–10% of cases from whom about half of the patients die (Büscher *et al*., 2017; Kennedy, 2013).

Despite the deleterious effects associated with the disease, there is erosion of research in terms of managing the disease. Additionally, the pharmaceutical companies have paid little attention to HAT due to the fact that the disease is more common in low-income population residing in the rural areas. This in turn has hindered the advancement in therapies (Siqueira *et al*., 2017). Moreover, the disease management focusing on the vector control has not been very successful due to the resistance to pesticides by the *Glossina spp*. On the other hand, the endemicity of HAT in rural set up of the tropical regions of Africa has attracted little attention by pharmaceutical companies and this is coupled by the little research in new ways to manage the condition. Despite the fact that HAT cause high morbidity and greater economic loss among the population residing in the rural set up in Africa, it has attracted little attention in new ways of managing the disease and this therefore underpins the urgency of developing more effective and safer drugs or alternative approaches. Therefore, the objective of this study was to determine the effects of cyanocobalamin in the regulation of immune response during *T. b. rhodesiense* infection in a mice model.

There is a need for a safe affordable treatment approach for HAT. In this study, vitamin B12 which is a complex essential water-soluble vitamin was investigated for use as an adjunct

therapy given its proven biological role in maintaining neuronal health, hematopoiesis, cellular energy production and immune modulation coupled with its anti-inflammatory and antioxidant properties (Green and Miller, 2022; Fang *et al*., 2017; O'Leary *et al*., 2010). Cobalamin deficiency may lead to the development of megaloblastic / macrocytic anemia and severe neurological disorders (Tamura *et al*., 1999).

Many forms of cobalamin have been reported to exist and they include: cyano-cobalamin, methyl-cobalamin, deoxyadenosyl-cobalamin and hydroxy-cobalamin forms. However, the cyano-cobalamin form still remains to be the most widely used form in supplements and prescription drug. Cyano-cobalamin can exist in the form of tablet, injection and nasal spray (O'Leary *et al*., 2010). Vitamin B12 improves immune function (Mikkelsen and Apostolopoulos, 2019; Tamura *et al*., 1999) and up-regulates glutathione (GSH) levels in sepsis/septic shock patients, an indication of a GSH-sparing effect that enhances the cytosolic bioavailability of the antioxidant (Manzanares *et al*., 2010). Vitamin B12 has also been shown to counter the cytokine storm, consequently attenuating lethal inflammation and oxidative stress, stimulating oxidative phosphorylation and eventually restoring optimal bacteriostasis and phagocytosis among covid-19 patients (Manzanares, 2020; Wheatley, 2006).

1.2: Problem statement

Human African Trypanosomiasis (HAT) is responsible for not only high morbidity and mortality but also a course for greater economic loss to pastoralist in the tropical regions of Africa. The development of vaccines against trypanosomes has not been successful due to the ever changing trypanosomal surface antigen called the Variant Surface Glycoprotein (VSG) that enables trypanosomes to dodge the host specific immune response. Few drugs that are currently available for HAT treatment are highly toxic and Melarsoprol is one of such drug which is preferred against the severe late stage HAT. Unfortunately, this drug causes the development of an extremely severe post-treatment reactive encephalopathy (PTRE) in about 10% of HAT patients, with 5% of them succumbing to PTRE-related deaths. Additionally, the disease management focusing on the vector control has not been very successful due to the resistance to pesticides by the tsetse fly (Glossina *spp*). Moreover, the endemicity of HAT in rural set up of the tropical regions of Africa has attracted little attention by pharmaceutical companies coupled by the little research in new ways to manage the condition. On the other hand, cyano-cobalamin (vitamin B12) has been used to improve various disease outcomes in a number of ailments due to its properties not limited to anti-inflammatory, anti-oxidant and erythropoietic properties. However, the role of vitamin B12 in mitigating deleterious and pathogenic events due to *T. b. rhodesiense* infection has not been determined.

1.3: Justification

Despite HAT being a neglected tropical disease, it is still one of the main contributor to the global parasitic disease burden, putting about 55 million people residing in rural areas with high tsetse fly infestation at risk in about 36 countries in sub-Saharan Africa with a 84% drop in the incidences (WHO, 2021). The development of vaccines against HAT has been a challenge due to the ever changing trypanosomal variant surface glycoprotein (VSG) that enables the parasites to evade the host immune system (Okomo *et al*., 1995). If HAT is left untreated or when the treatments are delayed, the patients may develop consciousness, incontinence, seizures and even death (Jamonneau *et al*., 2012). Even though few drugs are currently available for HAT treatment, intravenous melarsoprol remains the drug of choice presently available for the treatment of the late-stage HAT. However, this drug has been reported to be very toxic, and result in a severe post-treatment reactive encephalopathy (PTRE) which occur in about 5–10% of HAT patients (Büscher *et al*., 2017). Despite multiple deleterious events associated with the HAT (Siqueira *et al*., 2017), it has attracted little attention in new ways of management, thus underpins the urgency of developing more effective but safer drugs or alternative approaches. On the other hand, cyanocobalamin (vitamin B12) has been mentioned in a number of studies as a powerful anti-inflammatory and anti-oxidant agent against various pathological conditions (Mikkelsen and Apostolopoulos, 2019; Manzanares, 2020). In this study, vitamin B12 was investigated given its proven biological role in maintaining neuronal health, hematopoiesis, cellular energy production and immune modulation coupled with its anti-inflammatory and antioxidant properties (Green and Miller, 2022; Fang *et al*., 2017).

1.4: Objectives

1.4.1: Broad objective

To determine the effects of cyanocobalamin in the regulation of inflammatory responses, oxidative stress and pathological events during severe stage of Human African Trypanosomiasis.

1.4.2: Specific objectives

1. To determine the impact of cyanocobalamin on physiological and hematological indicators due to *T. b. rhodesiense* infection
2. To illustrate the impact of cyanocobalamin in the regulation of *T. b. rhodesiense*-induced inflammation and organ damage
3. To determine the ability of cyanocobalamin to ameliorate *T. b. rhodesiense*-driven organ pathology and oxidative stress

1.5: Research questions

1. In what ways does cyanocobalamin impact on the physiological and hematological indicators due to *T. b. rhodesiense* infection?
2. To what extent does cyanocobalamin administration influence inflammation and organ damage during *T. b. rhodesiense* infection?
3. How cyanocobalamin does ameliorate *T. b. rhodesiense*-driven organ pathophysiology and oxidative stress?

CHAPTER TWO

LITERATURE REVIEW

2.1: Etiology and epidemiology of Human African Trypanosomiasis (HAT)

Human African Trypanosomiasis (HAT) is caused by *T. b. gambianse* and *T. b. rhodesiense* both are unicellular flagellated protozoan parasites (Odiit *et al*., 1997). The burden of HAT (fig. 2.1) is in such a way that, the *T. b. gambiense* is responsible for the chronic form of the HAT and therefore, can last for several months to years before causing death to the mammalian host (Jamonneau *et al.*, 2012; Tomiotto *et al.,* 2017). HAT due to *T. b. gambianse* is more prevalent in the Western and Central parts of Africa. The *T. b. rhodesiense* is responsible for the acute form of sleeping sickness and therefore may cause death to mammalian host within weeks to few months of infection (Kato *et al.*, 2015). The *T. b. rhodesiense* sub-species of trypanosome are endemic in the Eastern and Southern parts of Africa (Tomiotto *et al.,* 2017). According to the WHO (2021) report on HAT, the incidences of *T. b. gambianse* were estimated to be about 2000 in Congo, while only 50 incidences of *T.b. rhodesiense* were reported during that period. Approximately 20,000 active cases of trypanosomiasis exist presently with about 65 million people being at risk (Tomiotto *et al.,* 2017). The majority of these incidences were reported in the D R Congo where 84% of the new cases were reported in 2015 (Franco *et al*., 2014). HAT has been ranked third most important contributor to the global parasitic disease burden after malaria and schistosomiasis (Cattand *et al*., 2001).

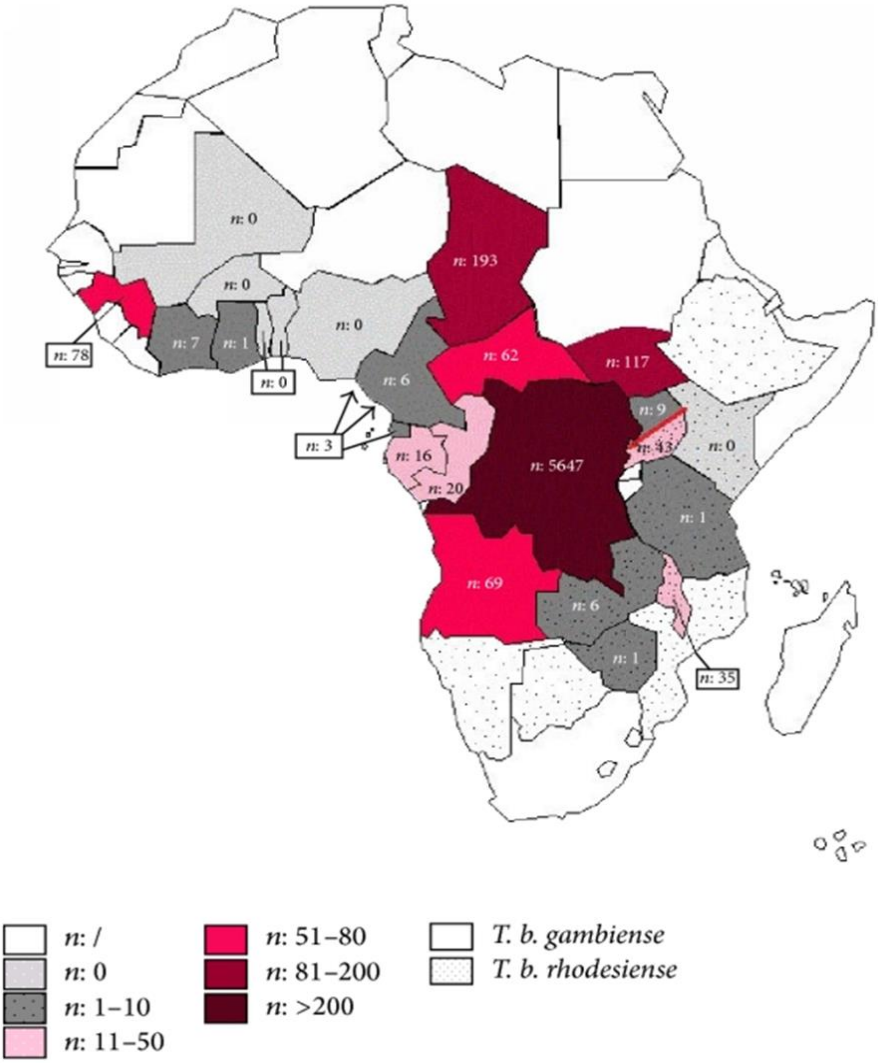

Figure 2.1 The burden of Human African Trypanosomiasis(WHO, 2021)

2.2: The vector and trypanosome life cycle

In both forms of trypanosomiasis, the animals are the main reservoirs of trypanosomes and human infections are incidental and can only take place when hematophagous tsetse fly (*Glossina* spp) bites humans when taking a blood meal (Alfituri *et al*., 2019; Franco *et al*., 2014). As shown in **figure 2.2**, the lifecycle of trypanosome naturally begins by the inoculation of metacyclic form of trypomastigotes into the vertebrate skin via the biting of the tsetse fly (Aksoy *et al*., 2014). The metacyclic forms of trypomastigotes then differentiate through binary fission into a long-slender form of trypomastigotes, which then proliferate and establish infections as they travel through the blood stream from which they actively colonize various tissues and organs like the lymph nodes through afferent lymphatic vessels of the vertebrate host (CDC, 2016).

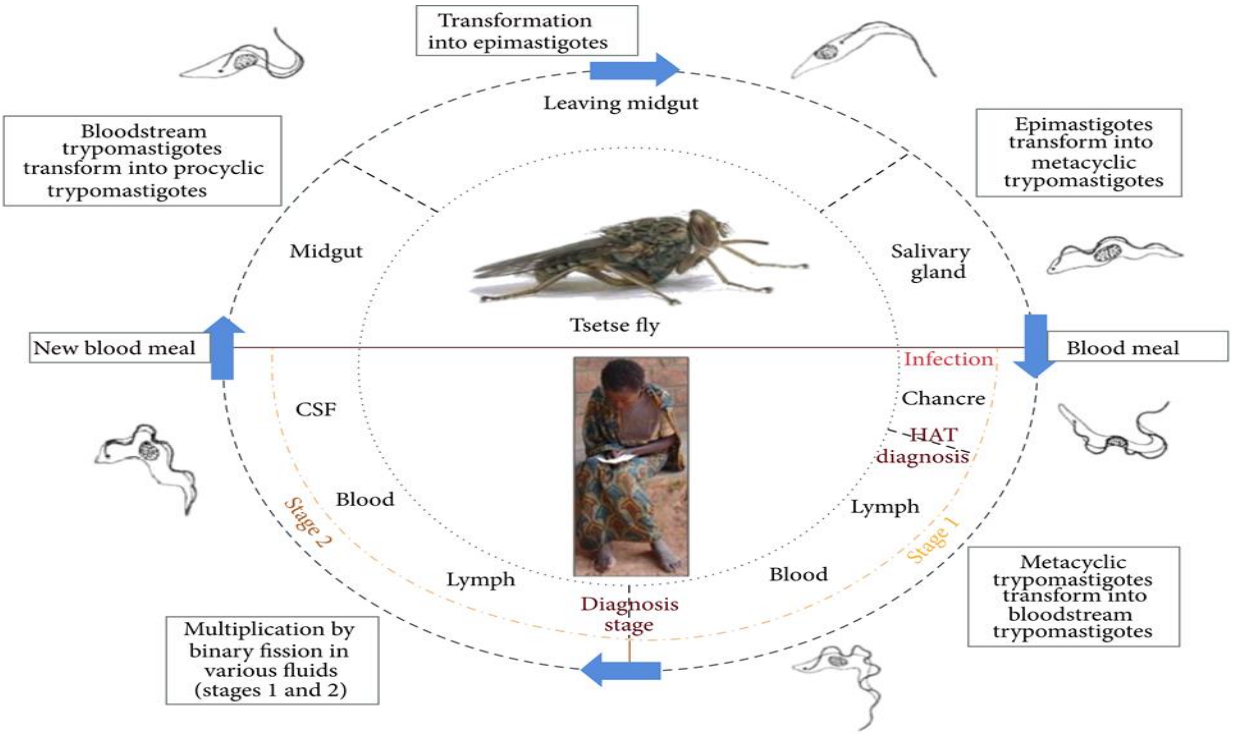

Figure 2.1: The life cycle of trypanosomes (Julien *et al*., 2015)

2.3: Host Immune Responses to Human African Trypanosomes

The host immune response is composed of both innate and the adaptive immune response. Previous immunological studies in mice artificially inoculated with trypanosomes via intra-peritoneal and intravenous routes have reported the role played by various immune cells like macrophages, monocytes, dendritic cells, neutrophils, and NK cells with regards to the control of the initial stages of trypanosomiasis (Bakari *et al.*, 2017). Large board of evidence has clearly demonstrated that the early host immune response against HAT occurs in less than two weeks and is characterized by the strong induction of pro-inflammatory cytokines such as IFN-γ, TNF and IL-6 alongside the production of nitric oxide (NO) which is also an inflammatory mediator (Baral, 2010; MacLean *et al.*, 2001). Additionally, the involvement of B cells activation leading to the production of antigen-specific antibodies such as IgM and IgG that target the immunodominant trypanosomal VSG which are released at peak parasitemia have also been documented (Bakari *et al.*, 2017; Magez *et al.*, 2002). Nonetheless, the artificial inoculation routes employed in these studies surpass the immunological events that take place in the skin during HAT infection.

2.4: Innate Immune Response during Human African Trypanosomiasis

During cellular innate immune response, trypanosomal factors activate various host immune cells prompting an acute inflammatory response as shown in **Figure 2.3** (Janeway *et al.*, 2002; Takeda *et al.*, 2003). The trypanosomal factors such as the trypanosomal DNA released from the dead parasites and trypanosomal VSG can directly activate the macrophages in a process called classical activation of macrophages (caMϕs) leading to the secretion of pro-inflammatory molecules like TNF-α, nitric oxide (NO), reactive nitrogen species (RNS) and reactive oxygen

species (ROS) (Shoda *et al*., 2001; Harris *et al*., 2006). The pattern recognition receptors (PRR) of neutrophils and NK cells usually interact with the trypanosomal pathogen associated molecular patterns (PAMPs) to become stimulated and hence promote the production of pro-inflammatory cytokines such as IFN-γ and TNF-α by the NK cells together with IL-1β and IL-6 by neutrophils. These cytokines can also bring about the classical activation of macrophages via the help of inducible nitric oxide synthase (iNOS) enzyme (Perobelli *et al*., 2015; MacLean *et al*., 2001). In addition, neutrophils can also control the parasitemia levels through phagocytosis or via the release of ROS and neutrophil extracellular traps (NETs) (Perobelli *et al*., 2015). But most importantly they are mediators of tissue repair and wound healing (Silva *et al*., 2016). Other studies have associated the NK cell-deficiency with uncontrolled levels of parasitemia and this could be due to lower levels of IFN-γ and TNF-α, and hence the rapid commencement of death (Onyilagha *et al*., 2019).

During the early onset of trypanosomiasis, a strong Th1 (pro-inflammatory type 1) immune response is initiated and characterized by increased levels of IFN-γ and IL-2 corresponding to the elevated numbers of macrophage in the spleen, liver, and bone marrow (Vincendaeu, 2006). Moreover, macrophages and liver-resident Kupffer cells phagocytose trypanosomes that are opsonized by parasite-specific immnoglobulins (Onyilagha *et al*., 2019; Guirnalda *et al*., 2007). Experiments in *T. brucei*-infected mice have revealed that Kupffer cells in the liver are involved in most of the parasite clearance that occurs *via* complement and antibody-mediated phagocytosis (Onyilagha *et al*., 2019).

Through the L-arginine metabolic pathway, classically activated macrophages can utilize iNOS enzyme to enhance the production of a very reactive and toxic NO (MacLean *et al*., 2001; Satriano, 2004; Wijnands *et al*., 2015). Additionally, these mononuclear phagocytes can further

hasten the production of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8, and IL-12 (Boulakirba *et al*., 2018). Interestingly, a number of pro-inflammatory cytokines have been documented to possess potent trypanostatic properties, further emphasizing the importance of these cytokines at the commensment of the infection (Namangala, 2012; Magez *et al*., 2007). Apart from pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8, and IL-12 produced by classically activated macrophages, studies involving the *T. congolense* and *T. brucei* have also reported the trypanostatic property of NO which is thought to inhibit the trypanosome growth and are also very crucial in the control of the initial wave of parasitemia (Lu *et al*., 2011). However, other studies have indicated the ability of NO to readily bind to hemoglobin and therefore, would be quickly quenched in the bloodstream, reducing its inhibitory effects (Mabbott *et al*., 1995).

Other studies have suggested that the involvement of the toll like receptor-2 (TLR2), toll like receptor-9 (TLR9) and Myeloid differentiation primary response 88 (MyD88) can excite the manifestation of pro-inflammatory molecules of both IFN α/β as well as TNF-α which hinders the disease progression by reducing the transmigration of leucocytes and parasites into the CNS (Amin *et al*., 2012). The antibody-opsonized parasites can be phagocytosed by the classically activated macrophages (caMϕs) (Shi *et al*., 2004). In addition, the caMϕs can also secrete some trypanotoxic molecules like TNF-α and NO that primarily controls the first peak of parasitemia (Kaushik *et al*., 2000).

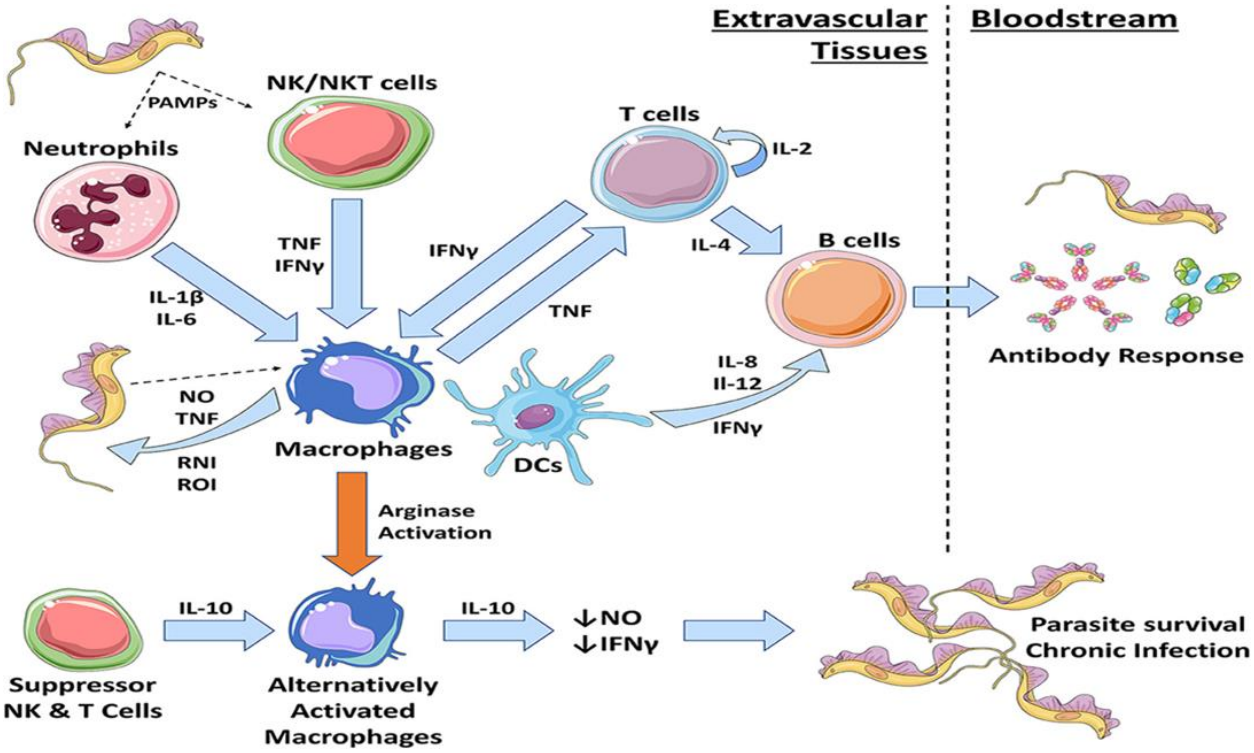

Figure2.2: The innate immune cells during Human African Trypanosomiasis (Alfituri *et al*., 2020)

2.5: Adaptive Immune Responses to Human African Trypanosomiasis

Despite the fact the initial induction of pro-inflammatory immune response is useful in the control of human African trypanosomiasis (HAT) infection during the early stage, the persistent and heightened secretion of pro-inflammatory cytokines can lead to the development of pathology on certain tissues and organs. Therefore, it is essential for the host immune cells to bring about the balance in the release of the pro-inflammatory cytokines (Kaushik *et al*., 2000) by way of down-regulating the activity of the classically activated macrophages and other immune cells that are co-producers of pro-inflammatory cytokines (Roszer, 2015; Murray *et al*., 2014). The Production of type II cytokines like IL-4, IL-10, IL-13 and TGF-β have been described in macrophage stimulation to become more anti-inflammatory type with concomitant

involvement in a prolonged survival of the host (Raes *et al*., 2007). The implications for this kind of immunological change is still under investigation, but studies have reported that the process of switching from classically activated macrophages (caMϕ) to alternatively-activated macrophages (aaMϕ) may contribute to the reduction of the deleterious events in the host due to a sustained inflammation and enhanced tissue repair and regeneration (Arango *et al*., 2014; Boulakirba *et al*., 2018). The hallmark of aaMϕ is the expression of arginases enzyme, which compete with iNOS enzyme resulting in the formation of ornithine and urea through the L-arginine pathway instead of NO and citrulline (Rath *et al*., 2014).

2.6: The inflammatory markers associated with trypanosome infection

2.6.1: Tumour necrosis factor-α (TNF-α)

Tumour necrosis factor-α (TNF-α) is a pro-inflammatory cytokine primarily produced by macrophages. This cytokine is involved in the innate immunity against intracellular pathogens. It is thought that TNF-α is secreted when the immunogen such as the soluble trypanosomal VSGs interacts with the cells of innate imunity (Magez *et al*., 1998). Studies in animal models have demostrated the lytic property of TNF-α and hence regarded as a key mediator in the control of *T. brucei* infections (Naesens *et al*., 2005). Studies from murine model involving trypanosomes in a TNF-α knockout mouse hhas shown a trypanosome-induced immunopathological features characterized by lymph-node associated immunosuppression and lipopolysaccharide hypersensitivity (Magez *et al*., 1999). Still, other studies have linked moderate to severe neuropathy in the brain to heightened levels of TNF-α (Sternberg *et al*., 2005). In staking contrast, studies in vervet monkey model have reported a non-significant level in TNF-α in CNS following *T. b. rhodesiense* infection and the clinical presentations were not in any way

associated with TNF-α level (Magez *et al*., 1999). It has also been shown that increased levels of TNF-α might be involved in associated with *T. b. rhodesiense* infections (Naesens *et al*., 2005). Notably, there is some evidence indicating a possible role of TNF-α in trypanosome transmigration across the blood brain barrier especially through Toll-like receptor (TLR)–MyD88–mediated signaling (Amin *et al*., 2012).

2.6.2: Interferon gamma (IFN-γ)

Interferon gamma (IFN-γ) is another pro-inflammatory cytokine secreted predominantly by T-cells and natural killer (NK) cells. IFN-γ plays a major role in both innate and adaptive immune response. Studies involving *T. b. brucei* in a murine model, has reported uncontrolled parasitemia and a significant reduction in the survival rate among IFN-γ knockout mice relative to the wild type mice (Mabbott *et al*., 1998), while other studies has linked the elevated levels of IFN-γ to a decline in parasitemia in a murine model infected with *T. b. rhodesiense* suggesting some form of resistance to infection (Namangala *et al*., 2001). The enhanced severity of neurological symptoms in a murine model infected *T. b. brucei* has been associated to the elevated levels of IFN-γ in the brain (Sternberg *et al*., 2005). Findings in other studies have established the essential role of IFN-γ in *T. b. brucei* parasite transmigration across the blood brain barrier (BBB) thereby exacerbating the HAT associated immunopathology (Rousset *et al*., 1992).

2.6.3: Interleukin-10 (IL-10)

IL-10 is one of the counter-inflammatory cytokine produced by a number of immune cells (innate and adaptive) such as macrophages and T-regulatory cells that ostensibly control the excessive inflammation (Sabat, 2010). Previous studies have demonstrated that IL-10 has a direct role in the up-regulation the production of antibodies and MHC class II expression on the B cells (Rousset *et al*., 1992). In parasitic diseases like malaria and toxoplasmosis, IL-10 has been reported to play some defensive roles (Hunt and Grau, 2003; Sarciron and Gherardi, 2000). In yet another study, IL-10 knockout mice infected with *T. b. brucei* registered a reduced survival time (Namangala *et al*., 2001). Likewise, elevated levels of IL-10 in trypanosome infected mice has been linked to a noticeably reduction in IFN-γ concentrations and subsequent longer survival time than infected control animals (Magez *et al*., 2007; MacLean *et al*., 2001; Namangala *et al*., 2001). Avalanche body of evidence have equally demonstrated that *T. b. rhodesiense* and in *T. b. gambiense* infection in a murine model resulted in the up-regulation of IL-10 levels in plasma and CNS particularly in the late stage disease (Lejon *et al*., 2002; MacLean *et al*., 2001).

2.6.4: Interleukin-1 beta (IL-1β)

Interleukin-1 beta (IL-1β) is another pro-inflammatory cytokine primarily produced by monocytes and macrophages (Schindler *et al*., 1990), and has been shown to play certain roles in both innate and adaptive immune responses (Dinarello *et al*., 2009; Ludigs *et al*., 2012). Studies in inflammatory disorders have mentioned IL-1β to have some potential role in the dysfunction of the blood brain barrier (Argaw *et al*., 2006). Studies involving a *T. b. brucei* cloned stabilate (GVR35/C1.8) in a murine model have reported an apparent elevation in the levels of plasma IL-1β relative to the controls (Sternberg *et al*., 2005). While other studies have found no correlation

of IL-1β levels to the degree of neuro-inflammation, some studies have demonstrated that the levels of IL-1β, TNF-α and IFN-γ in the brain was responsible for about 94.8 % of the variations in the neuropathology (Charles *et al*., 2016). Patients in Tororo and Soroti (Ugandan) with *T. b. rhodesiense* form of HAT reported non significant differences in plasma IL-1β levels despite the variations in the disease progression and severity between the two foci (MacLean *et al*., 2007). However, the available literature does not clearly outline the role played by IL-1β during HAT pathogenesis.

2.6.5: Transforming growth factor-beta (TGF-β)

Transforming growth factor- beta (TGF-β) is a pluripotent cytokine and posses pro- and counter-inflammatory effects subject to its environment and concentration. High concentration of TGF-β has been reported to suppress the synthesis of pro- inflammatory cytokines and in particular the TNF-α and IFN-γ by blood mononuclear cells and peritoneal-derived macrophages (Wahl *et al*., 1989; Espevik *et al*., 1987). Though the role of TGF-β in HAT progression is scanty, studies have proposed that TGF-β could have some influence on the pathogenesis of *T. b. rhodesiense* form of HAT. The *T. b. rhodesiense*-driven cytokine profile comparisson study in the two distinct geographical HAT foci (Uganda and Malawi) reported a significant elevation in plasma TGF-β levels among patients in Malawi comparative to the patients in Uganda. The authors associated the increased plasma TGF-β levels among the Malawi patients to a reduced pathology and prolonged survival time. However, the specific role of TGF-β in HAT pathogenesis was not demonstrated (MacLean *et al*., 2004). On the other hand, Maclean *et al*. (2007) reported that there wasn't any significant difference in plasma TGF-β levels in other two HAT foci in Uganda despite the disease being more severe in Tororo than Soroti.

2.6.6: Interleukin-6 (IL-6)

This is a multi-functional inflammatory cytokine that possess both pro and anti-inflammatory properties with diverse implications in pathophysiology of some neurological and inflammatory ailments. Studies investigating the disease conditions in rats have demonstrated that IL-6 possesses beneficial properties in metabolic control, neuronal survival, neuro-protective and analgesic effects (Pedersen *et al*., 2009; Oka *et al*., 1995; Kushima and Hatanaka, 1992). On contrary, destructive properties of IL-6 such as neuronal degeneration and cell death in degenerative disorders have been reported (Gadient and Otten, 1997). Additionally, studies in the neuropathological disorders in mice have reported that hightened proliferation of IL-6 was linked to the enhanced permeability of blood brain barrier coupled with increased neuropathological abnormalities (Brett *et al*., 1995). Additional findings in studies involving *T. b. rhodesiense* in a vervet monkey and mice models reported an elevation in the levels of IL-6 in cerebrospinal fluid (CSF) during the late stage HAT (Nyawira *et al*., 2013; Sternberg *et al*., 2005). Similarly, HAT patients have been reported to exhibit elevated levels of IL-6 in CSF during late stage HAT of both *T. b. rhodesiense* and *T. b. gambiense* disease (MacLean *et al*., 2012; Courtin *et al*., 2006).

2.7: Mechanisms employed by trypanosomes to evades mammalian host immunity and oxidative stress

Once the tse-tse fly inoculates the trypanosomastigotes into the mammalian host, the parasites then encounter the first barrier which is the host innate immunity (Janeway *et al*., 2002). Human and primates serum has been reported to contain certain protein such as apolipoprotein A1 (APOA1), apolipoprotein L1 (APOL1) and haptoglobin related protein (HRP). These proteins has trypanolytic activities and therefore, capable of killing the trypanosomes (Molina *et al*.,

2008; Vanhollebeke *et al*., 2006). However, both *T. b. rhodesiense* and *T. b. gambiense* have developed a mechanism to overcome the activities of APOA1, APOL1 and HRP of the mamalian host serum. For instance, *T. b. rhodesiense* has been shown to possess the serum resistance antigen (SRA) gene which neutralizes the activity of serum trypanolytic factors (TLF), thereby conferring resistance to lysis (Takeda *et al*., 2003; Capewell *et al.*, 2015).

Besides the SRA gene, the trypanosomes can escape the recognition of host immunity through antigenic variation of the membrane bound variant-specific surface glycoprotein (VSG) which prevents the interaction between the components of the immune responses and the underlying plasma membrane of the parasite (Pays, 2006). Other studies have also reported that trypanosomes are not only invades phagocytic cells through classic phagocytosis as seen in classically activated macrophages (caMϕ), but can also actively invade mammalian non-professional phagocytic cells by induced phagocytosis as seen in alternatively actiated macrophages (aaMϕ) (Maul *et al*., 1996). The main mammalian host cells targeting trypanosomes at the site of infection are macrophages and dendritic cells, both of which play a key role in the trypanosomal immune response as they are specialized antigen presenting cells (Geiger *et al*., 2016). Despite the fact that macrophages play an important role as effector cells that control and kill the intracellular form of the parasite via oxidative and non- oxidative mechanism, they also serve as long-term host cells that facilitate the replication and survival of the pathogens (Bogdan and Rollinghoff, 1999).

The activation of the macrophage membrane-associated nicotinamide adenine dinucleotide phosphate (NADPH) oxidase during trypomastigote phagocytosis usually leads to the production of a stable superoxide radical anion ($O_2^{\cdot -}$) which can then be transformed to hydrogen peroxide (H_2O_2) by the action of superoxide dismutase (SOD) (Piacennza *et al*., 2013; Alvarez *et al*.,

2011; Fridovich, 1995). The pro-inflammatory cytokines particularly the IFN-γ and TNF-α proliferated during trypanosome infection via the action of inducible nitric oxide synthase (iNOS) stimulate the macrophages to produce high amounts of NO (MacLean *et al*., 2001). The $^{\cdot}NO$ generated reacts with $O_2^{\cdot -}$ to produce peroxynitrite ($ONOO^-$), which is a potent oxidant with cytotoxic properties against trypanosomes (Radi, 2013; Alvarez *et al*., 2004). Studies have shown that trypanosomes possess some antioxidant enzymes like peroxidases (Tables 2.1) and iron superoxide dismutases (Tables 2.2) located at different subcellular compartments of the parasite. These antioxidant enzymes diminish the equivalents from NADPH thus protect the parasite against host oxidative stress and eventually propagate the survival of the parasites within the mammalian host (Piacenza *et al*., 2013).

Table 2.1:Peroxidases of Trypanosoma cruzi.

Peroxidase	**Subcellular location**	**Activity**
Tryparedoxin peroxidase (TcCPX)	Cytosol	Detoxify $ONOO^-$, H_2O_2 and small-chain organic hydroperoxides (Pineyro *et al*., 2005; Trujillo *et al*., 2004)
Tryparedoxin peroxidase (TcMPX)	Mitochondria	
Glutathione peroxidase I (TcGPXI)	Cytosol glycosome	Hydroperoxidases (Wilkinson *et al*., 2002)
Glutathione peroxidase II (TcGPXII)	Endoplasmic reticulum	Lipid hydroperoxidases (Wilkinson *et al*., 2002)
Ascorbate-dependent heme peroxidase (TcAPX)	Endoplasmic reticulum	Resistance H_2O_2 (Piacanza *et al*., 2008)

Table 2.2: Iron superoxide dismutase (FeSODs) of Trypanosoma cruzi.

Iron superoxide dismutase (FeSODs)	**Subcellular location**	**Specificity**
TcSODB1	Cytosol	Detoxify $O_2^{\cdot -}$ and hence the formation of $ONOO^-$ (Mateo *et al*., 2008)
TcSODB1-2	Glycosome	
TcSODA	Mitochondria	
TcSODB	Mitochondria	

2.8: Immunosuppression due to Human African Trypanosomiasis

The outstanding distinctive feature in trypanosomiasis is the ability of trypanosome to suppress the host immune responses leading to an enhanced susceptibility to the opportunistic infections. The HAT-driven immune suppression may affect both the humoral (B cell) and the cellular (T-cell and macrophage) mediated immune functions and subsequently leads to the immunopathological incidences associated with trypanosome infection (Darji *et al*., 1992; Sileghem *et al*., 1991; Taylor, 1998). Some studies have suggested that the macrophages and the T-cells might be implicated in the HAT-driven immunosuppression (Tabel *et al*., 2008), while other studies involving trypanosomes has reported that stimulated suppressive macrophages play certain role in the immunosuppression (Schleifer and Mansfield, 1993; Borowy *et al*., 1990). The inhibition of T-cell proliferation may cause the decreased production and expression of IL-2, thereby enhacing the occurrence of immunosuppression during HAT (Darji *et al.*, 1992). Other studies in a mouse model with *T. brucei* have shown that the proliferation of T-cell in the spleen, peritoneal cavity and lymph nodes especially during the early stages of HAT were hindered by the prostaglandins and nitric oxide (NO) which are thought to be up regulated by TNF and IFN-γ (Schleifer *et al*., 1993; Mabbott *et al*., 1998; Magez *et al*., 1999; MacLean *et al*., 2001). Furthermore, the TNF encourages the growth of suppressive cells by inducing the production of IFN- γ in the lymph nodes as demonstrated in the study involving mice infected with *T. brucei* (Darji *et al*., 1996). Meanwhile, during severe late stage of HAT, the inhibition of T-cell proliferation in the lymph nodes occur as a result of NO and prostaglandin, but involving an independent pathway, such that the IFN-γ released by CD8+ T-cell plays a crucial role (Beschin *et al*., 1998; Darji *et al*., 1993). Molecules secreted by the host macrophages particularly the IL-

10 have been revealed not only to inhibit antigen presentation but also contributes to the impairment of T-cell activation (Ding *et al*., 1993).

2.9: Immunopathology during Human African Trypanosomiasis

Uncontrolled type I immune response due to HAT may lead to pathological events that contributes to the neurological disorders causing deregulation of sleep pattern. This is very common in the late stage HAT hence the name sleeping sickness. However, in experimental trypanosomiasis the pathological indicators observed are mainly the loss of body weight (cachexia), reduced locomotory activity, fever, splenomegally and the liver damages (D'Ieteren *et al*., 1998). has also been reported to be most common pathological types seen in human, bovine and the experimental murine trypanosomiasis, and the extent of is regarded as the main indicator of the HAT severity (D'Ieteren *et al*., 1998). In bovine trypanosomiasis, one feature of the trypano-tolerance is the capacity to control the infection-associated and consequently the loss of productivity of the host (Naessens, 2006). The trypanosomiasis associated may be as a result of either loss of RBCs, for example, the cytokine-activated macrophages are thought to be responsible for the improved phagocytosis of parasites and the RBCs or due to failure to mount a strong compensatory erythropoietic reaction (Stijlemans *et al*., 2008; Akinbamijo *et al*., 1998).

Some studies have shown that the levels of serum TNF has got influence on the severity of neuropathology during the late stage of HAT (Okomo-Assoumou *et al*., 1995). However, some studies reported a non-relationship between the TNF serum level and HAT pathology (Lejon *et al*., 2002; MacLean *et al*., 2001). Other reports have shown that the improved manifestation of TNF mRNA in the brain of *T. brucei*-infected mice (Hunter *et al*., 1991; Hunter and Kennedy, 1992) and the association in trypanosome-infected cattle between TNF production by monocytes

correlates with the severity of HAT-associated (Sileghem *et al*., 1994). Therefore, the accrued understanding about the trypanosome-stimulated prolification of TNF indicates that the cytokine might be beneficial in the early stage of HAT through its role in parasite clearance.

2.10: Haematological changes during Human African Trypanosomiasis

Haematological changes during HAT are well documented and enormously influence the disease pathogenesis, usually indicated by the critical features such as the rapid decline in red blood cell (RBC) counts, haemoglobin (Hb) concentration and packed cell volume (PCV) in the infected mammalian hosts, hence a confirmation of anemia due to HAT (John *et al*., 2001). Studies in rodents infected with either *T.b. rhodesiense* or *T.b. gambiense* have shown the incidences of either macrocytic normochromic or microcytic hypochromic anemia (Stephen, 1986). The microcytic hypochromic form of anemia has been previously linked to iron deficiency since there is possiblity of failure to incorporate iron into the red cell precursors even in presence of adequate iron storage during HAT (John *et al*., 2001). However, the variation in the kind of anemia due to HAT could be attributed to a number of factors such as the stage of disease, pathogenicity of trypanosomes together with the host species (Buscher *et al*., 2017).

Studies have not reached a consensus in the pattern of leucocyte changes during HAT. The findings from the livestock have reported a distinct HAT-leukocytopenia characterized with neutrocytopenia and lymphocytopenia (Stephen, 1986). The neutrocytopenia due to HAT has been associated with increased susceptibility of infected animals to concurrent infections (Stephen, 1986). The occurrence of leukopenia has also been attributed to factors like leukophagocytosis due to trypanosomal antigen coating of leukocytes and depression of the production of leukocytes (Mackenzie *et al*., 1978). Conversely, studies involving *T. brucei*

infections in mice and rabbits have reported leukocytosis which indicates the active role played by WBCs in immunopathogenesis of trypanosomosis (Emeribe *et al*., 1991; Paling *et al*., 1991). Therefore, the leucocyte response during trypanosomosis is essentially determined by the stage of disease, trypanosome species, and host involved (Emeribe *et al*., 1991).

Thrombocytopenia has also been reported as a significant feature of trypanosomiasis (Stephhen, 1986; Wellde *et al*., 1978). The significant fall in platelets counts during trypanosome infection has been described in a number of studies (Stephen, 1986; Robins *et al*., 1975). The low platelet count together with increase in mean platelets volume (MPV) indicates the hyper-destruction of platelets by trypanosomal toxic products (Dow, 1994). The low platelet counts could be as a result of other factors like the pooling of blood in the spleen, removal of platelets by mononuclear phagocytic system and increased 'consumption' of platelets by disseminated intravascular coagulation reaction which have been widely reported in mammalian hosts infected with trypanosomes (Robins *et al*., 1975). The proliferation in MPV has been linked to an increased growth of megakaryocytes in response to thrombopoetic stress particularly when there is peripheral destruction of platelets (Thompson and Jakubowski, 1988).

2.11: Laboratory Diagnosis of Human African Trypanosomiasis

The diagnosis of HAT involves identification of trypanosomes in body fluids collected from a chancre, lymph node aspirate, blood and bone marrow aspirate. But when the late stage HAT is suspected, cerebrospinal fluid (CSF) is recommended. Wet smear preparations is ideal for the motile trypanosomes and smears should be fixed and then stained with Giemsa (or Field) stain and finally examined under light microscope (Brun *et al*., 2010). The concentration of trypanosomes in blood may be low but concentration techniques such as centrifugation,

miniature anion-exchange centrifugation and quantitative buffy coat technique may be used to enhance sensitivity (Kennedy, 2006). Since the seroconversion has been reported to occur during the onset of symptoms, the antibody detection assays may not be very useful clinically during HAT. However, a card agglutination test may be necessary during mass screening programs to identify the candidates for microscopic examination for HAT (Kennedy, 2013; Brun *et al*., 2010).

When the late stage HAT is suspected, a lumbar puncture should be performed in patients to enhance the collection of CSF. Such CSF sample has been shown to have elevated levels of lymphocytes (≥6 cells/mcL), total protein and non-specific IgM. In addition to trypanosomes, the characteristic Mott cells may be present (WHO, 1998). Other, nonspecific laboratory findings may include, monocytosis coupled with significant elevation in the serum polyclonal IgM levels (Kennedy, 2013; Brun *et al*., 2010).

2.12: Current Treatment of Human African Trypanosomiasis

There are approximately five different drugs currently available for the treatment HAT and they include: pentamidine, suramin, melarsoprol, eflornithine and nifurtimox. These drugs come as a donation by the pharmaceutical companies in collaboration with other non-governmental organizations (Graf *et al*, 2013). Studies have reported that therapeutic for the early-stage HAT is relatively effective and less toxic compared to the drugs used in the late-stage therapy (Büscher *et al.,* 2017; Kennedy, 2013). The pentamidine drug is administered through intramuscular or intravenous is somehow effective for both stages of *T. b. rhodesiense* form of HAT and the late stage of *T. b. gambiense* HAT. Pentamidine drug has been reported to have side effects such as hypotension, aberrations in glucose metabolism, renal dysfunction and gastro-intestinal

symptoms (Atouguia *et al*., 2000). Additionally, suramin therapy is used in the early-stage of *T. b. rhodesiense* HAT and is administered through the intravenous route. Even though it is somehow effective therapy, suramin has been associated with mild renal dysfunction, peripheral neuropathy, anaphylactic reactions and bone marrow toxicity leading to peripheral blood abnormalities (Kennedy, 2008; Kennedy, 2004).

Intravenous melarsoprol drug remains the prefered treatment for the severe late-stage HAT due to *T. b. rhodesiense* and *T. b. gambiense.* Though very effective against HAT, melarsoprol has been shown to be very painful when administered into the HAT patients, coupled with high toxicity levels associated with the development of severe post-treatment reactive encephalopathy (PTRE) that occur in about 10% of HAT patients of whom 5–9% succumb due to melarsoprol-induced PTRE (Büscher *et al.,* 2017; Kennedy, 2013).

A number of new therapeutics targeting the late-stage HAT are under evaluation with a lot of interest being focused on the fexinidazole therapy (a drug of the nitro-heterocyclic group). Some recent publications on the HAT treatment success rate between oral fexinidazole and NECT therapy in late-stage *T. b. gambiense* have reported a 91% success rate during the 18 month assessment point for oral fexinidazole, while NECT has a treatment success rate of 98% under similar duration. The report also indicated that the side effects for both oral fexinidazole and NECT therapy are similar (Mesu *et al*., 2017). This is evidently a major progress in HAT treatment but the efficacy of fexinidazole in the late-stage *T. b. rhodesiense* HAT remains to be seen.

2.13: Cyanocobalamin (Vitamin B12)

Cyanocobalamin (vitamin B12) is one of the essential water-soluble vitamins which is highly complex and contains the mineral cobalt. Vitamin B12 exists in many forms such as the cyano-cobalamin, methyl-cobalamin, deoxyadenosyl-cobalamin and hydroxy-cobalamin. However, the cyano-cobalamin form remain the most commonly used in supplementation and prescription drugs available in the form of tablet, injection, and nasal spray (O'Leary, 2012; Zhang *et al*., 2013). Vitamin B12 is produced naturally by the certain gut bacteria (Fang *et al*., 2017) and is essential for DNA synthesis, erythropoeiosis, cellular energy production and maintaining neuronal health (Green *et al*., 2017).

2.14: Clinical manifestations of vitamin B12 deficiency

The subclinical deficiency of vitamin B12 is defined as 119–200 pmol/L of serum vitamin B12 (Green *et al*., 2017; Hannibal *et al*., 2016). This deficiency could occasion some deleterious events to macromolecules such as nucleic acids, proteins and lipids though the affected individuals apparently tend to remain asymptomatic (Ames, 2006). Studies have reported that the vitamin B12 stored in the hepatic system immensely exceed the daily loss of the vitamin, the deficiencies can therefore remain clinically unexpressed for a number of years (Carmel, 2013). The subclinical vitamin B12 deficiency can be caused by inadequate intake, increased body demand, mal-absorption and to some extent the genetic causes (Thakkar and Billa, 2015). Studies have revealed some connections between subclinical vitamin B12 deficiency and serum metabolic markers associated with the reduction in the mitochondrial function, myelin stability, and peripheral neuron function and enhanced oxidative stress (Brito *et al*., 2017). Subclinical vitamin B12 deficiency has also been implicated in a number of age-related diseases like

schizophrenia, type 2 diabetes (T2D), Alzheimer's disease, and Parkinson's disease all of which share oxidative stress as a commonality in their pathophysiology (Firth *et al*., 2017; Di Meo *et al*., 2016; McCaddon *et al*., 2013; McCaddon *et al*., 2002).

2.15: Vitamin B12 as anti-inflammatory and anti-oxidant agent

Cyanocobalamin (vitamin B12) has been mentioned in a number of studies as a powerful anti-inflammatory and anti-oxidant agent against various pathological conditions (Mikkelsen and Apostolopoulos, 2019; Manzanares, 2020). Studies in human cells inparticular the aortic endothelial cells, neuronal cells and other cell-free systems have demonstrated that supplementation of physiologically relevant concentrations of vitamin B12 greatly down-regulates the levels of superoxide in the cytosol and the mitochondria (Chan *et al*., 2017; Moreira *et al*., 2011), though the mechanism involved in this action has not been clearly established. Similar reports were also made in rats where superoxide bursts in retinal ganglion cells were significantly down-regulated by vitamin B12 treatment and hence the improved cell survival. Additionally, the authors further aleuded that the enzymatically processed vitamin B12 acted as a direct superoxide scavenger (Moreira *et al*., 2011; Chan *et al*., 2017).

Vitamin B12 may indirectly stimulate the scavenging of ROS by preservation of glutathione, through some complicated network of reactions that has not been fully elucidated (Karamshetty *et al*., 2016; Manzanares and Hardy, 2010). Besides the modulation of the oxidative stress, vitamin B12 has been reported to play some role in the modulation of immune responses. For instance, vitamin B12 deficient Alzheimer patients have been found with increased production of basal interleukin-6 relative to the vitamin B12 competent Alzheimer patients (Politis *et al*., 2010). Studies in humans and rats with vitamin B12-deficiency have also reported upsurge in

tumour necrosis factor alpha (TNF-α) and decline in the levels of transforming growth factor-beta (TGF-β) in comparisson to their controls, further suggesting the ability of vitamin B12 in the protection against the low-grade inflammation via modulating the expression of cytokines and growth factors (Green and Miller, 2022; Fang *et al*., 2017; Birch *et al*., 2009). Vitamin B12 has been shown to be an important cofactor in homocysteine (Hcy) metabolism since the subclinical vitamin B12 deficiency is associated with reduced conversion of Hcy to methionine leading to the elevation of intracellular Hcy (Green *et al*., 2017). Homocysteine (Hcy) is capable of facilitating the accumulation of ROS through a number of mechanisms leading to production of hydrogen peroxide (H_2O_2) (Fang *et al*., 2017; Tyagi, 2005; Loscalzo, 1996).

2.16: Immune modulation by Vitamin B12

The deficiency of cyanocobalamine in the body may lead to the development of conditions like megaloblastic and peripheral nervous system disorder. Studies have reported that administration of vitamin B12 can restore the immune parameters particularly in patients with megaloblastic by balancing the CD4+/CD8+ ratio and elevation of CD3 and CD19 (Tamura *et al*., 1999; Bunting *et al*., 1997; Imamura *et al*., 1984). According to Ingram *et al.* (1997), cyanocobalamine has been shown to suport the humoral immunity and cellular immunity by enhancing the levels of serum IgG, IgA, and IgM. The study also suggested that the lymphocyte apoptosis can be prevented by the administration of cyanocobalamin which in turn may improve the immunologic abnormalities witnessed in patients with pernicious anemia. Other studies have also suggested that the ineffective hematopoiesis due to vitamin B12 deficiency concerned primarily CD8+ cells (Green and Miller, 2022; Fang *et al*., 2017).

The cyanocobalamin have also been reported to facilitate the recovery from depressed NK cell function whereby the functions of lymphocyte and NK cell are completely reinstated and serum levels of cyanocobalamin returned to normal (Tamura *et al.,* 1999). Other studies have also reported an elevation in the total numbers of cells that have strong NK cell activity such as CD3–, CD16+, and CD57+ cells after cyanocobalamin therapy and hence improved antitumor activity (Hsing *et al.*, 1993). Studies involving cyanocobalamin deficient rats reported a decline in the levels of serum C3, IgG, and IgM, this condition was restored upon administration of cyanocobalamin (Funada *et al.*, 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1: Study Area

This study was conducted at the laboratories of the Technical University of Kenya, School of Health Sciences and Technology, department of Biomedical Science and Technology located at the Nairobi central business district (CBD) along Haile Selassie, avenue factory road. Besides the animal house where various laboratory animals are kept, these laboratories are well fitted with modern laboratory equipments such as microscopes, iosafety cabinets and ELISA machines among others.

3.2: Ethics Statement

The experimental guidelines and procedures pertaining to the use of mice were strictly observed and approval was obtained from the Institute of Primate Research (IPR) ethics committee (ISERC/08/2017). Humane endpoints were observed throughout the study. This research was conducted in accordance with the internationally accepted principles for laboratory animal use and care, as stipulated in the Institutional Animal Care and Use Committee (IACUC) and the ethical review committee for the use of laboratory animals.

3.3: Experimental Animals, Parasites and cyanocobalammin

Female adult Wild Type (WT) Swiss-white mice weighing between 23-26g and aged 6-8-weeks were procured from department of vetenary services (DVS) Kabete Laboratories in Nairobi, Kenya. The mice were then maintained on mice pellets that are commercially available from Unga Feeds Company and water *ad libitum*. The mice were then housed in standard Tecniplast

cages with wood chippings as bedding materials in pathogen-free conditions at 23-25℃ room temperature with a 12 hr light/dark cycle.

The initial weight of each mouse was established by the analytical electronic balance (Mettler PM34, DoltaRange®). Subsequently, the mice were subcutaneously injected with 0.02ml dose of ivermectin (Ivermectin®, Anupco, Suffolk, England) with the aim to eradicate both ecto and endo parasites. The picric acid was used to label the mice accordingly and finally, the mice were allowed to acclimatize for one week. The *T. b. rhodesiense* KETRI 2537 was used in this study. Four female Swiss white mice served as a donor for the propagation of the stock of trypanosomes stabilate of *T.b.rhodesiense* and each experimental mouse was infected with 5.0 x 10^4 trypanosomes. The vitamin B12 powder (98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). A dosage of 8mg/kg of vitamin B12 (Wang *et al*., 2019) was administered orally via gavage.

3.4: Sample size

Given that the study employed one-way ANOVA for group comparison, resource equation approach was used to determine the maximum number of animals needed for this study.

$n = DF/k + 1$

Where:

- n= number of animals per group
- DF = degrees of freedom (minimum=10 and maximum=20)
- *k* = number of experimental groups (in this case, 3 experimental groups)

n = (20/3) + 1 = 6.7+1 = 7.7 = 8 mice/group. For uniformity, the naïve (non-experimental) group was also also assigned equal number of mice as experimental group

Total number of subjects (N) = n x 4 = 8 x 4 = 32 mice

A replica of each experimental group (8 x 3 = 24 mice) was used for the survival analysis. Additionaly, 4 mice were used as parasite donor mice for the experimental groups. Therefore, the grand total (N') = 32 + 24 + 4 = 60 mice.

3.5: Experimental design

Four mice were randomly picked and infected with 5 x 10^4 cryo-preserved clone of *T. b. rhodesiense* to ascertain the viability and thereafter, they were then used as the parasite donor mice. The remaining mice were randomly assigned into four groups with n=8 mice per experimental group and then left to acclimatize for a week before the commencement of the experiments. Each group was then subjected to treatments as described in the *table 3.1* below. The remaining mice were subjected to similar treatment and used for the survival analysis for the group 2, 3 and 4. The mice were monitored daily and sacrificed at 40^{th} days post infection (dpi).

Table 3.1: ***Experimental groups and treatments***

Mice group	**Sample size (n=8)**	**Treatment**
Group 1	8	Naïve animals (Negative controls)
Group 2	8	Mice were infected with 5 x 10^4 *T. b. rhodesiense* but not challenged with vitamin B12 (positive controls)
Group 3	8	Mice were exposed to 8mg/kg of vitamin B12 oral supplementation from 14^{th} days prior to inoculation of 5 x 10^4 *T. b. rhodesiense* and thereafter until the end of the study.
Group 4	8	Mice were challenged with 8mg/kg of vitamin B12 supplementation from the 4^{th} days post-infection with 5.0 × 10^4 *T. b. rhodesiense* upto 39^{th} day of the study

3.6: Determination of survival rate and parasitaemia levels

Survival rate was determined by monitoring the experimental mice daily for HAT-related clinical manifestation, the time and the day on which each mouse died was recorded. The parasitaemia level for each infected mouse was determined after every two days by microscopic examination of a blood smear collected by tail snip employing the rapid matching method as described by Herbert and Lumsden (1976).

3.7: Determination of neurological integrity

Rapid murine coma and behavior scale (RMCBS) is a quantitative tool usually used in the determination of the general health and well-being of the animal including the neuropathologic injury in motor and cognition systems. Briefly, the individual mouse was scored according to the 10 parameters that included: gait, body balance, body position, touch escape, aggression, grooming, motor performance, limb strength, pinna reflex and the toe pinch as described by

Carroll *et al*. (2010). The mice were routinely checked after every 2 days for clinical symptoms which were scored in a scale of 2-0 for each parameter.

3.8: Evans Blue Assay:

Evans blue assay was performed at the terminal stage of the study (40 dpi) to assess the stability of the blood brain barrier (BBB) by intravenously injecting the mouse from each group with 200µl of 2% Evans blue dye prepared in 0.95% NaCl (w/v) and then allowed to rest for two hours before they were sacrificed. Brains were harvested and photographs were taken against a white background. The brains were then incubated in 2ml formamide for 48 hrs at 37°C on a shaker followed by quantification of the extravated dye spectrophotometrically at 620nm.

3.9: Determination of the change in body weights and relative organ weights (ROW)

The general body weight of mice was determined after every two days using an analytical electronic balance (Mettler PM34, DoltaRange®). This was followed by the determination of the change in the body weight of each individual mouse by subtracting the initial body weight from the final body weight. Subsequently, the relative organ weight of each individual organ was determined by dividing the organ weight by the final body weight and expressed as a percentage.

3.10: Sample collection

The blood samples for hematological assay were collected from individual mice and placed in heparinized tubes while the blood sample for serum was collected in microfuge tubes and left at room temperature for 30 min before being centrifuged at 10,000 rpm for 5 min and stored at 4°C for immunological and biochemical assays. The kidney, brain, liver, spleen and heart were also extracted for both biochemical and histopathological analysis.

3.11: Determination of haematological values

The blood samples from each experimental mouse were analyzed using an automated Bechman Coulter counter machine for a complete blood haemogram that generated the following: hemoglobin (Hb) levels, red blood cell (RBC) count and its indices, white blood cell (WBC) count and subtypes, platelets and indices. Some portion of blood from each mouse was collected into 100 µL microhaematocrit capillary tubes for the determination of PCV as described by Woo, (1970). In brief, the capillary tubes were sealed with plastic at one end and centrifuged in a haematocrit centrifuge at 10 000 rpm for 5 min. PCV for the individual mouse was then read using a micro-haematocrit reader and expressed as a percentage (%) of the total blood volume.

3.12: The Cytokine assays

The levels of the inflammatory cytokines (TNF-α, IL-10 and IFN-γ) in serum were spectrophotometrically measured by sandwich enzyme linked immune sorbent assay ELISA (Multiskan ex-355, Thermo Electron Corporation, Waltham, Massachusetts, USA) (sand-wich ELISA) using cytokine-specific kit procured from Invitrogen (Thermo Fischer Scientific, California, USA) as per the manufacturer's protocol. The spectrophotometric readings were performed by the use of ELISA optical reader (Multiskan ex-355, Thermo electron corporation, Waltham, Massachusetts, USA) at 450nm absorbancy.

3.13: Serum analysis for Liver and Kidney function and Lipid profiles

Serum from each individual mouse was fed into an auto-analyzer machine for the estimation of the levels of Aspartate amino-transferase (AST), alanine amino-transferase (ALT), Bilirubin and alkaline phosphate transferase as a marker of liver function. Creatinine, urea and uric acid levels were considered as a marker of kidney function. Moreover, the levels of high density lipoproteins, cholesterol and triglycerides were also estimated and considered as lipid profiles.

3.14: Determination of Nitric Oxide (NO) levels

Griess reagent was made by mixing equal volumes of solution A and B in an Eppi. Next, 10 µl of Griess reagent was mixed with 75 µl of serum samples from each mouse and 65 µl of solution A in triplicate and then incubated for 30 min. Next, a blank (row H_{1-3}) was prepared by mixing 10 µl of Griess reagent with 140 µl of soltion A followed by the preparation of standard by diluting at 1/10 (eg: 50 µl sdt in 450 µl A. dest) in order to get a concentration of 100 µM in row A_{1-3}. This was followed by making a serial dilution from row B-G_{1-3} (75 µl of A_{1-3} + 150 µl of 100 µM sdt in row A) and then10 µl of Griess reagent +65 µl of A. dest were added to the standard wells. Finally the readings were measure at 548 nm.

3.15: Reduced glutathione (GSH) assay

The homogenization of the individual organ samples were done using an ice-cold homogenizing buffer (0.5 ml of 0.25M sucrose, 5mM Hepes-Tris pH 7.4 with protease inhibitor) and aliquoted into 0.5 cryovial tubes. Subsequently, the homogenates were kept at -80°C awaiting analyses. About 50µl of solution A (5%w/v of Sulphosalicyclic acid and 0.25mM ethylene diamine tetra acetic acid (EDTA) were mixed with 50µl of the individual organ homogenates (brain, liver,

spleen and kidney) and sbsequently centrifuged at 8000 x g for 10 minutes at 4°C. Next, 200µmol of GSH standard solution was prepared in 0.5% sulphosalicylic acid (SSA) and serial dilutions made using the same solution (0.5% SSA) to final concentrations of 100, 50, 25, 12.5, 6.25, 3.13 and 1.56µmol of 5.5 Dithiobis (2-nitrobenzoic acid (DTNB)). The Ellman's reagent was prepared by dissolving 0.1M potassium phosphate buffer into a 5mM of EDTA disodium salt, pH 7.5 (KPE buffer) to a final concentration of 0.6mg/ml. Finally, the first two rows of a 96-well microtitre plate were loaded with 25µl of each standard followed by aliquoting 25µl of the sample into the remaining wells in triplicates. 100µl of freshly prepared DTNB was added to each well and the absorbance measured at 450nm at an interval of 30 seconds using a multi-detection microtitre plate reader (R & D Systems, Minneapolis, MN).

3.16: Determination of malondialdehyde levels (MDA)

To determine lipid peroxidation levels in murine, malondialdehyde levels were measured by assays of thiobarbituric acid reactive species (TBARS) (Draper and Hadley, 1990). Serum samples were mixed with an equal volume of thiobarbituric acid 0.67% and heated at 92-96^{o}C for 30 min. Thiobarbituric acid reactive species production was quantified at 535nm using a spectrometer. Results were expressed as malondialdehyde per milligram of protein (Puhl *et al*., 1994). . The levels of lipid peroxidation were determined by mixing serum from the individual mouse with an equal volume of thiobarbituric acid 0.67% and the resultant mixture was then heated at 92-96^{o}C for 30 min. The quantification of the Thiobarbituric acid reactive species (TBARS) was quantified by a spectrometer set at 535nm.

3.17: Histopathological analysis

The extracted organ samples (brain, liver, kidney and spleen) were fixed in 4% neutral buffered formalin dehydrated in absolute ethanol and subsequently embedded in paraffin wax blocks. The prepared tissues were sectioned at 5μm and subsequently stained using hematoxylin and eosin for microscopic examination. The severity of the pathological injury on the said tissues were graded on a scale of 0–4 where 0 represented normal pathology with no indications of inflammation and 4 indicated a severe tissue inflamation characterized by the presence of high amount of inflammatory cells in the tissues.

3.18: Statistical analysis

Statistical analysis was done using the GraphPad Prism software package (Version 5.0). One-way ANOVA was used to compare the treatment groups with controls. For internal comparisons, Turkey's post hoc test was used. Log-rank (Mantel-Cox) test was also employed in this study for the survival analysis. The results were given as a mean ±SEM with the level of significance set at $P<0.05$.

CHAPTER FOUR

RESULTS

4.1: The impact of vitamin B12 on physiological and hematological indicators due to *T.b.rhodesiense* infection in mice

4.1.1: Effects of vitamin B12 on the pre-patent period due to *T.b.rhodesiense* infection in mice

The result from the current study demonstrates that the mice infected with *T.b.rhodesiense* registered a pre-patent period that ranged between 4 to 5 days and a mean ±SEM of 4.6 ± 0.25. However, the *T.b.rhodesiense* infected mice that were pre exposed to vitamin B12 registered a pre-patent period that ranged from 4-7 days with a mean ±SEM of 5.0 ± 0.65. Additionally, the *T.b.rhodesiense* infected mice that were exposed to vitamin B12 post infection recorded a pre-patent period that ranged from 4-6 days and a mean ± SEM of 5.0 ± 0.55 (Table 4.1). Despite the extension observed in the pre-patent period, these alterations were not statistically significant ($p>0.05$).

Table 4.1: The pre-patent period of experimentally innoculated *T.b.rhodesiense* in a mice model.
Treatment marked with the same letters are not statistically significant different at p<0.05

Mice Group	**Mean Pre-patent period (days) ± SEM**	**Range**
WT - T.b.r controls	4.65 ± 0.25^{a}	4 - 5 days
Vit B12 - WT-T.b.r	5.0 ± 0.65^{a}	4 - 7 days
WT-T.b.r - Vit B12	5.0 ± 0.55^{a}	4 - 6 days

4.1.2: Effects of vitamin B12 on the survival rate of *T.b.rhodesiense* infected mice

As shown in (Fig. 4.1), mice infected with *T.b.rhodesiense* depicted a significant (P<0.0128) reduction in the survival rate since only 2/8 (25%) mice survived beyond 40 days post infection. In contrast, pre or post exposure to Vitamin B12 in *T.b.rhodesiense* infected mice resulted in a significant (P<0.0128) improvement in the survival rate since 5/8 (62.5%) survived past 40 days post infection. Meanwhile, *T.b.rhodesiense* infected mice had a median survival time of 39 dpi relative to the mice that were pre or post exposed to vitamin B12 which registered a median survival time of 47 & 43 dpi respectively.

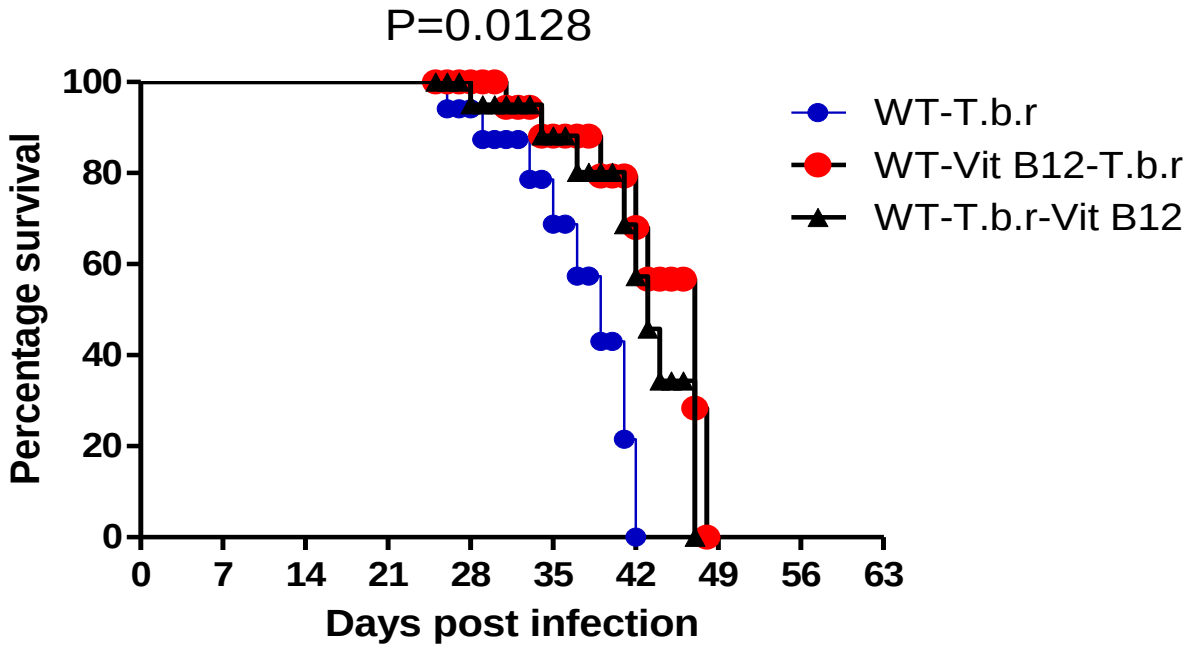

Figure 4.3: Effects of vitamin B12 on the survival rate among the *T.b.rhodesiense* infected mice. Analysis was done using ANOVA with Tukey's test for group comparisons. The indicated level of significance P<0.0128.

4.1.3: The effects of vitamin B12 on parasitaemia during *T.b.rhodesiense* infection in mice

The findings from this study revealed that the mice that were infected with *T.b.rhodesiense* alone and the ones that were subjected to the daily oral supplementation with vitamin B12 from 4th dpi registered a very sharp and steady exponential parasitaemia levels with the first peak at 7th dpi followed by a decline in the parasitaemia levels running to the 11th dpi and a subsequent rise leading to a 2nd peak at 15th dpi. On the other hand, the mice that were exposed to vitamin B12 prior to *T.b.rhodesiense* inoculation showed a reduced but steady exponential parasiteamia levels with a 1st peak registered at 7th dpi followed by a relatively sharp decline to the 10th day with a subsequent gradual increase up to the 15th dpi where the 2nd peak was witnessed. Consequently, there were comparable parasitaemia levels and peaks across all the groups at the 15th dpi further suggesting that the parasiteamia levels were independent of vitamin B12 treatment (Fig. 4.2).

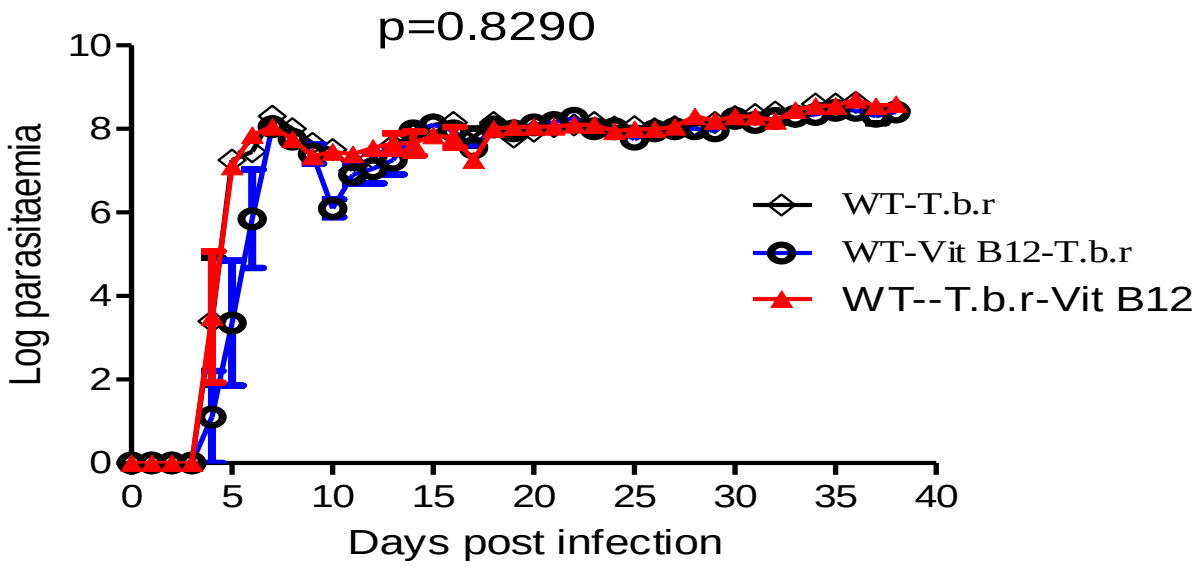

Figure 4.4: Effects of vitamin B12 on parasitaemia levels during *T.b.rhodesiense* infection in mice. Analysis was done using ANOVA with Tukey's test for group comparisons. The indicated level of significance P = 0.8290

4.1.4: Effects of Vitamin B12 on the *T.b.rhodesiense* induced neurological injury in mice model

Results from the present study clearly demonstrates that mice infected with *T.b.rhodesiense* registered a significant (P<0.0001) neurological injury in comparison to the naïve group. The observed *T.b.rhodesiense*-driven neurological injury was characterized by deterioration of general health of mice which was coupled with diminished overall scores in RMCBS. However, pre or post exposure of *T.b.rhodesiense* infected mice to vitamin B12 led to a significant improvement in neurological performance as evidenced by higher scores in RMCBS (Fig.4.3).

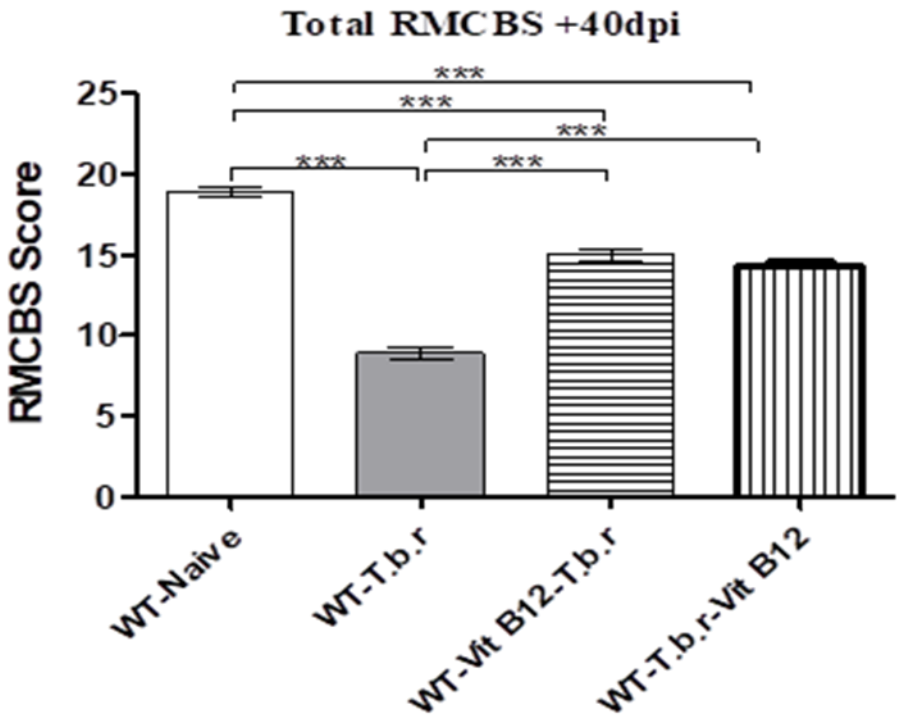

Figure 4.5: Effects of vitamin B12 on neurological function and general well-being of mice following *T.b.rhodesiense* infection. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons. The asterisks indicated the level of significant differences: ***P<0.0001 while the bars represent mean ± SEM.

4.1.5: Effects of vitamin B12 on individual RMCBS scores during *T.b. rhodesiense* infection in mice

It is evident from the results obtained from this study that mice infected with *T.b. rhodesiense* alone elicited a marginal reduction in the aggression, grooming, pinna reflex and gait scores, though not statistically significant (P >0.05). Nevertheless, pre or post administration of vitamin B12 restored these individual RMCBS scores further suggesting some improvements associated with the neurological function during *T.b. rhodesiense* infection (Fig.4.4).

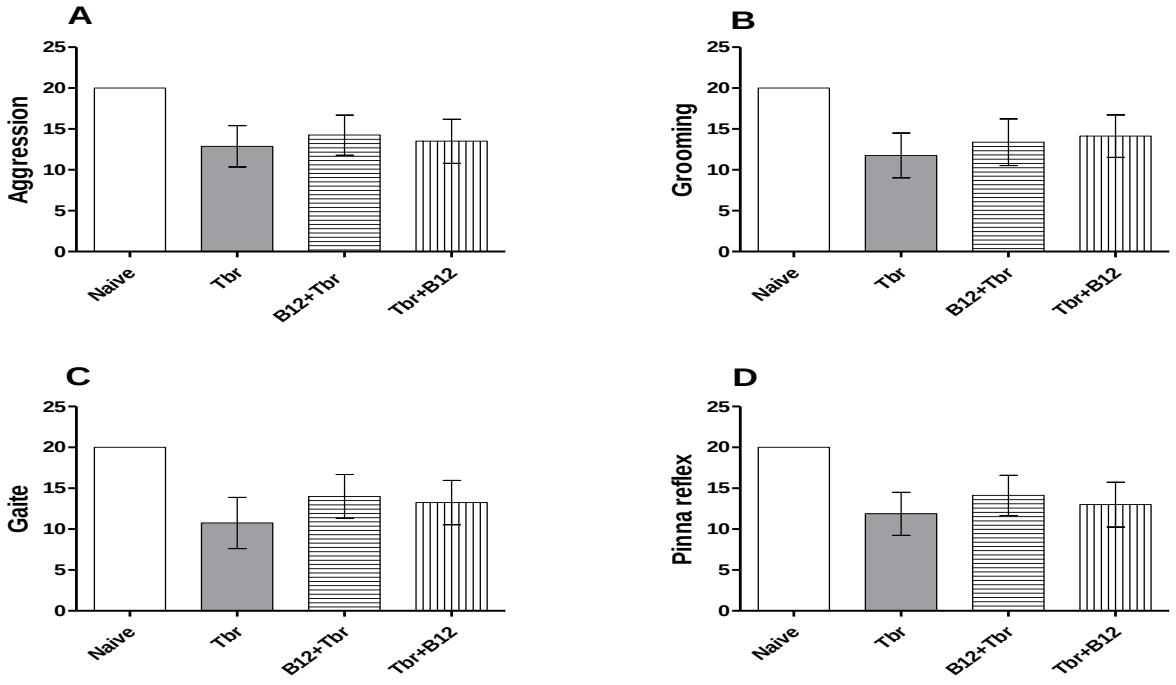

Figure 4.6: Effects of vitamin B 12 on individual RMCBS scores among *T.b.rhodesiense* infected mice. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons. Bars represent mean ± SEM..

4.1.6: Effects of Vitamin B12 on the integrity of blood-brain barrier during the *T.b.rhodesiense* infection in mice

The findings from this study revealed that mice infected with *T.b.rhodesiense* had a disrupted blood-brain barrier when compared with the mice in the naïve group. This was achieved by quantification of the amount of Evans blue dye that penetrated the blood-brain barrier of *T.b.rhodesiense* infected mice. The results displayed a significant ($P<0.05$) elevations of Evans blue dye in the brain relative to the naïve group. However, pre or post oral supplementation with vitamin B12 resulted in a significant reduction in the levels of Evans blue dye in brain of *T.b.rhodesiense* infected mice (Fig. 4.5A). In contrast, the concentration of the amount of Evans blue dye retained in the blood was significantly ($P<0.05$) reduced in *T.b.rhodesiense* infected mice relative to other groups (Fig. 4.5B). Additionally, This *T.b.rhodesiense*- driven disruption of blood-brain barrier was exhibited by extraversion of Evans blue dye which conspicuously dark stained the brain of the mice infected with *T.b.rhodesiense* comparative to the brain harvested from the naïve group and groups exposed to vitamin B12 as shown below (Fig. 4.5C).

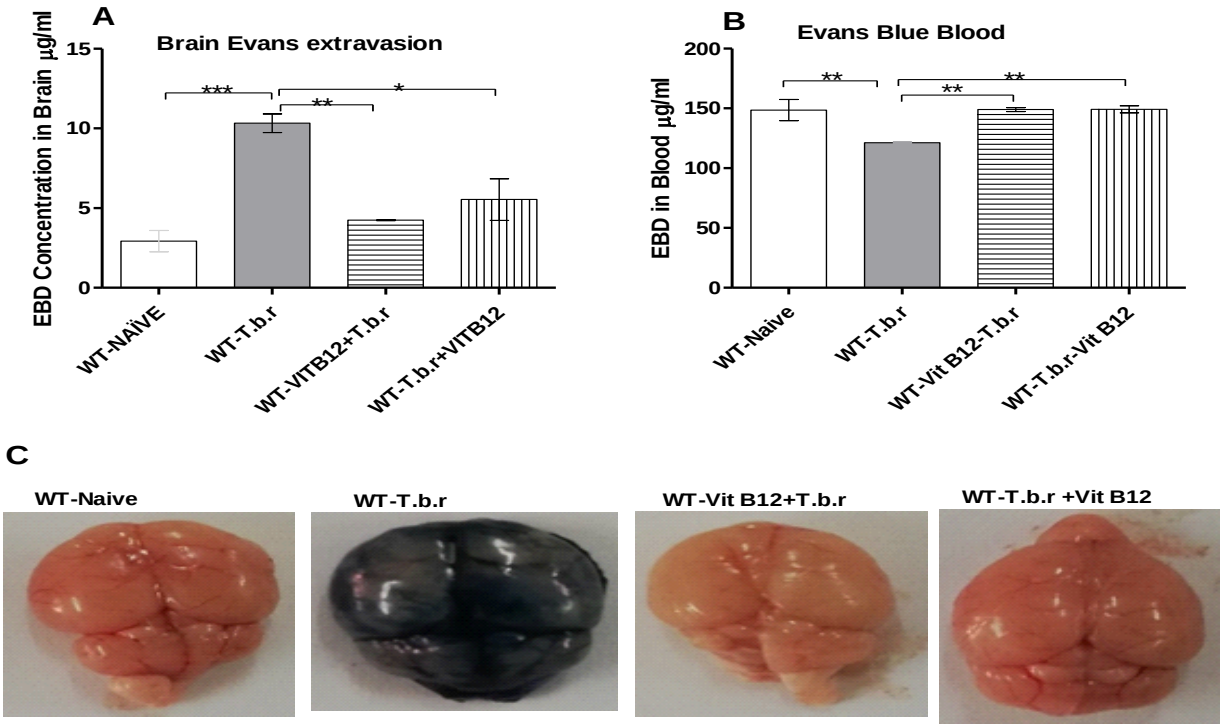

Figure 4.7: Effect of vitamin B12 on the integrity of blood-brain barrier of mice experimentally infected with *T. brucei rhodesiense*. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons. (Asterisks indicates the level of significant differences: *P<0.05; *P<0.001; ***P<0.0001). Bars represent mean ± SEM. Photos of brain harvested from mice after the injection with Evans blue dye for the test of the integrity of blood-brain barrier.

4.1.7: Effects of Vitamin B12 on *T.b.rhodesiense*-induced weight loss in mice

The *T.b.rhodesiense* infected mice registered a significant (P<0.05) reduction in body weight when compared to the naïve group. Noteworthy, pre or post exposure with vitamin B12 significantly (P<0.05) ameliorated the *T.b.rhodesiense* -driven body weight loss, denoting protection from *T.b.rhodesiense* -induced body weight loss (Fig. 4.6).

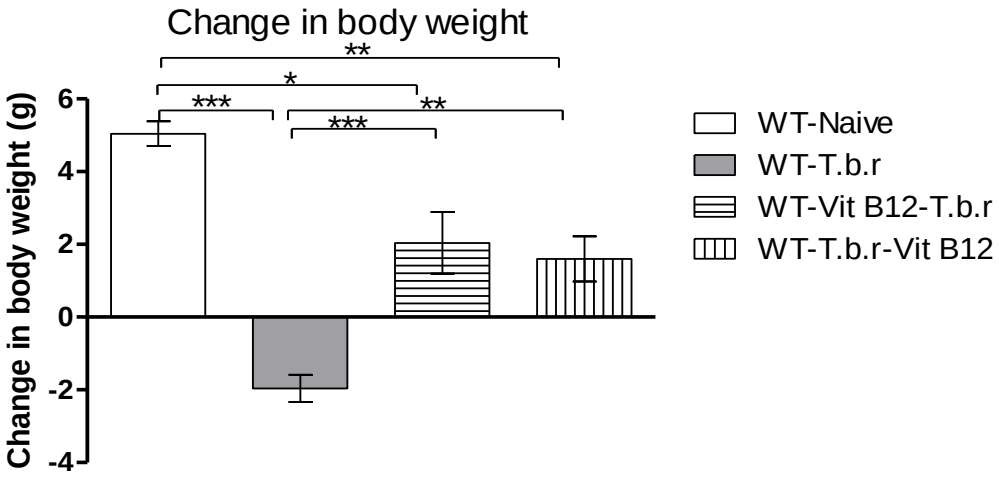

Figure 4.8: Effect of vitamin B12 on the mean weight of mice experimentally infected with *T. brucei rhodesiense* and their controls. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons. The asterisks indicate the level of significant differences: *P<0.05; **P<0.001; ***P<0.0001. Bars represent mean ± SEM.

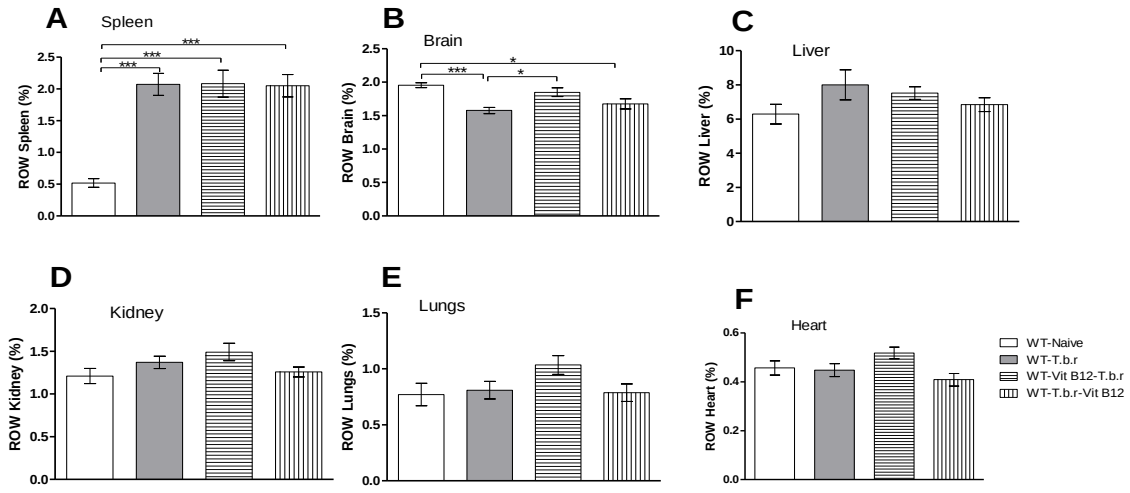

Figure 4.9: Effect of vitamin B12 on the mean relative organ weight of mice experimentally infected with *T. brucei rhodesiense* and their controls. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons (Asterisks indicate the level of significant differences: *P<0.05; ***P<0.0001). Bars represent mean ± SEM.

4.1.9: Effects of Vitamin B12 on the levels of hematocrit, RBC and hemoglobin during *T.b.rhodesiense* infection in mice

Infection of mice with *T.b. rhodesiense* significantly (P<0.05) suppressed the levels of hematocrit (HCT), red blood cells (RBC) with a concomitant decrease in the levels of hemoglobin (HGB) (P<0.05) (Fig. 4.8 A - C respectively). However, pre or post oral supplementation with vitamin B12 occasioned a significant elevation in the levels of HCT, RBC and HGB among the *T.b.rhodesiense* infected mice.

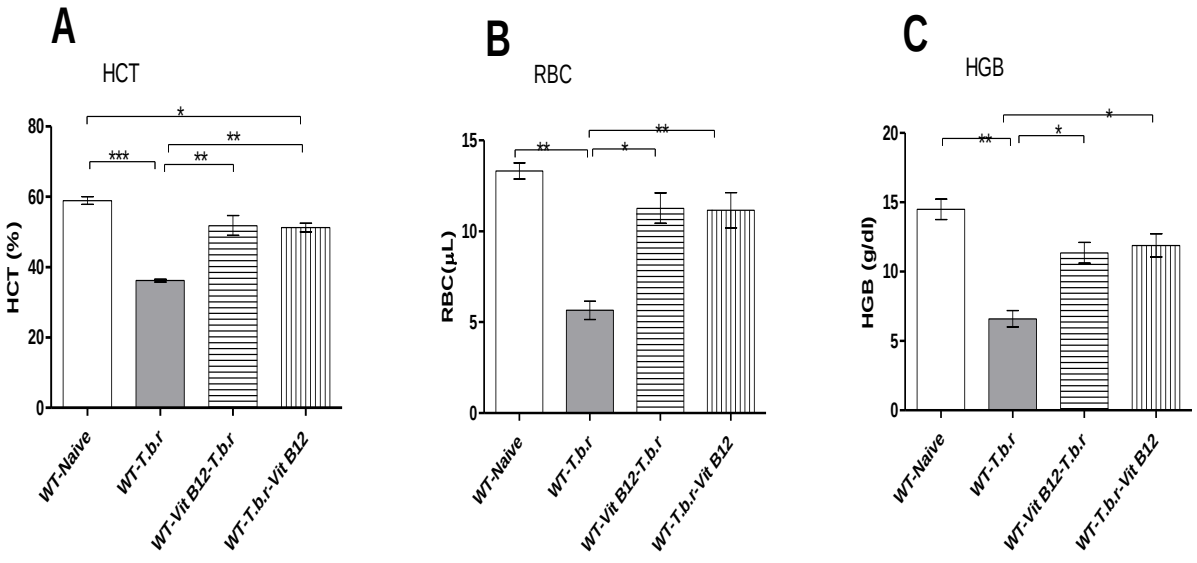

Figure 4.10: Effect of vitamin B12 on the mean HCT, RBC and HGB in mice experimentally infected with *T. brucei rhodesiense* and their controls. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons (Asterisks indicate the level of significant differences: *P<0.05; **P<0.001; ***P<0.0001). Bars represent mean ± SEM.

4.1.10: Effects of Vitamin B12 on RBC indices following *T.b.rhodesiense* infection in mice

Infection of mice with *T.b.rhodesiense* resulted in significant (P<0.05) suppressions of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and an increase in the red cell distribution width standard deviation (RDW-SD) and in the percentage red cell distribution width coefficient of variation (RDW-CV) (Figs 4.9A-D). Importantly, *T.b.rhodesiense* induced suppression and augmentation of RBC indices were abrogated due to pre or post exposure to vitamin B12

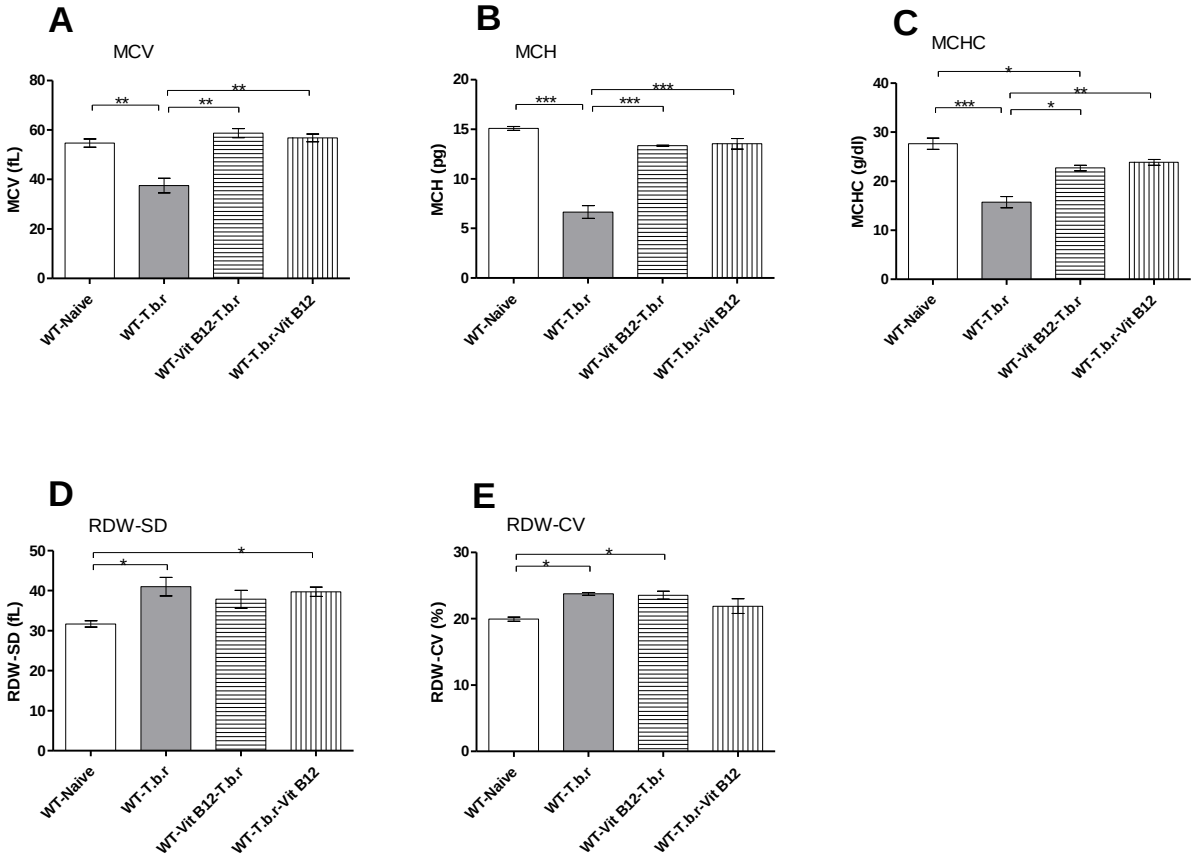

Figure 4.11: Effect of vitamin B12 on the mean HCT, RBC and HGB in mice experimentally infected with *T. brucei rhodesiense* and their controls. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons (Asterisks indicate the level of significant differences: *P<0.05; **P<0.001; ***P<0.0001). Bars represent mean ± SEM.

4.1.11: Effects of Vitamin B12 on WBC and sub types during *T.b.rhodesiense* infection in mice

The results obtained herein revealed that group of mice infected with *T.b.rhodesiense* had significantly ($P<0.05$) elevated levels of WBC count and lymphocytes in comparison to the naïve group (Fig.4.10A-B). Notably, pre or post oral supplementation with Vitamin B12 considerably down regulated this *T.b.rhodesiense*-induced leukocytosis and lymphocyte count levels. On contrary, there was significant ($P<0.05$) reduction in the levels of neutrophils, elevation in the levels of monocytes following infection of mice with *T.b.rhodesiense* (Fig.4.10C-D). Importantly, the mentioned *T.b.rhodesiense* induced neutropenia and monocytosis were restored in the presence of vitamin B12. Additionally, there was a significant ($P<0.05$) elevation in the levels of eosinophils and basophils following infection of mice with *T.b.rhodesiense* (Fig.4.10E-F). Worthy of note, pre or post administration with vitamin B12 up-regulated even further the levels of eosinophils and basophils.

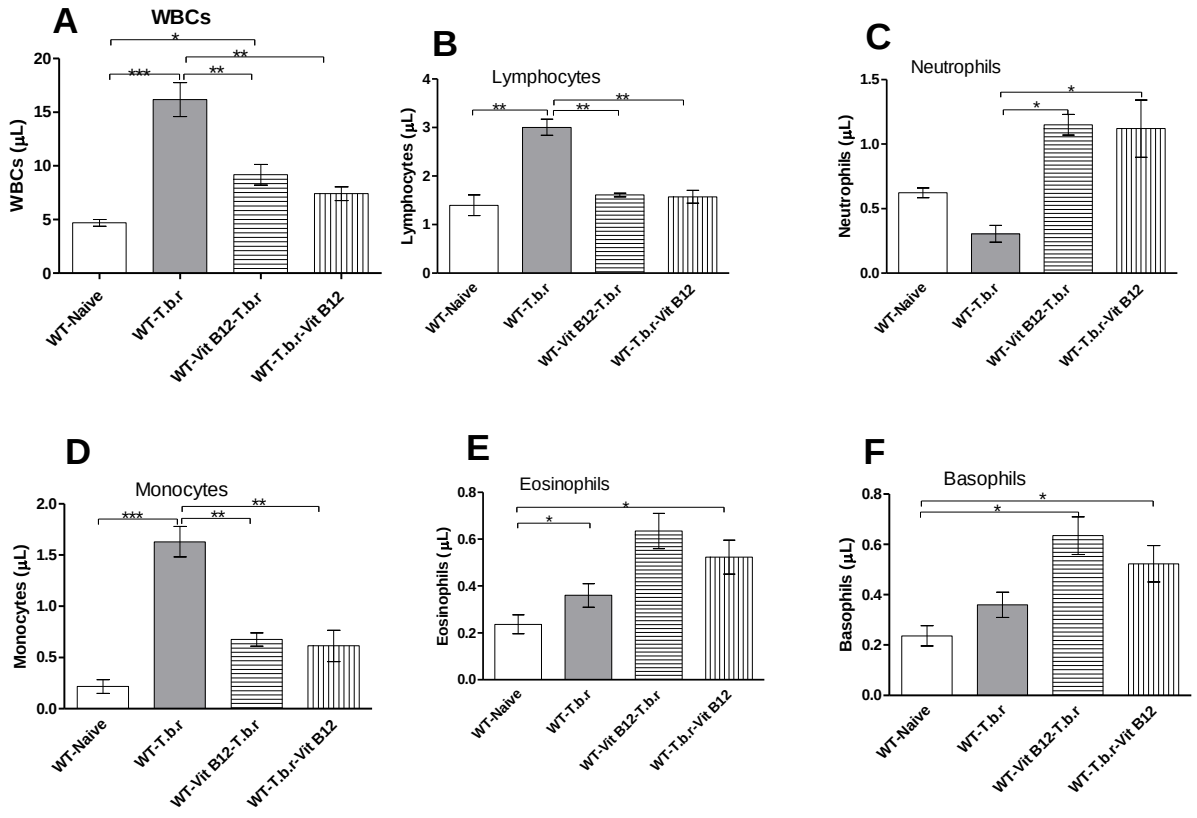

Figure 4.12: Effect of vitamin B12 on the WBCs and sub types in mice experimentally infected with *T. brucei rhodesiense* and their controls. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons (Asterisks indicate the level of significant differences: *P<0.05; **P<0.001; ***P<0.0001). Bars represent mean ± SEM.

4.1.12: Effects of Vitamin B12 on platelets and its indices during *T.b.rhodesiense* infection in mice

A comparative analysis of platelet and platelet indices following infection of mice with *T.b.rhodesiense* revealed comparable results in platelet levels and its indices like P-LCR, PCT, PDW and MPV across all the experimental groups (Fig. 4.11A-E respectively).

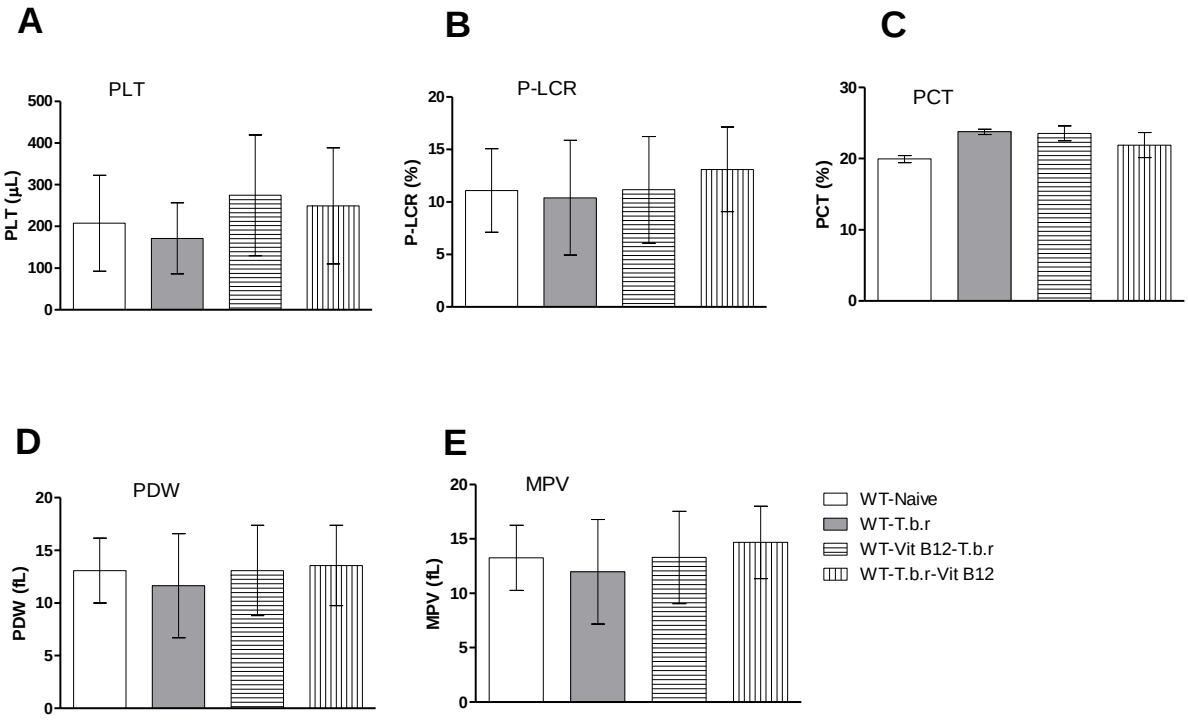

Figure 4.13: Effect of vitamin B12 on the platelets and indices in mice experimentally infected with *T. brucei rhodesiense* and their controls. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons. Bars represent mean ± SEM.

4.2: The impact of vitamin B12 in the regulation of *T.b.rhodesiense* -induced immune response and organ damage

4.2.1: Effects of Vitamin B12 on the levels of immunoglobulins following *T.b.rhodesiense* infection in mice

A significant (P<0.05) elevation in the levels of total immunoglobulins was registered by group of mice infected with *T.b.rhodesiense* in comparison to the naïve group of mice (Fig.4.12). Remarkably, pre or post administration of Vitamin B12 further enhanced the levels of total immunoglobulins among the *T.b.rhodesiense* infected mice.

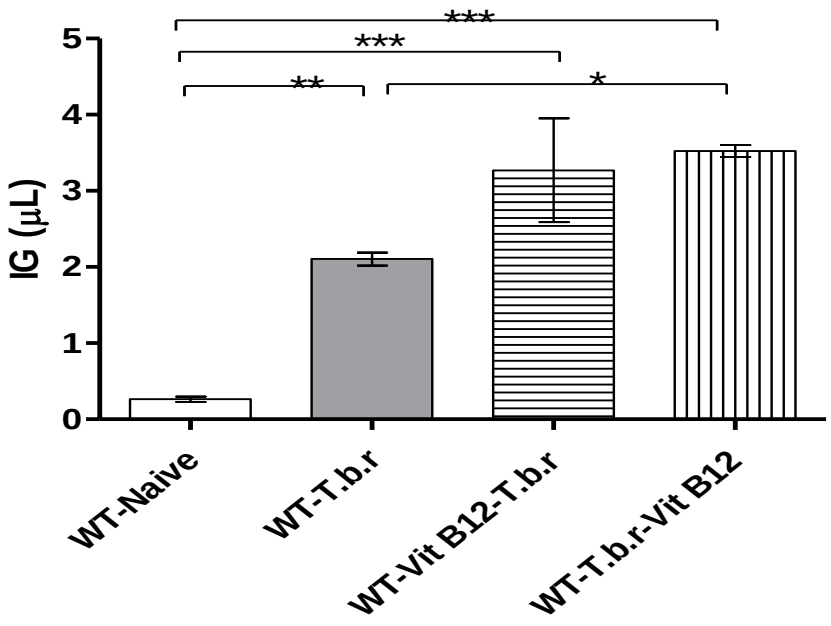

Figure 4.14: Effect of vitamin B12 on the mean immunoglobulin's of mice experimentally infected with *T. brucei rhodesiense*. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons (Asterisks indicate the level of significant differences: *P<0.05; **P<0.001; ***P<0.0001). Bars represent mean ± SEM.

4.2.2: Effects of Vitamin B12 on the levels of inflammatory cytokines following *T.b.rhodesiense* infection in mice

The serum levels of inflammatory cytokines were determined and the results from this study clearly indicates that the levels of pro-inflammatory cytokines (TNF-α and IFN-γ) were significantly ($P<0.05$) elevated in *T.b.rhodesiense* infected mice relative to the naïve control group of mice (Fig. 4.13A-B). Importantly, this heightened levels of pro-inflammatory cytokines was nullified by either pre or post exposure with vitamin B12. On the other hand, infection of mice with *T.b.rhodesiense* culminated in the suppression of the anti-inflammatory cytokine interleukin-10 (IL-10) (Fig. 4.13C). However, pre or post vitamin B12 administration significantly ($P<0.05$) up-regulated the levels of IL-10. Remarkably, pre or post vitamin B12 administration showed a tendency to reverse the IL-10 suppression. An analysis of the ratios of the pro-inflammatory cytokines versus the anti-inflammatory cytokines revealed that there was higher levels in *T.b.rhodesiense* infected group of mice (Fig. 4.13D-E), indicative of *T.b.rhodesiense*-induced inflammation which was attenuated with pre or post administration with vitamin B12.

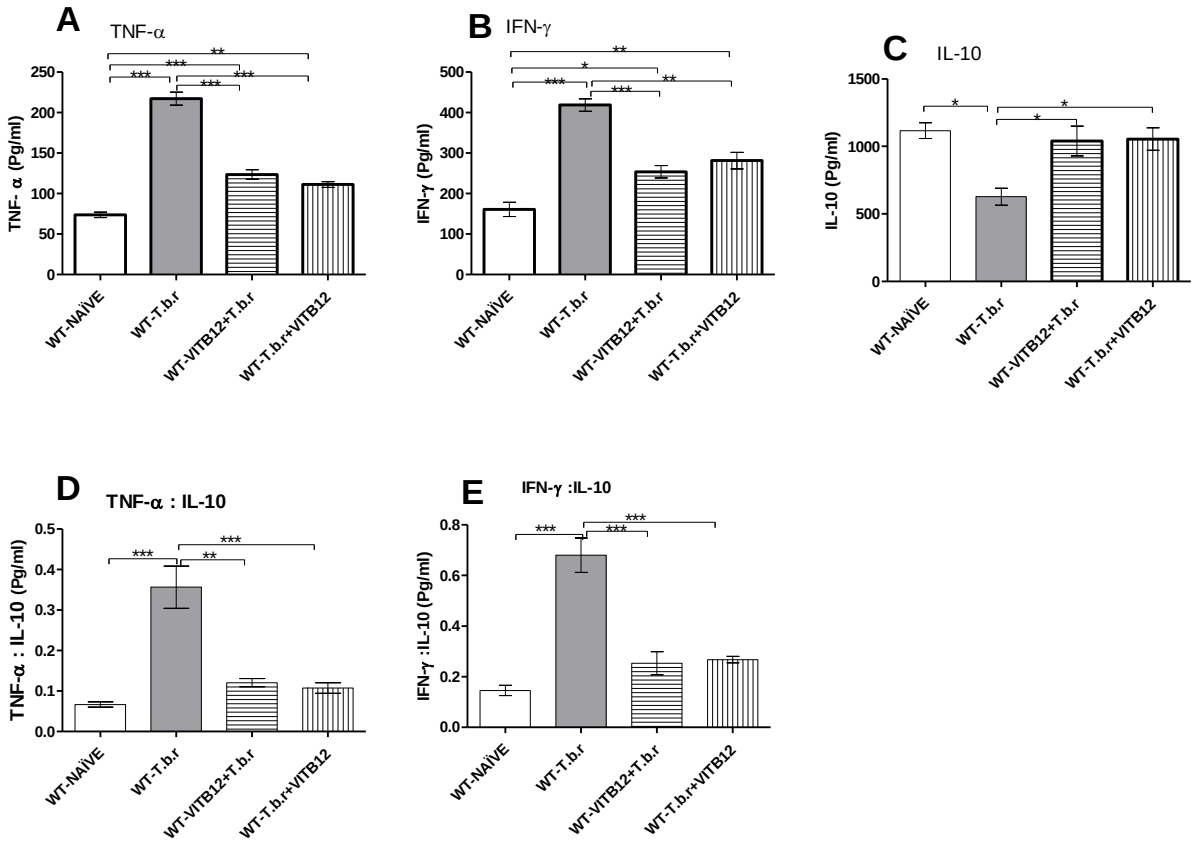

Figure 4.15: Effect of vitamin B12 on the mean inflammatory cytokines of mice experimentally infected with *T. brucei rhodesiense*. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons (Asterisks indicate the level of significant differences: *P<0.05; **P<0.001; ***P<0.0001). Bars represent mean ± SEM.

4.2.3: The effects of vitamin B12 on the mean nitric oxide (NO) during *T.b.rhodesiense* infection in mice

The result obtained herein established that *T.b.rhodesiense* infection in mice induced a significant (P<0.0001) elevation in the levels of NO in comparison to the naïve group (Fig. 4.14), denoting *T.b.rhodesiense*-induced inflammation. Strikingly, pre or post oral supplementation with vitamin B12 significantly (P<0.0001) down-regulated the levels of NO among the *T.b.rhodesiense* infected mice.

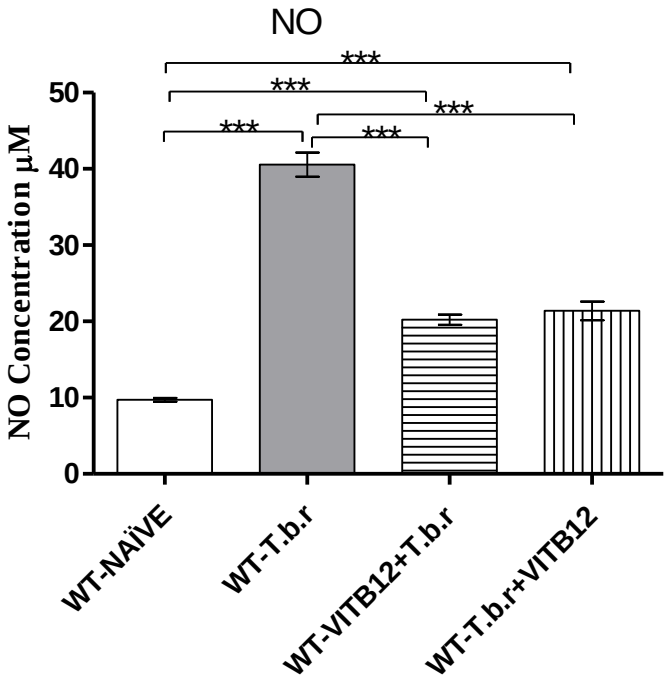

Figure 4.16: Effect of vitamin B12 on the mean nitric oxide of mice experimentally infected with *T. brucei rhodesiense* and their controls. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons (Asterisks indicate the level of significant differences: ***P<0.0001). Bars represent mean ± SEM.

4.2.4: Effects of Vitamin B12 on *T.b.rhodesiense* -induced liver injury

The results from the present study indicate that *T.b.rhodesiense* infection in mice resulted in significant (P<0.05) elevations of the liver biomarkers alanine aminotransferase (ALT) (Fig 4.15A), aspartate aminotransferase (AST) (Fig 4.15B), alkaline phosphatase (Fig 4.15C) and total bilirubin (Fig 4.15D) signaling *T.b.rhodesiense* -induced liver damage. Exceptionally, these *T.b.rhodesiense*-induced liver biomarkers were abolished in the presence of vitamin B12. Nevertheless, the levels of GGT were comparable across all the groups (Fig. 4.13E).

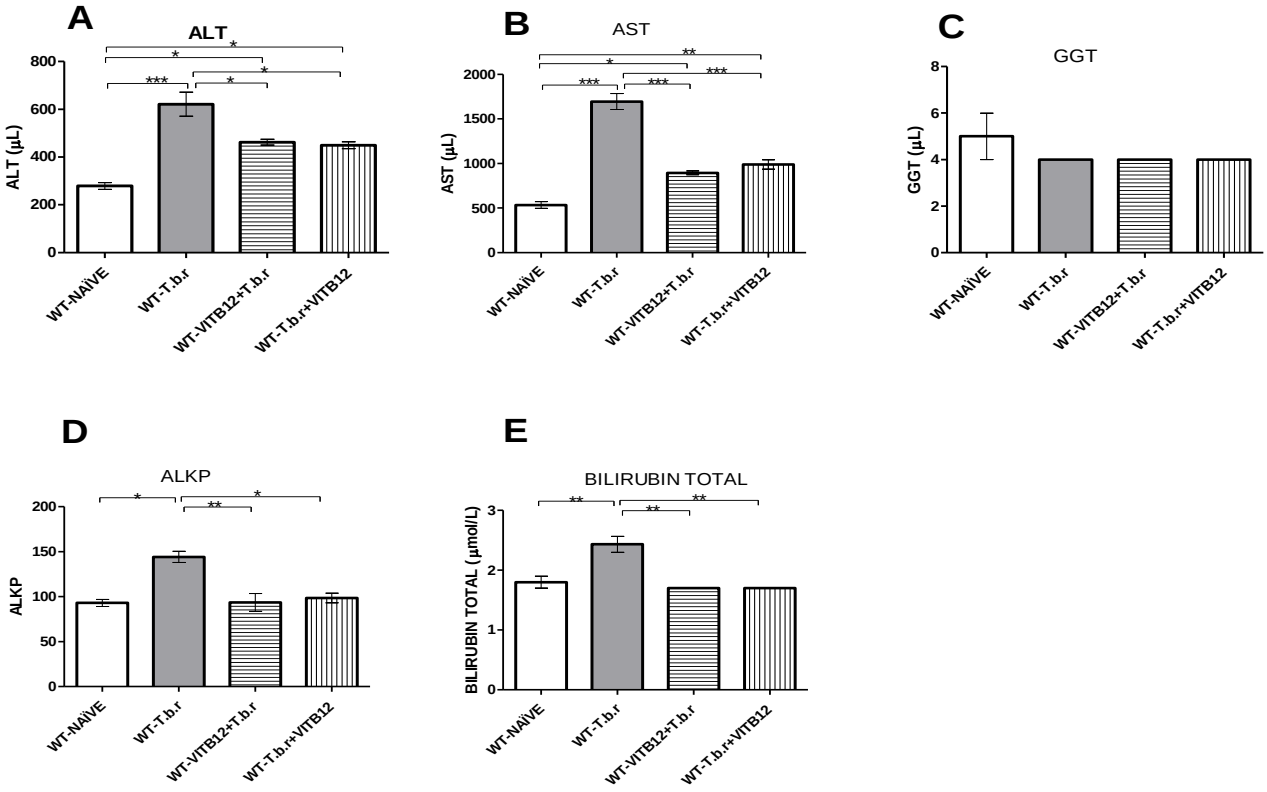

Figure 4.17: Effect of vitamin B12 on the liver function indicators in mice experimentally infected with *T. brucei rhodesiense*.Analysis was done using ANOVA with Tukey's post hoc test for group comparisons (Asterisks indicate the level of significant differences: *P<0.05; **P<0.001; ***P<0.0001). Bars represent mean ± SEM.

4.2.5: Effects of Vitamin B12 on *T.b.rhodesiense* –induced alteration of total protein and albumin

Mice infected with *T. brucei rhodesiense* registered a significant (P<0.001) decline in the albumin levels compared to the naïve group (Fig. 4.16 A), suggesting the damage of both liver and kidney. However, pre or post exposure to vitamin B12 significantly (P<0.001) up-regulated the albumin levels in the *T. brucei rhodesiense* infected mice. On the other hand, the levels of total protein were comparable across the groups (Fig. 4.16B).

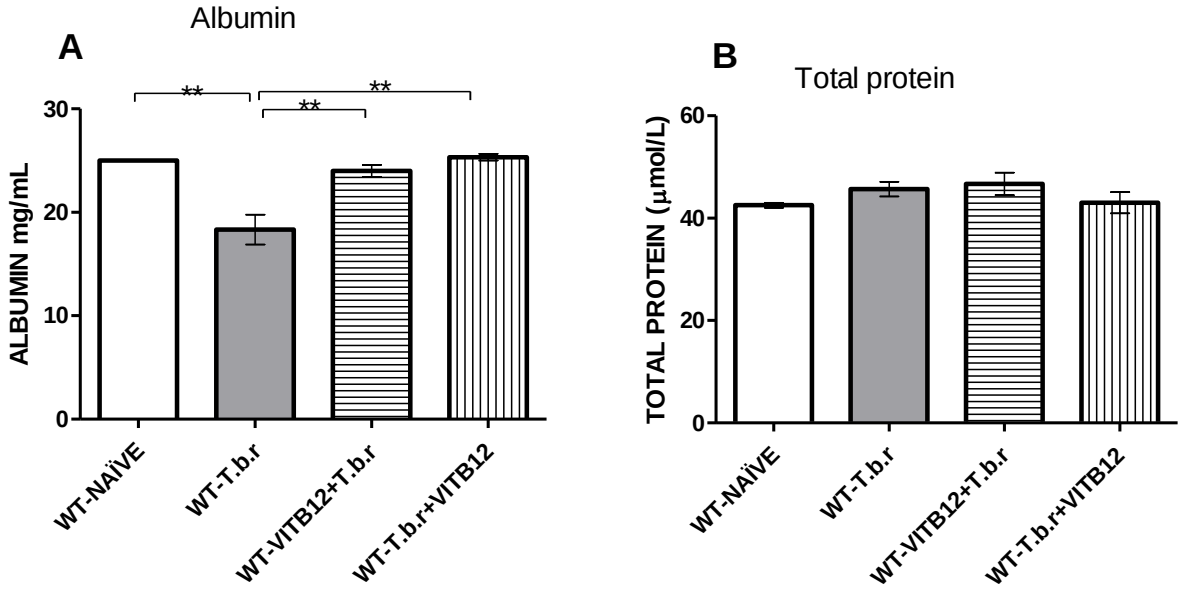

Figure 4.18: Effect of vitamin B12 on the albumin and total protein of mice experimentally infected with *T. brucei rhodesiense*. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons (Asterisks indicate the level of significant differences: **P<0.001). Bars represent mean ± SEM.

4.2.6: Effects of Vitamin B12 on *T.b.rhodesiense* -induced kidney damage

The kidney enzyme biomarker creatinine, and metabolic product like urea were significantly ($P<0.05$) elevated following infection of mice with *T.b.rhodesiense* (Fig. 4.17A-B), indicative of *T.b.rhodesiense* -induced kidney inflammation. Noticeably, the rise in creatinine and urea was curtailed in the presence of Vitamin B12.

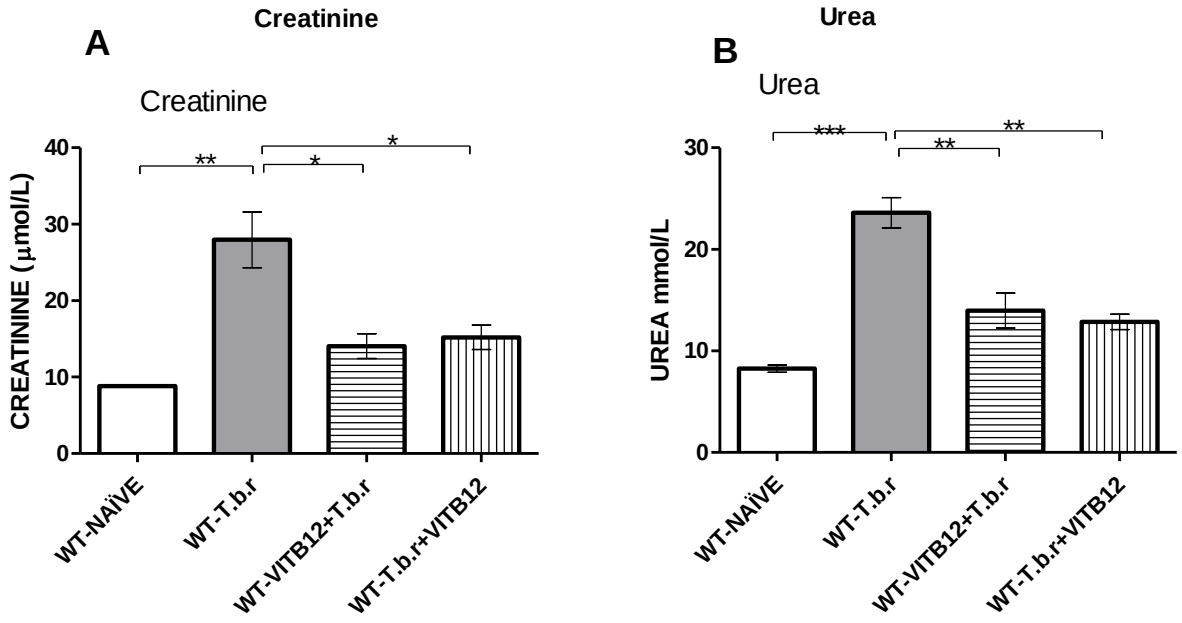

Figure 4.19: Effect of vitamin B12 on the mean indicators of kidney function in mice experimentally infected with *T. brucei rhodesiense*. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons (Asterisks indicate the level of significant differences: *P<0.05; **P<0.001; ***P<0.0001). Bars represent mean ± SEM.

4.2.7: Effects of vitamin B12 on uric acid following *T. brucei rhodesiense* infection in mice

Mice infected with *T. brucei rhodesiense* registered a significant (P<0.05) elevation in the levels of uric acid relative to the naïve group. Conversely, oral supplementation with vitamin B12 caused a significant (P<0.05) reduction in the levels of uric acid among the *T. brucei rhodesiense* infected mice.

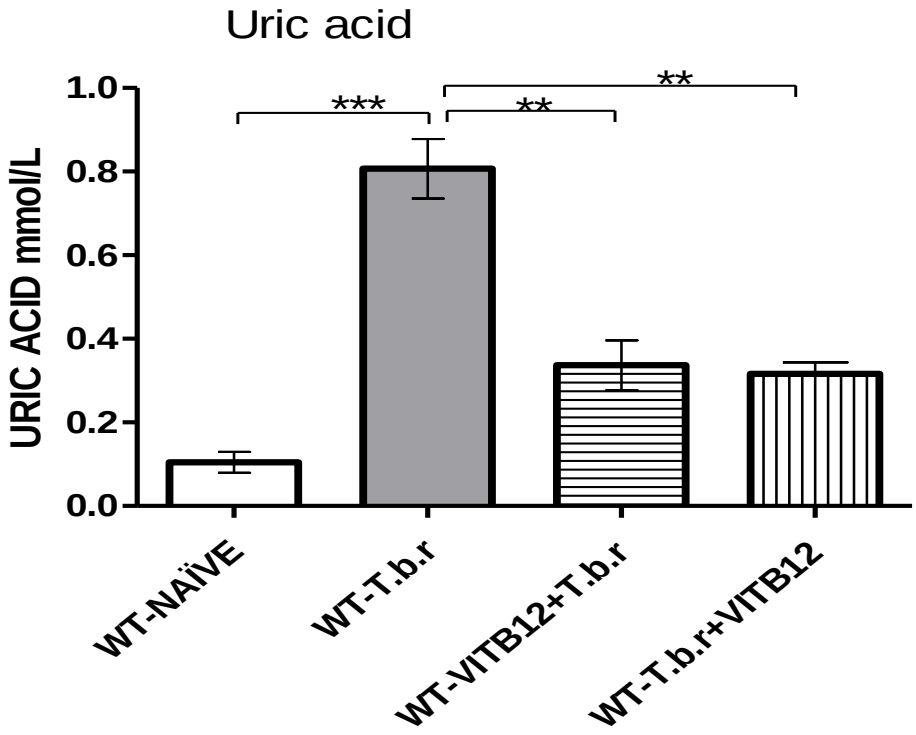

Figure 4.20: Effect of vitamin B12 on the mean uric acid levels in mice experimentally infected with *T. brucei rhodesiense* and their controls. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons (Asterisks indicate the level of significant differences: **P<0.001; ***P<0.0001). Bars represent mean ± SEM.

4.2.8: Effects of Vitamin B12 on lipid profile following *T.b.rhodesiense* infection in mice

Results from this study revealed that mice inoculated with *T.b.rhodesiense* registered a significant (P<0.05) elevation in serum levels of triglycerides and total cholesterol when compared to the naïve group (Fig. 4.19A-B). However, pre or post administration with vitamin B12 significantly (P<0.05) down regulated the *T.b.rhodesiense*-driven elevated levels of triglycerides and total cholesterols. On the other hand, the *T.b.rhodesiense* infection in mice triggered a significant (P<0.05) depletion in the levels of high density lipoproteins comparative to the naïve group (Fig. 4.19C). Notably, pre or post exposure to vitamin B12 significantly (P<0.05) restored the levels of HDL among the *T.b.rhodesiense* infected mice.

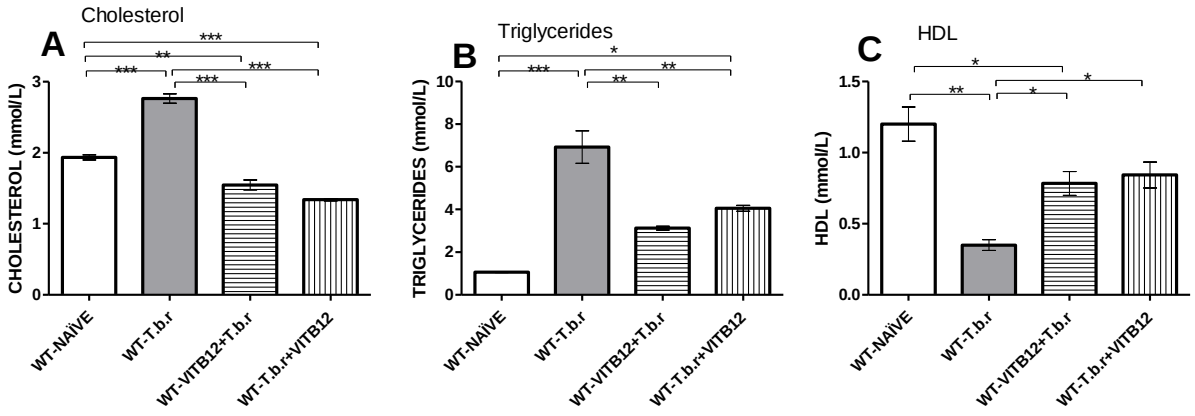

Figure 4.21: Effect of vitamin B12 on the lipid profiles in mice experimentally infected with *T. brucei rhodesiense*. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons (Asterisks indicate the level of significant differences: *P<0.05; **P<0.001; ***P<0.0001). Bars represent mean ± SEM.

4.3: Administration of vitamin B12 ameliorates the *T.b.rhodesiense* -driven organ pathology and oxidative stress

4.3.1: The effects of vitamin B12 on liver tissues following *T. brucei rhodesiense* infection in mice

In this study, the liver histological tissues from the mice infected with *T.b.rhodesiense* registered a diffuse congestion of hepatic vessels (C), Focal infiltration of liver parenchyma with mononuclear cells (Star) and multifocal hemorrhages relative to the naïve group suggesting hepatic injury. However, pre or post exposure to vitamin B12 confers some protection from *T.b.rhodesiense*-driven liver injury (Fig. 4.20).

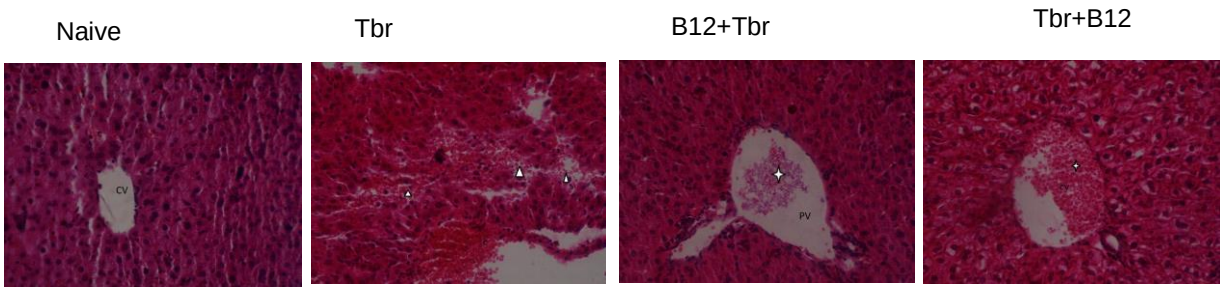

Figure 4.22: Effects of vitamin B12 on liver tissue sections following *T.b. rhodesiense* infection in mice. Histological examination was done using H and E staining and X400 magnification. PV-portal vein, CV-central vein, C-congestion. Arrows indicate hepatocyte cytoplasm vacuolation.

4.3.2: The effects of vitamin B12 on brain cells following *T. brucei rhodesiense* infection in mice

The brain sections from mice infected with, *T.b.rhodesiense* revealed chronic brain injury that was characterized by the congestion of meningeal blood vessels (C) and mononuclear infiltration of the meninges (arrow). However, pre or post exposure to oral supplementation with B12 showed an improved recovery from the *T.b.rhodesiense*-driven brain injury (Fig. 4.21).

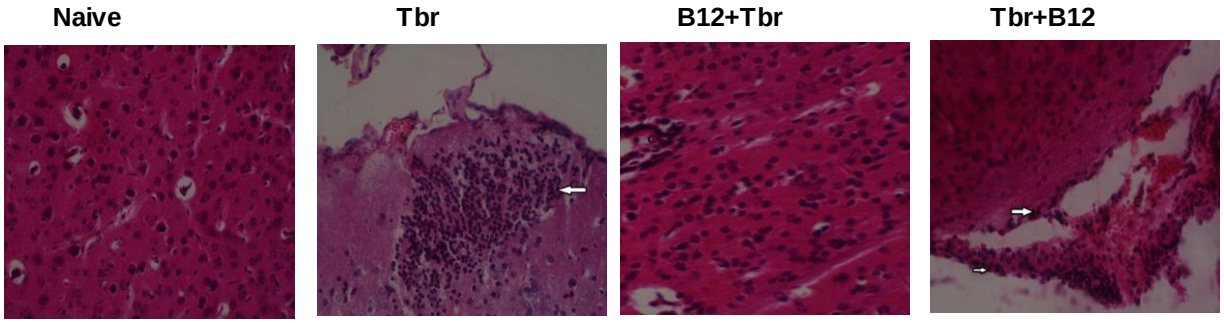

Figure 4.23: Effects of vitamin B12 on brain tissues sections following *T.b.rhodesiense* infection in mice. Histological examination was done using H and E staining and X400 magnification. BV-blood vessels, Arrows indicated areas of pathology.

4.3.3: The effects of vitamin B12 on the cells of spleen following *T. brucei rhodesiense* infection in mice

Further histological examinations were done on spleen and the result revealed a comparable *T.b.rhodesiense* –driven congestion across the groups relative to the naïve group (Fig. 4.22).

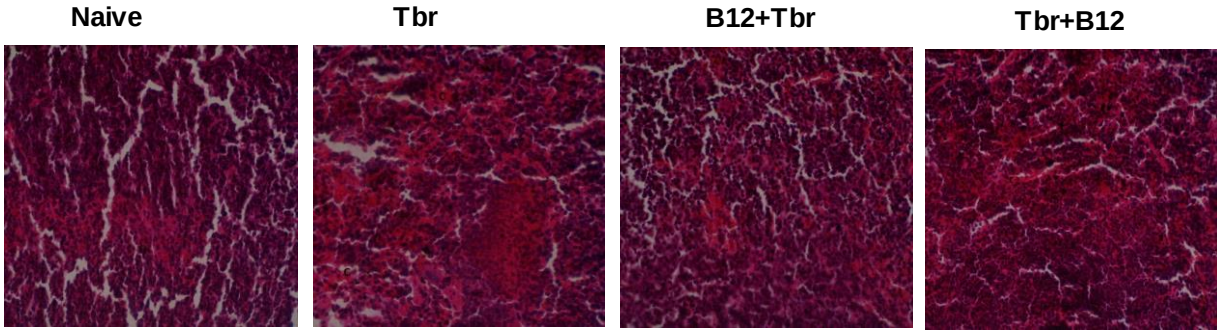

Figure 4.24: Effects of vitamin B12 on spleen tissue sections following *T.b.rhodesiense* infection in mice. Histological examination was done using H and E staining.

4.3.4: Effects of Vitamin B12 on glutathione (GSH) levels following *T.b.rhodesiense* infection in mice

Infection of mice with *T.b.rhodesiense* resulted in significant ($P<0.05$) depletion of reduced glutathione (GSH) in the spleen (Fig 4.23A), brain (Fig 4.23B) and liver (Fig 4.23C). Interestingly, the presence of vitamin B12 among the *T.b.rhodesiense* infected mice blocked the GSH depletions. In the kidney and lungs, infection of mice with *T.b.rhodesiense* induced a significant ($P<0.05$) elevation of GSH levels. Pre or post oral supplementation with vitamin B12 significantly ($P<0.05$) restored the levels of GSH in the kidney and the lungs (Fig 4.23D-E). Additional findings, reveals that the GSH level in the heart were unaffected during the infection process (Fig 4.23F).

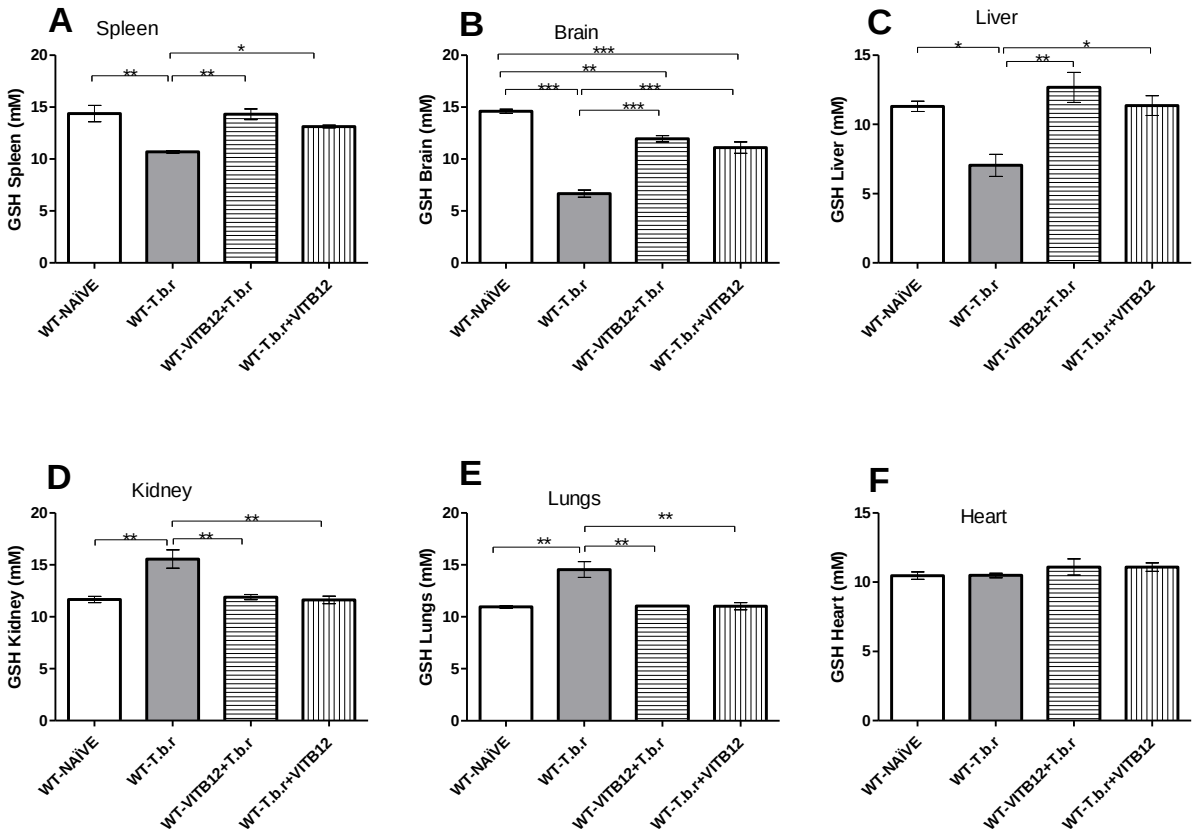

Figure Error! No text of specified style in document.**4.25: Effect of vitamin B12 on the *T.b.rhodesiense*-induced oxidative stress in mice.** Analysis was done using ANOVA with Tukey's post hoc test for group comparisons (Asterisks indicate the level of significant differences: *P<0.05; **P<0.001; ***P<0.0001). Bars represent mean ± SEM.

4.3.5: Effects of Vitamin B12 on melandialdehyde (MDA) levels during *T.b.rhodesiense* infection in mice

The result herein revealed that *T.b.rhodesiense* infection in mice prompted a significant (P<0.0001) elevation in the levels of MDA in comparison to the naïve group (Fig. 4.24). Remarkably, the MDA levels were significantly (P<0.0001) reduced in the presence of Vitamin B12.

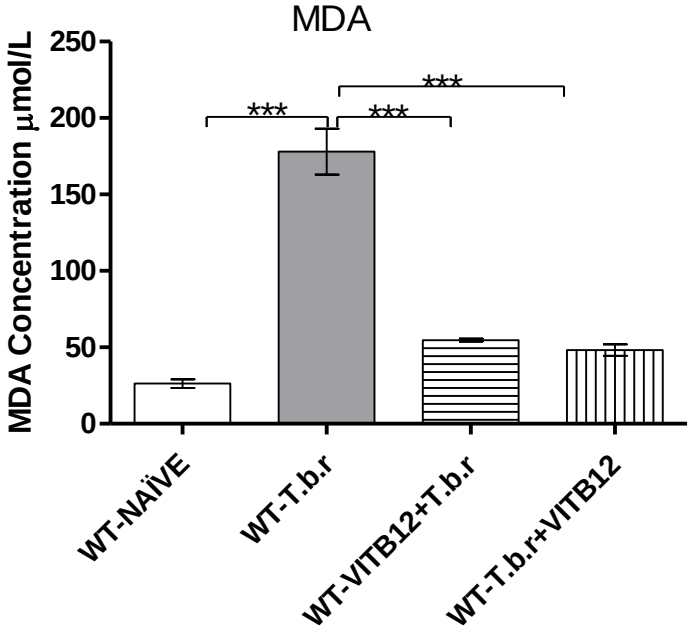

Figure 4.26: Effect of vitamin B12 on the MDA in mice experimentally infected with *T. b. rhodesiense*. Analysis was done using ANOVA with Tukey's post hoc test for group comparisons (Asterisks indicate the level of significant differences: ***P<0.0001). Bars represent mean ± SEM.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMENDATIONS

5.1 DISCUSSION

5.1.1. Effects of vitamin B12 on the pre-patent period of *T. b. rhodesiense* in mice

In the present study, it has been established that pre-patent period of *T.b.rhodesiense* in mice ranged between 4 to 5 days with a mean ± SEM of 4.6 ± 0.25. This result is in agreement with some previous studies in which the mean ± SEM of pre-patent period in mice infected with *T.b.rhodesiense* was reported to be between 4 ± 0.0 to 5 ± 0.0 (Ndungu *et al*., 2020). However, administration of vitamin B12 marginally prolonged the pre patent period of *T. b. rhodesiense* in mice but the mechanism involved is not yet clear. Other studies have reported the anti-parasitic property of vitamin B12 which is thought to have some influence on the pre patent period (Ciccareli *et al*., 2012), while other studies have linked the non-statistical variation in the pre-patent period to various isolate forms of *T. b. rhodesiense* (Ndungu *et al*., 2020; Magez *et al*., 2008).

5.1.2. Effects of vitamin B12 on the survival rate in *T. b. rhodesiense* infected mice

From the current study, it is evident that oral supplementation with vitamin B12 protected the mice against *T. b. rhodesiense*-induced deleterious events associated with severe late-stage of HAT such as the breach of the blood brain barrier, loss of weight, anemia, inflammation and oxidative stress. The survival rate of mice post-infection was indeed used as the main indicator of the role played by vitamin B12 during *T. b. rhodesiense* infection. Notably, vitamin B12 significantly enhanced the survival rate among the *T. b. rhodesiense* infected mice. This

beneficiency may be attributed to its anti-inflammatory, antioxidant and erythropoietic properties; that perhaps delayed the onset of the pathophysiology characteristic present during the severe late stage of HAT. This observation corroborates previous studies in which vitamin B12 was reported to improve health outcomes in a number of diseases (Young *et al*., 2020; Van de Lagemaat *et al*., 2019; Al-Daghri *et al*., 2016). Therefore, vitamin B12-driven improvement in survival in *T. b. rhodesiense* infected mice was a major finding raising possibilities for new adjunct therapy that assuage inflammation and oxidative stress.

5.1.3: The effects of vitamin B12 on parasitaemia during *T. b. rhodesiense* infection in mice

Substantial and progressive parasitaemic waves observed in the present study occurred independent of vitamin B12 treatment. This observation may be linked to the ability of the parasites to evade the host immune system through the change of trypanosomal variant surface glycoproteins (VSG) or the ability of trypanosomes to invade the extravascular spaces and organs leading to un even trypanosome population in peripheral blood circulation as reported in other studies (Ndungu *et al*., 2020; Stijlemans *et al*., 2018; Matthews *et al*., 2015). This observation contradicts the findings by Ciccarelli *et al*. (2012), who reported a marked anti-parasitic activity of vitamin B12 against trypomastigotes. Other studies involving *T. b. rhodesiense* infection in rats and pigs have also reported fluctuations in the peak of parasitemia levels in peripheral blood in circulation and linked this phenomenon to the ability of trypanosomes to enter the extravascular regions and organs leading to uneven trypanosome population in peripheral blood circulation (Oparah *et al*., 2017; Morrison *et al*., 2010). The fluctuations in parasitemia levels were thought to be as a result of expression of a series of antigen variant surface glycoproteins (VSG) on trypanosomal cell membrane thus exposing the

mamalian host to a series of variant antigen types which eventually leads to unsuccessfully control of parasitaemia by the host's immune cells (Anderson, 1996; Road, 2001).

5.1.4: Effects of vitamin B12 on *T. b. rhodesiense*-induced neuronal injury in mice

Further analysis showed *T. b. rhodesiense*-driven decrease in the total rapid murine coma and behavioral scale scores (RMCBS), a clear evidence of diminished general health and neurological integrity. Similar findings has been reported in the previous study that implicated *T. b. rhodesiense* in disruption of myelin sheath resulting in a profound neurological deficits characterized by very low degree motor performance, aggression and touch response behavior in rats (Shipton and Thachil, 2015). Administration of vitamin B12 resulted in a significant improvement in the total RMCBS; perhaps due to the ability of vitamin B12 to support vital physiological and biochemical processes such as homocysteine (Hcy) and nerve metabolism, fatty acid and nucleic acid synthesis and energy production and DNA synthesis. Previously, Calderón & Nava, (2020) and Gröber *et al*. (2013) demonstrated involvement of vitamin B12 in fatty acid and nucleic acid synthesis, homocysteine (Hcy) and nerve metabolism, energy production as well as cell maturation processes, with unique role in maintenance of an intact gastro-intestinal mucosa.

5.1.5; Effects of vitamin B12 on the integrity of blood brain barrier during *T. b. rhodesiense* infection.

During the meningoencephalitis stage, the trypanosomes cross the blood brain barrier and accumulate in the cerebrospinal fluid (CSF) and brain, resulting in neuronal dysfunction (Kristensson *et al*., 2010). The *T. b. rhodesiense*-induced breach of the blood brain barrier has been linked to the trypanosomal factors and the effects of nitric oxide and other inflammatory

mediators acting upon the cerebral endothelial cells leading to an enhanced permeability of blood brain barrier; favoring the migration of cells and other materials to the brain. High levels of TNF-α in the brain promote trypanosome-driven breach of the blood brain barrier resulting in severe neuropathy (Kato *et al*., 2016; Kennedy, 2004). In the current study, the impact of vitamin B12 on the blood brain barrier during *T. b. rhodesiense* infection in mice was determined, and the findings revealed that *T. b. rhodesiense* infection occasioned an immense degradation of blood brain barrier, suggesting trypanosomal factors acting upon the cerebral endothelial cells leading to an enhhanced permeability of blood brain barrier that eventually favor the migration of cells and other materials to central nervous system (CNS). This observation is in agreement with the findings by Kennedy *et al.* (2004) who reported the breach of blood brain barrier and linked it to the effects of nitric oxide and other inflammatory mediators on cerebral endothelial cells. Remarkably, vitamin B12 completely blocked the *T. b. rhodesiense*-driven breach of the blood brain barrier. Though the exact mechanism involved in this phenomenon is not clear at this point, it is possible that vitamin B12 protects the blood brain barrier via stabilization of the trypanosomal factors as well as its positive modulation of the inflammatory mediators. This is a crucial finding that opens possibilities for further studies on the exact molecular mechanism underlying the capacity of vitamin B12 to block the *T. b. rhodesiense*-induced disruption of the blood brain barrier.

5.1.6: Effects of vitamin B12 on *T. b. rhodesiense*-induced weight loss in mice

In this study, the *T. b. rhodesiense* infection in mice resulted in the development of cachexia, which may be attributed to loss of appetite or alteration of fatty acid oxidation leading to exploitation of the fat reservoirs as reported elsewhere by Trinidade *et al*. 2016. Alternatively, this phenomenon could as well be due to parasitic driven disruption of normal growth and

development (Barai *et al*., 2017). Other studies have linked the *T. b. rhodesiense*-induced weight loss to persistent expression of TNF-α which in turn correlates with the severity of the disease (Kamidi *et al*., 2018). Notably, oral supplementation with vitamin B12 significantly attenuated the *T. b. rhodesiense*-induced cachexia in mice; perhaps due to down-regulation of the β oxidative pathway (Calderón and Nava, 2020). Further analysis was performed on the relative organ weight to ascertain whether there was any significant difference in these vital organs. The *T. b. rhodesiense* infected mice had increased spleen weight irrespective of vitamin B12 treatment. This *T. b. rhodesiense*-induced splenomegaly could be due to the extensive proliferation of macrophages within the splenic sinusoids, with heightened inflammation (Nyariki, *et al*., 2014).

5.1.7: Effects of vitamin B12 on hematological parameters during *T. b. rhodesiense* infection

The occurrence of anemia during *T. b. rhodesiense* infection is well documented and has been linked to a number of factors such as enhanced production of ROS, that causes RBCs damage, expression of trypanosomal toxins and metabolites, idiopathic serum factors and bone marrow nitric oxide changes that inhibits erythropoiesis (Musaya *et al*., 2015; Neves *et al*., 2021). Other studies have also manifested a constant and inevitable haemolytic anemia in trypanosomiasis with severity pegged on the virulence of *trypanosome species* and host susceptibility (Mbaya *et al*., 2010; Morrison *et al*., 2010). The mechanism to which *T. b. rhodesiense* -induced anemia develops may include the destruction of the erythrocytes through the lashing action of the trypanosome flagella or due to the release of pyrogens following the strong parasitaemia wave during trypanosomosis (Morrison *et al.*, 2010). In the current study, *T. b. rhodesiense*-induced anemia was characterized by decreased RBCs, HCT and haemoglobin levels, a phenomena that

has been reported in other studies (Mbaya *et al*., 2012; Neves *et al*., 2021). Administration of vitamin B12 assuaged the *T. b. rhodesiense*-induced anemia, suggesting hematopoiesis property of vitamin B12. Similar observation have been reported previously in a study on *T. brucei brucei* infection in rats whereby the significantly reduced levels of HCT, RBC and HGB were reversed upon administration of trypanocidal drug (Nwoha and Omamegbe, 2015). Additionally, this study further investigated the kind of anemia that was at play and the results revealed the existence of *T. b. rhodesiense*-driven microcytic hypochromic anemia as indicated by significantly reduced levels of MCV, MCH and MCHC among the *T. b. rhodesiense* infected mice a phenomena that has been observed in other studies (Mbaya *et al*., 2012; Neves *et al*., 2021). Oral supplementation with vitamin B12 assuaged the *T. b. rhodesiense*-induced microcytic hypochromic anemia, reinforcing previous findings that have identified vitamin B12 as having an important role in erythropoesis (Calderón and Nava, 2020; Ciccarelli *et al*., 2012).

5.1.8: Effects of vitamin B12 on platelets during *T. b. rhodesiense* infection in mice.

This study fuerther investigated the effects of vitamin B12 on the platelets following *T. b. rhodesiense* infection in mice. The results obtained herein revealed comparable levels in platelet counts across all the groups. This observation was in contradiction to the findings in previous studies involving *trypanosome* infection in both domestic and wild livestock which have reported *trypanosoma*-driven thrombocytopenia associated with many factors such as haemorrhage, vasoconstriction or tissue damage and increased splenic sequestration of platelets (Oparah *et al*., 2017). The non-significantly elevated levels in platelet counts following administration of vitamin B12 among *T. b. rhodesiense* infected mice was an indication that vitamin B12 can positively influence the factors associated with thrombocytopenia during trypanosomiasis though this may require further investigation.

5.1.9: Effects of vitamin B12 on WBCs and sub-types during *T. b. rhodesiense* infection in mice.

The presence of the trypanosomal variant surface glycoprotein (VSG) on *T. b. rhodesiense* membrane is known to activate the host immune cells leading to leukocytosis, usually indicated by elevations in total leucocyte counts (Oparah *et al*., 2017; Adeyemi & Adenike, 2012).

In the current study, *T. b. rhodesiense* infection resulted in heightened levels of total WBC, lymphocytes and monocytes, with a significant decline in neutrophil levels. This finding was expected and constitutes a normal immune response to *T. b. rhodesiense* infection as reported elsewhere by Nantes *et al*. 2019 and Oparah *et al*. 2017. this observation was also in contrast with the findings in a previous study involving *T. brucei* infection in rats, which reported a significant elevations in the total leucocyte counts and attributed it to the enhanced host immune action leading to an improved mop up of antigens in the body system (Ekanem & Yusuf, 2007). The decrease in neutrophils during *T. b. rhodesiense* infection could enhance the vulnerability of the infected mice to bacterial and fungal infections. Notably, vitamin B12 significantly nullified *T. b. rhodesiense*-driven changes to total WBCs, lymphocytes, neutrophils and monocytes. This is clear evidence for vitamin B12-driven immune modulation; in particular, the crucial role of vitamin B12 to facilitate the production of T-lymphocyte recruitment during a cellular immune response (Happi *et al*., 2016; Lewicki *et al*., 2014; Erkurt *et al*., 2008).

5.1.10: The effects of vitamin B12 on total immunoglobulin's during *T. b. rhodesiense* infection in mice

In the present study, it has been shown that the *T. b. rhodesiense* infected mice registered a significant increase in the levels of total immunoglobulins suggesting the host immune response to parasite. This *T. b. rhodesiense*-driven immunoglobulin concentration was linked to high parasiteamia levels which corresponds to the frequent change in trypanosomal VSG that causes the persistence of antigen and eventually account for heightened and prolonged immunoglobulin response (Jeffery *et al*., 2010). On the other hand, oral supplementation with vitamin B12 further enhanced the proliferation of *T. b. rhodesiense*-driven total immunoglobulin suggesting the crucial role played by vitamin B12 in the synthesis of immunoglobulins (Jeffery *et al*., 2010). This observation is consistent with the findings in a previous study that reported that vitamin B12 favors both humoral and cellular immunity through enhancement of T cell proliferation and pokeweed mitogen-dependent immunoglobulin synthesis in B cells (Erkurt *et al*., 2008).

5.1.11: The effects of vitamin B12 on *T. b. rhodesiense*-induced inflammation in mice

The trypanosomal VSG triggers severe inflammatory immune responses which are mediated by the release of interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-2 (IL-2) and interleukin-6 (IL-6) with concomitant depletion of anti-inflammatory cytokine interleukin-10 (IL-10) (Musaya *et al*., 2015; Karori *et al*., 2008). In the current study, *T. b. rhodesiense* infection in mice resulted in heightened levels of pro-inflammatory cytokines IFN-γ and TNF-α in serum, indicating the occurrence of *T. b. rhodesiense*-induced tissue inflammation associated with disease severity. Similar observations were reported in a previous study where infection with trypanosomes triggered a strong cytokine response (Musaya *et al*.,

2015). Remarkably, vitamin B12 ameliorated the trypanosome-induced inflammation in mice, as indicated by a significant down regulation in both IFN-γ and TNF-α, suggesting the anti-inflammatory effect of vitamin B12, that eventually led to an improved health outcomes among the *T. b. rhodesiense* infected mice. Similar observations have been reported by Tourkochristou *et al*., 2021 and Al-Daghri *et al*., 2016. On the other hand, the anti-inflammatory cytokine IL-10 was overwhelmed in *T. b. rhodesiense* infected mice, an indication that IL-10 plays a regulatory role in the equilibrium between pathology and trypanotolerance by slowing down excessive inflammatory responses, thereby influencing the survival of *T. b. rhodesiense* infected mice (Care and Manuel, 2021; Van de Lagemaat *et al*., 2019; Musaya *et al*., 2015). However, in the presence of vitamin B12, *T. b. rhodesiense*-driven down-regulation of IL-10 was significantly blocked, further signifying the ability of vitamin B12 to modulate the inflammatory cytokines which correlates with improved treatment outcomes during *T. b. rhodesiense* infection in mice. To validate the existence of *T. b. rhodesiense*-driven inflammation, the ratios between pro-inflammatory and anti-inflammatory cytokines were investigated in this study, and the result demonstrated excessive pro-inflammatory response attributable to *T. b. rhodesiense* infection and consequently echoes intensified inflammation. The ratios between pro-inflammatory and ant-inflammatory cytokines demonstrate a healthy balance in the presence of vitamin B12. In the absence of vitamin B12, pro-inflammatory cytokines were significantly elevated (Al-Daghri *et al*., 2016). From these findings, it is evident that the anti-inflammatory power of vitamin B12 is able to counter severe inflammation during an active *T. b. rhodesiense* infection.

5.1.12: Effects of vitamin B12 on nitric oxide during *T. b. rhodesiense* infection in mice

To further validate the occurrence of inflammation, the levels of nitric oxide (NO) was measured since it has been widely mentioned as a marker of inflammation. In the current study, the *T. b. rhodesiense* infected mice recorded a significant elevation in the levels of NO, signifying the development of *T. b. rhodesiense*-induced inflammation and probably oxidative stress. The observed *T. b. rhodesiense*-induced inflammation could be as a result of massive production of pro-inflammatory cytokine (IFN-γ and TNF) response and a Th1-driven macrophage activation; both known to enhance the proliferation of NO from macrophages (Tourkochristou *et al*., 2021; Van de Lagemaat *et al*., 2019). Similar observation have been reported in other studies where NO and its derivative like peroxynitrites ($OONO^-$) were proposed to induce lipid peroxidation leading to the rapid detrimental effects of host tissues and cells (Al-Daghri *et al*., 2016). Other studies in mice infected with *Trypanosoma congolense* have also implicated NO as a key mediator of the cytokine response due to its cytostatic and cytolytic properties (Qureshi *et al*., 2012). However, the exposure to vitamin B12 reversed the *T. b. rhodesiense*-induced elevation of NO levels, signifying the anti-inflammatory property of vitamin B12. This finding is quite significant because it suggests that vitamin B12 is able to affect NO synthase via a pathway that is not yet known. This is an important finding that requires further scrutiny.

5.1.13: Effects of vitamin B12 on *T. b. rhodesiense*-driven liver damage in mice

HAT is associated with the liver injuries. Transaminases enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALKP) and gamma glutamine transaminase (GGT) have been used as diagnostic markers for liver injury (Akinseye & Angela, 2020; Umar *et al*., 2007). In the present study, *T. b rhodesiense* infection in mice resulted in

significant elevation of serum ALT, AST, ALKP and GGT, suggesting a *T. b. rhodesiense*-induced liver damage. Similar observations were reported in animals experimentally infected with *T. b. brucei,* where these liver biomarkers in serum were elevated and associated with liver injury due to inflammation or oxidative stress (Oparah *et al*., 2017; Farsi *et al*., 2016; Molla *et al*., 2015). Interestingly, exposure to vitamin B12 greatly down-regulated these liver function biomarkers in *T. b rhodesiense* infected mice, suggesting the capacity of vitamin B12 to assuge hepatocellular injury during *T. b rhodesiense* infection.

To validate the *T. b rhodesiense*-induced hepatocellular injury, total bilirubin and albumin levels were determined. High parasitaemia during trypanosomiasis coupled with heightened pro-inflammatory cytokines and oxidants, induces hemolysis of RBCs leading to the breakdown of heme and finally the production of unconjugated bilirubin which is subsequently transported to the liver for conjugation with glucuronic acid and excretion (Giannini *et al*., 2005). In this study, *T. b rhodesiense* infection in mice resulted in elevated levels of total bilirubin, an indication that the liver excretory capacity was impaired due to hepatocellular injury. Notably, vitamin B12 significantly down-regulated the levels of total bilirubin. Once again, suggesting the ability of vitamin B12 to assuage the *T. b rhodesiense*-driven liver injury.

Production of albumin can be stimulated by the action of insulin and growth hormones. High levels of pro-inflammatory mediators like TNF-α, IL-6 and IL-1 can promote liver inflammation and eventually inhibit the synthesis of albumin (Giannini *et al*., 2005). In the current study, the *T. b. rhodesiense* infection in mice caused a significant decline in the levels of albumin, further suggesting liver damage. Vitamin B12 protected the mice from the *T. b. rhodesiense*-driven decrease in albumin, thus implies that vitamin B12 protected the mice from *T. b. rhodesiense*-driven hepatocellular injury.

5.1.14: Effects of vitamin B12 on the *T. b. rhodesiense*-driven kidney damage in mice

Creatinine is a product formed when creatinine phosphate is broken down in the muscles, and usually produced at constant rate. Urea on the other hand, is a waste product of many living organism and the major organic component of human urine. It is formed when amino acids are broken down to form proteins. All these renal function markers are primarily removed by the kidney through glomerular filtration (Allam *et al*., 2011). In the current study, renal injury biomarkers like creatinine, urea and uric acid were determined and the results revealed the *T. b. rhodesiense*-induced kidney injury as characterized by elevated levels of creatinine, urea and uric acid. This finding was in agreement with the findings by Akinseye & Angela, (2020) who reported an elevated urea and creatinine levels in monkey and mice infected with *T.b.rhodesiense* and *T. b. gambiense* respectively. Notably, vitamin B12 significantly blocked *T. b. rhodesiense*-driven changes to creatinine, urea and uric acid, indicating the ability of vitamin B12 to alleviate the deleterious effect on the kidney and the glomerular filtration rates during *T. b. rhodesiense* infection.

5.1.15: Effects of vitamin B12 on lipid profiles during the *T. b. rhodesiense* infection in mice

Infection of mice with *T. b. rhodesiense* resulted in elevated levels of serum total cholesterol and triglycerides with a declined levels in serum HDL- cholesterol, an indication that acute *T. b. rhodesiense* infection caused some defective degradation of lipid metabolism leading to lipolysis and hypertriglyceridemia and possibly making the free fatty acid unavailable for importation into hepatocytes despite the increase in serum triglyceride concentration. This finding was in conformity with the observations made by Igbokwe *et al.* (2009) and Nakamura, (1998) who reported an increase in the levels of serum total cholesterol, triglycerides and LDL- cholesterol in

T. brucei infection of rabbits and rats respectively. However, contradictory findings were reported in a study where *T. congolense* infection of donkey resulted in lowered values of serum HDL- cholesterol, LDL-cholesterol and total cholesterol (Samuel *et al*., 2018). Meanwhile, exposure to vitamin B12 to *T. b. rhodesiense* infected mice led to a significant reduction in the serum total cholesterol and triglycerides with significant elevation in the levels of HDL. This suggests that vitamin B12 enhanced the regulation of lipid metabolism. Nevertheless, this observation merits further investigation.

5.1.16: The effects of vitamin B12 on *T. b. rhodesiense*-induced oxidative stress in mice

The ability of *T. b rhodesiense* to induce oxidative damage was also investigated in the current study. Consequently, reduced glutathione (GSH) levels in various organs were determined given that GSH is a potent endogenous antioxidant (Care & Manuel, 2021; Misra *et al*., 2017). The development of oxidative damage in various organs can either be indicated by the elevation or reduction in the GSH levels (Dukhande *et al*., 2006). The findings herein showed that GSH was depleted in the spleen, liver and brain suggesting severe *T. b. rhodesiense*-induced elevation of ROS and oxidative stress. This observation is in agreement with the findings in other studies which associated the depletion of cellular GSH with severe oxidative stress (Tourkochristou *et al*., 2021; Nyariki *et al*., 2019). On the other hand, the GSH levels were elevated in the kidney and lungs suggesting a vital role of the cellular antioxidant system in the pathogenesis of HAT. This observation contradicts the findings by Umar *et al*., (2010) who reported a significant depletion of GSH in the kidney of mice experimentally infected of *T. congolense.* Notably, in the presence of vitamin B12, *T. b rhodesiense*-driven oxidative stress was nullified, as indicated by a significant modulation of GSH levels, implying a stable anti-oxidant system. This is quite intriguing, suggesting that the severe and damaging depletion of GSH during *T. b rhodesiense*

can be controlled if a powerful anti-oxidant and anti-inflammatory molecule is deployed alongside the conventional treatment.

5.1.17: The effects of vitamin B12 on *T. b rhodesiense*-driven lipid peroxidation

To validate the occurrence of oxidative stress, melandialdehyde (MDA) concentration (lipid peroxidation index) was determined since it involves the production of pro-oxidants which correlates with lipid membrane damage and eventually the deterioration of membrane integrity (Eze *et al*., 2008; Ekmekci and Terzioglu, 2005). *T. b rhodesiense* infection in mice resulted in significant elevations of MDA levels, an indication of *T. b rhodesiense*-driven lipid peroxidation. Indeed, oxidative stress has been observed previously to correlate with the production of pro-oxidants that causes lipid membrane damage and deterioration of membrane integrity (Eze *et al*., 2008; Ekmekci and Terzioglu, 2005). This observation may not be surprising as trypanosomes have been reported to elicit the production of free radicals such as nitric oxide (NO) (Eze *et al.*, 2008). This is very crucial due to importance of ROS and severe inflammation in late stage HAT (Ayo *et al*., 1999; Igbokwe *et al*., 1992; Bulkley, 1983). Generation of enhanced ROS leading to membrane peroxidation and tissue injury has also been reported in diseases such as malaria, haemorrhagic shock and acquired immunodeficiency syndrome (Alho and Leinonen, 1999). This increase in the lipid peroxidation index was significantly reversed by oral supplementation with vitamin B12 further giving credence to the role of vitamin B12 in alleviating the *T. b rhodesiense*-driven oxidative stress in mice. Similar observations were made by Eze *et al*. (2008) who reported that severe vitamin B12 deficiency, induces oxidative stress in mice.

5.1.18: Effects of vitamin B12 on liver, spleen and brain sections following *T. b rhodesiense* infection in mice.

The histological examination revealed that *T. b rhodesiense* infection-induced chronic liver damage as characterized by diffuse congestion of hepatic vessels and focal infiltration of liver parenchyma with mononuclear cells. The brain from the *T. b rhodesiense* infected mice exhibited congestion of meningeal blood vessels and mononuclear infiltration of the meninges. Similar observations has been previously reported, where the activation of astrocytes and microglia were linked to severe inflammatory cell infiltration in the brain of trypanosome infected mice, hence the increased number of necrotic cells (Maloba *et al*., 2012; De sausa *et al*., 2011). The *T. b rhodesiense* infection has been known to induce severe meningitis, prominent perivascular cuffing by lymphocytes and macrophages, reactive gliosis, hemorrhage (RBC present in the parenchyma), encephalitis and marked increase in the cellularity infiltration; post treatment reactive encephalitis has been a key pathology after treatment with melarsoprol (Nyariki *et al*., 2018). In this study, it is evident that supplementation with vitamin B12 significantly assuaged the *T. b rhodesiense*-induced liver damage and brain injury. Treatment with vitamin B12 appear to protect against multiple deleterious events like inflammatory responses, oxidative stress and other adverse neurological changes associated with severe late-stage HAT. These findings open possibilities for further studies on the potential use of vitamin B12 as an adjunct therapy alongside the standard treatment regime especially for the late stage HAT.

5.2: CONCLUSIONS

From the current study, the following conclusions were drawn:

1. Vitamin B12 greatly improved the *T. b rhodesiense*-induced weight loss, RMBC scores and the integrity of blood-brain barrier but no effect on the parasitemia levels in mice. It also alleviated the *T. b rhodesiense*-driven anemia.
2. Vitamin B12 modulated the *T. b rhodesiense*-induced inflammatory cytokine, improved total immunoglobulin levels and also relieved the liver and kidney damage in mice.
3. This study demonstrated that *T. b rhodesiense* infection in mice occasioned massive tissue damage, significant high concentration of serum MDA, and low concentrations of GSH. However the exposure to vitamin B12 reduced the *T. b rhodesiense* driven tissue damage, lipid peroxidation as well as modulation of GSH among the *T. b rhodesiense* infected groups an indication of strong anti-oxidant property of vitamin B12.

5.3 RECOMMENDATION

1. Further studies may be considered to establish the effects of vitamin B12 on the specific *T. b rhodesiense*-driven immunoglobulin's and other inflammatory cytokines

2. Further studies on the potential use of vitamin B12 as an adjunct therapy alongside the standard treatment regime, especially for severe late-stage HAT

3. Further studies on the exact mechanism involved in the phenomenon where vitamin B12 blocked the *T. b rhodesiense* -driven breach of the blood-brain barrier

4. Further scrutiny on the pathway in which vitamin B12 affects NO synthase during *T. b rhodesiense* infection

5. Further investigation on how vitamin B12 enhanced the regulation of lipid metabolism during *T. b rhodesiense* infection

REFERENCES

Adeyemi, O. S., & Adenike, F. (2012). Biochemical and morphological changes in Trypanosoma brucei brucei - infected rats treated with homidium chloride and diminazene aceturate. *23*(4), 179–183. https://doi.org/10.1515/jbcpp-2012-0018

Akinbamijo, O. O., Bennison, J. J., Jaitner, J., & Dempfle, L. (1998). Haematological changes in N'Dama and Gobra Zebu bulls during Trypanosoma congolense infection maintained under a controlled feeding regimen. *Acta Tropica*, *69*(3), 181-192.

Akinseye, O. R., & Angela, A. N. (2020). Biochemical indicators in trypanosomiasis infections. *9*(1), 11–14. https://doi.org/10.15406/japlr.2020.09.00346

Aksoy, S., Attardo, G., Berriman, M., Christoffels, A., Lehane, M., Masiga, D., & Toure, Y. (2014). Human african trypanosomiasis research gets a boost: Unraveling the tsetse genome: E2624. *PLoS Neglected Tropical Diseases*, *8*(4), e2624. doi:https://doi.org/10.1371/journal.pntd.0002624

Al-Daghri, N. M., Rahman, S., Sabico, S., Yakout, S., Wani, K., Al-Attas, O. S., Saravanan, P., Tripathi, G., McTernan, P. G., & Alokail, M. S. (2016). Association of vitamin B12 with pro-inflammatory cytokines and biochemical markers related to cardiometabolic risk in Saudi subjects. *Nutrients*, *8*(9), 1–8. https://doi.org/10.3390/nu8090460

Alfituri, O. A., Quintana, J. F., MacLeod, A., Garside, P., Benson, R. A., Brewer, J. M., Mabbott, N. A., Morrison, L. J., & Capewell, P. (2020). To the Skin and Beyond: The Immune Response to African Trypanosomes as They Enter and Exit the Vertebrate Host. *Frontiers in immunology*, *11*, 1250. https://doi.org/10.3389/fimmu.2020.01250

Alho, K., Medvedev, S. V., Pakhomov, S. V., Roudas, M. S., Tervaniemi, M., Reinikainen, K., ... & Näätänen, R. (1999). Selective tuning of the left and right auditory cortices during spatially directed attention. *Cognitive Brain Research*, *7*(3), 335-341.

Allam, L., Ogwu, D., Agbede, R. I. S., & Sackey, A. K. B. (2011). Hematological and serum biochemical changes in gilts experimentally infected with Trypanosoma brucei. *81*(5), 597–609.

Alvarez MN, Peluffo G, Piacenza L, Radi R. (2011). Intraphagosomal peroxynitrite as a macrophage-derived cytotoxin against internalized Trypanosoma cruzi: Consequences for oxidative killing and role of microbial peroxiredoxins in infectivity. *The Journal of Biological Chemistry*.;286(8):6627-6640. DOI: 10.1074/jbc.M110.167247

Alvarez MN, Piacenza L, Irigoin F, Peluffo G, Radi R. (2004). Macrophage-derived peroxynitrite diffusion and toxicity to Trypanosoma cruzi. *Archives of Biochemistry and Biophysics*. 432(2):222-232. DOI: 10.1016/j.abb.2004.09.015

Ames B.N. (2006). Low micronutrient intake may accelerate the degenerative diseases of aging through allocation of scarce micronutrients by triage. *Proc. Natl. Acad. Sci. USA*.;103:17589–17594. doi: 10.1073/pnas.0608757103. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Amin DN, Vodnala SK, Masocha W, Sun B, Kristensson K, Rottenberg ME. (2012). Distinct toll-like receptor signals regulate cerebral parasite load and interferon α/β and tumor necrosis factor α–dependent t-cell infiltration in the brains of *Trypanosoma brucei*—Infected Mice. *J Infect Dis*.;205(2):320–32. doi:10.1093/infdis/jir734.

Anderson, R. M. (1996). Antigenic variation and the within-host dynamics of parasites. *93*(February), 985–989.

Arango Duque, G., & Descoteaux, A. (2014). Macrophage cytokines: involvement in immunity and infectious diseases. *Frontiers in immunology*, *5*, 491.

Argaw AT, Zhang Y, Snyder BJ, Zhao ML, Kopp N, Lee SC, et al. (2006). IL-1beta regulates blood-brain barrier permeability via reactivation of the hypoxia-angiogenesis program. *J Immunol (Baltimore, Md: 1950).* 177(8):5574–84.

Atouguia JLM, Kennedy PGE. (2000). Neurological aspects of human African trypanosomiasis. In: Davies LE, Kennedy PGE, editors. Infectious Diseases of the Nervous System. Oxford: Butterworth-Heinemann; p. 321–72

Ayo, J. O., Oladele, S. B., Ngam, S., Fayomi, A., & Afolayan, S. B. (1999). Diurnal fluctuations in rectal temperature of the Red Sokoto goat during the harmattan season. *Research in Veterinary Science*, *66*(1), 7-9.

Bakari, S. M., Ofori, J. A., Kusi, K. A., Aning, G. K., Awandare, G. A., Carrington, M., & Gwira, T. M. (2017). Serum biochemical parameters and cytokine profiles associated with natural African trypanosome infections in cattle. *Parasites & vectors*, *10*, 1-13.

Barai, P., Higa, K., & Srinivasan, V. (2017). Lithium dendrite growth mechanisms in polymer electrolytes and prevention strategies. *Physical Chemistry Chemical Physics*, *19*(31), 20493-20505.

Baral, T. N. (2010). Immunobiology of African trypanosomes: need of alternative interventions. *BioMed Research International*, *2010*.

Birch C.S., Brasch N.E., McCaddon A., Williams J.H. (2009). A novel role for vitamin B(12): Cobalamins are intracellular antioxidants in vitro. *Free Radic. Biol. Med*.;47:184–188. doi: 10.1016/j.freeradbiomed.2009.04.023. [PubMed] [CrossRef] [Google Scholar]

Bogdan C, Rollinghoff M. (1999). How do protozoan parasites survive inside macrophages? *Parasitology Today*.;15(1):22-28

Borowy, n. K., sternberg, j. M., schreiber, d., nonnengasser, c., & overath, P. (1990). Suppressive macrophages occurring in murine Trypanosoma brucei infection inhibit T-cell responses in vivo and in vitro. *Parasite immunology*, *12*(3), 233-246.

Boulakirba, S., Pfeifer, A., Mhaidly, R., Obba, S., Goulard, M., Schmitt, T., ... & Jacquel, A. (2018). IL-34 and CSF-1 display an equivalent macrophage differentiation ability but a different polarization potential. *Scientific reports*, *8*(1), 256.

Brett FM, Mizisin AP, Powell HC, Campbell IL. (1995). Evolution of neuropathologic abnormalities associated with blood-brain barrier breakdown in transgenic mice expressing lnterleukin-6 in astrocytes. *J Neuropathol Exp Neurol*. 54(6):766–75.

Brito A., Grapov D., Fahrmann J., Harvey D., Green R., Miller J.W., Fedosov S.N., Shahab-Ferdows S., Hampel D., Pedersen T.L., et al. (2017). The human serum metabolome of vitamin B-12 deficiency and repletion, and associations with neurological function in elderly adults. *J. Nutr*.147:1839–1849. doi: 10.3945/jn.117.248278. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Brun R, Blum J, Chappuis F, Burri C. (2010). Human African trypanosomiasis. *Lancet* 375:148–59. 10.1016/S0140-6736(09)60829-1

Brun R, Blum J. (2012). Human African trypanosomiasis. Infect Dis Clin North Am. Jun;26(2):261-73. doi: 10.1016/j.idc.2012.03.003. Epub 2012 Apr 17. PMID: 22632638.

Bucheton B, MacLeod A, Jamonneau V. (2011). Human host determinants influencing the outcome of *Trypanosoma brucei gambiense* infections. *Parasite Immunol*. 33(8):438–47.

Bulkley, B. H., D'Amico, B. A. R. B. A. R. A., & Taylor, A. L. (1983). Extensive myocardial fiber disarray in aortic and pulmonary atresia. Relevance to hypertrophic cardiomyopathy. *Circulation*, *67*(1), 191-198.

Bunting RW, Seling MK, Dickersin GR (1997). Apoptotic cells in peripheral blood from patients with low serum cobalamin. J Submi-crosc Cytol Pathol; 29: 223–227.

Burden, F. A., Mulugeta, G., Patrick, V., Trawford, R., & Brooks, H. W. (2016). Reference intervals for biochemical and haematological parameters in mature domestic donkeys (Equus asinus) in the UK. *28*, 134–139. https://doi.org/10.1111/eve.12512

Büscher P, Cecchi G, Jamonneau V, Priotto G. (2017). Human African trypanosomiasis. Lancet 390:2397–2409. 10.1016/S0140-6736(17)31510-6

Calderón-Ospina, C. A., & Nava-Mesa, M. O. (2020). B Vitamins in the nervous system: Current knowledge of the biochemical modes of action and synergies of thiamine, pyridoxine, and cobalamin. *CNS Neuroscience and Therapeutics*, *26*(1), 5–13. https://doi.org/10.1111/cns.13207

Capewell P, Cooper A, Clucas C, Weir W, Macleod A. (2015). A co-evolutionary arms race: trypanosomes shaping the human genome, humans shaping the trypanosome genome. Parasitology 142(Suppl. 1):S108–19. 10.1017/S0031182014000602

Care C. & Manuel, H. D. C. (2021). *1* , 2 1. 4*(march), 1–10. https://doi.org/10.35454/rncm.v4n1.167

Carmel R. (2013). Diagnosis and management of clinical and subclinical cobalamin deficiencies: Why controversies persist in the age of sensitive metabolic testing. *Biochimie*.95:1047–1055. doi: 10.1016/j.biochi.2013.02.008. [PubMed] [CrossRef] [Google Scholar]

Carroll, R. W., Wainwright, M. S., Kim, K. Y., Kidambi, T., Gomez, N. D., Taylor, T., & Haldar, K. (2010). A rapid murine coma and behavior scale for quantitative assessment of murine cerebral malaria. *PloS one*, *5*(10), e13124.

Chan W., Almasieh M., Catrinescu M.-M., Levin L.A. (2017). Cobalamin-associated superoxide scavenging in neuronal cells is a potential mechanism for vitamin B12–deprivation optic neuropathy. *Am. J. Pathol*.188:160–172. doi: 10.1016/j.ajpath.2017.08.032.

Charles D. Kato, Enock Matovu, Claire. M. Mugasa, Ann Nanteza & Vincent P. (2016). Alibu Allergy, Asthma & Clinical Immunology volume 12, *Article number*: 4

Ciccarelli, A. B., Frank, F. M., Puente, V., Malchiodi, E. L., Batlle, A., & Lombardo, M. E. (2012). Antiparasitic effect of vitamin B12 on Trypanosoma cruzi. *Antimicrobial Agents and Chemotherapy*, *56*(10), 5315–5320. https://doi.org/10.1128/AAC.00481-12

Courtin D, Jamonneau V, Mathieu JF, Koffi M, Milet J, Yeminanga CS, et al. (2006). Comparison of cytokine plasma levels in human African trypanosomiasis. *Trop Med Int Health*. 11(5):647–53.

Darji A, Sileghem M, Heremans H, Brys L, and De Baetselier P. (1993). Inhibition of T-cell responsiveness during experimental infections with *Trypanosoma brucei*: active involvement of endogenous gamma interferon," *Infection and Immunity*, vol. 61, no. 7, pp. 3098–3102,.

Darji A., Beschin A., Sileghem M., Heremans H., Brys L., and De Baetselier P. (1996).In vitro simulation of immunosuppression caused by *Trypanosoma brucei*: active involvement of

gamma interferon and tumor necrosis factor in the pathway of suppression. *Infection and Immunity*, vol. 64, no. 6, pp. 1937–1943.

De Sousa PiresI, V. L., de SouzaII, J. R. F., GuimarãesIII, S. B., da Silva FilhoIV, A. R., GarciaV, J. H. P., & de VasconcelosVI, P. R. L. (2011). Preconditioning with L-alanyl-L-glutamine in a Mongolian Gerbil model of acute cerebral ischemia/reperfusion injury1. *Acta Cirúrgica Brasileira*, *26*(1).

Denicola A, Rubbo H, Rodriguez D, Radi R. (1993). Peroxynitrite-mediated cytotoxicity to Trypanosoma cruzi. *Archives of Biochemistry and Biophysics*. 304(1):279-286. DOI: 10.1006/abbi.1993.1350

Di Meo S., Reed T.T., Venditti P., Victor V.M. (2016). Role of ROS and RNS sources in physiological and pathological conditions. *Oxid. Med. Cell. Longev*.2016:1245049. doi: 10.1155/2016/1245049. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

d'Ieteren, G. D., Authié, E., Wissocq, N., & Murray, M. (1998). Trypanotolerance, an option for sustainable livestock production in areas at risk from trypanosomosis. *Revue scientifique et technique (International Office of Epizootics)*, *17*(1), 154-175.

Dinarello CA. (2009). Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol. 27:519–50.

Ding L, Linsley P. S, Huang L. Y, Germain R. N, and Shevach E. M. (1993). IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. *Journal of Immunology*, vol. 151, no. 3, pp. 1224–1234.

Dow RB. (1994). The clinical and laboratory utility of platelet volume parameters. *Australia Journal of Medical Science*. 15: 1-8.

Draper, H. and Hadley, M. (1990) Malondialdehyde Determination as Index of Lipid Peroxidation. Methods in Enzymology, 186, 421-431. http://dx.doi.org/10.1016/0076-6879(90)86135-I

Ekanem, J. T., & Yusuf, O. K. (2007). Some liver function indices and blood parameters in T . brucei -infected rats treated with honey Trypanosomosis Research Unit , Department of Biochemistry , University of Ilorin , PMB 1515 , Ilorin , Nigeria Received 8 June 2007. 19(2), 81–86.

Ekmekci Y, Terzioglu S. (2005). Effects of oxida-tive stress induced by paraquat on wild and cultivated wheat. *Pest Biochem Physiol.* 83:69-81.

Emeribe AO, and Anosa VO. (1991). Haematology of experimental Trypanosoma brucei gambiense infection. II. Erythrocyte and leucocyte changes. *Revue d'Elevage et de*

Medicine
Veterinaire des Pays Tropicaux. 44: 53-57.

Espevik T, Figari IS, Shalaby MR, Lackides GA, Lewis GD, Shepard HM, et al. (1987). Inhibition of cytokine production by cyclosporin A and transforming growth factor β. *J Exp Med*. 166(2):571–6.

Eze, J. I., Anene, B. M., & Chukwu, C. C. (2008). Determination of serum and organ malondialdehyde (MDA) concentration , a lipid peroxidation index , in Trypanosoma brucei -infected rats. 67–72. https://doi.org/10.1007/s00580-008-0722-6

Fang H, Kang J, Zhang D. (2017). Microbial production of vitamin B12: a review and future perspectives. *Microb Cell Fact*. Jan 30;16(1):15. doi: 10.1186/s12934-017-0631-y. [Article]

Ferrante A, Allison A. (1983). Alternative pathway activation of complement by African trypanosomes lacking a glycoprotein coat. *Parasite Immunol.* 5(5):491–8.

Figarella K., Uzcategui N. L., Beck A., Schoenfeld C., Kubata B. K., Lang F. and M Duszenko M. (2006). Prostaglandin-induced programmed cell death in *Trypanosoma brucei* involves oxidative stress. *Cell Death and Differentiation* 13: 1802–1814.

Firth J., Stubbs B., Sarris J., Rosenbaum S., Teasdale S., Berk M., Yung A.R. (2017). The effects of vitamin and mineral supplementation on symptoms of schizophrenia: A systematic review and meta-analysis. *Psychol. Med*.47:1515–1527. doi: 10.1017/S0033291717000022. [PubMed] [CrossRef] [Google Scholar]

Franco, J. R., Simarro, P. P., Diarra, A., & Jannin, J. G. (2014). Epidemiology of human African trypanosomiasis. *Clinical epidemiology*, *6*, 257–275. https://doi.org/10.2147/CLEP.S39728

Frenkel D, Zhang F, Guirnalda P, Haynes C, Bockstal V, Radwanska M, et al. (2016). *Trypanosoma brucei* co-opts NK cells to kill splenic B2 B cells. *PLoS Pathog*.12:e1005733

Fridovich I. (1995). Superoxide radical and superoxide dismutases. *Annual Review of Biochemistry*. 64:97-112. DOI: 10.1146/annurev.bi.64.070195.000525

Funada U, Wada M, Kawata T, Mori K, Tamai H, Kawanishi T, Kunou A, TanakaN, Tadokoro T, Maekawa A (2000). Changes in CD4+CD8–/CD4–CD8+ ratio and humoral immune functions in vitamin B 12 -deficient rats. Int J Vitam Nutr Res 2000; 70: 167–171.

Gadient RA, Otten UH. (1997). Interleukin-6 (IL-6)—a molecule with both beneficial and destructive potentials. *Prog Neurobiol*. 52(5):379–90.

Gaillot K, Lauvin MA, Cottier JP (2017) Vertical transmission of human African trypanosomiasis: clinical evolution and brain MRI of a mother and her son. PLoS Negl Trop Dis 11(7):e0005642. https://doi.org/10.1371/journal.pntd.0005642

Geiger A, Bossard G, Sereno D, Pissarra J, Lemesre JL, Vincendeau P, et al. (2016). Escaping deleterious immune response in their hosts: Lessons from trypanosomatids. Frontiers in Immunology.7:212. DOI: 10.3389/fimmu.2016.00212

Graf, F. E., Ludin, P., Wenzler, T., Kaiser, M., Brun, R., Pyana, P. P., & Mäser, P. (2013). Aquaporin 2 mutations in Trypanosoma brucei gambiense field isolates correlate with decreased susceptibility to pentamidine and melarsoprol. *PLoS neglected tropical diseases*, *7*(10), e2475.

Green R, Miller JW (2022). Vitamin B12 deficiency. Vitam Horm. 119:405-439.

Green R., Allen L.H., Bjørke-Monsen A.-L., Brito A., Guéant J.-L., Miller J.W., Molloy A.M., Nexo E., Stabler S., Toh B.-H., et al. (2017). Vitamin B12 deficiency. *Nat. Rev. Dis. Prim*.3:17040. doi: 10.1038/nrdp.2017.40. [PubMed] [CrossRef] [Google Scholar]

Gröber, U., Kisters, K., & Schmidt, J. (2013). Neuroenhancement with Vitamin B12-underestimated neurological significance. *Nutrients*, *5*(12), 5031–5045. https://doi.org/10.3390/nu5125031

Guirnalda, P., Murphy, N. B., Nolan, D., & Black, S. J. (2007). Anti-Trypanosoma brucei activity in Cape buffalo serum during the cryptic phase of parasitemia is mediated by antibodies. *International journal for parasitology*, *37*(12), 1391-1399.

Gutierrez FR, Mineo TW, Pavanelli WR, Guedes PM, Silva JS. (2009). The effects of nitric oxide on the immune system during Trypanosoma cruzi infection. *Memórias do Instituto Oswaldo Cruz*. 104(Suppl 1):236-245

Gutteridge JMC. (1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clin Chem*; 41:1819–28.

Hannibal L., Lysne V., Bjørke-Monsen A.-L., Behringer S., Grünert S.C., Spiekerkoetter U., Jacobsen D.W., Blom H.J. (2016). Biomarkers and algorithms for the diagnosis of vitamin B12 deficiency. *Front. Mol. Biosci*.3:27. doi: 10.3389/fmolb.2016.00027. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Harris, T. H., Cooney, N. M., Mansfield, J. M., & Paulnock, D. M. (2006). Signal transduction, gene transcription, and cytokine production triggered in macrophages by exposure to trypanosome DNA. *Infection and Immunity*, *74*(8), 4530-4537.

Herbert, W.J. and Lumsden, W.H.R. (1976) Trypanosoma brucei: A Rapid Matching Method for Estimating the Host's Parasitemia. Experimental Parasitology, 40, 427-432. http://dx.doi.org/10.1016/0014-4894(76)90110-7

Hertz CJ, Mansfield JM. (1999). IFN-γ-dependent nitric oxide production is not linked to resistance in experimental African trypanosomiasis. *Cell Immunol.* 192(1):24–32.

Huet, G., Lemesre, J. L., Grard, G., Noireau, F., Boutignon, F., Dieu, M. C., & Jannin, J. (1990). AND Serum lipid and lipoprotein trypanosomiasis abnormalities in human African. 792–794.

Hunt NH, Grau GE. (2003). Cytokines: accelerators and brakes in the pathogenesis of cerebral malaria. *Trends Immunol*. 24 (9):491–9.

Hunter CA, Jennings FW, Kennedy PG, Murray M. (1992). Astrocyte activation correlates with cytokine production in central nervous system of Trypanosoma brucei brucei-infected mice. *Lab Invest.* 67(5):635–42.

Hunter, C. A., Gow, J. W., Kennedy, P. G., Jennings, F. W., & Murray, M. (1991). Immunopathology of experimental African sleeping sickness: detection of cytokine mRNA in the brains of Trypanosoma brucei brucei-infected mice. *Infection and Immunity*, *59*(12), 4636-4640.

Hunter, C. A., Jennings, F. W., Kennedy, P. G., & Murray, M. (1992). Astrocyte activation correlates with cytokine production in central nervous system of Trypanosoma brucei brucei-infected mice. *Laboratory investigation; a journal of technical methods and pathology*, *67*(5), 635-642.

Igbokwe IO, Lafon JY, Umar IA, Hamidu LJ. (1998). Erythrocyte and hepatic glutathione concentrations in acute Trypanosoma brucei infection of rats. *Trop Veter*; 16:81–3.

Igbokwe IO, Umar IA, Omage JJ, Ibrahim NDG, Kadima KB, Obagaiye OK, Saror DI, Esievo KAN. (1996). Effect of acute Trypanosoma vivax infection on cattle erythrocyte glutathione and susceptibility to in vitro peroxidation. *Veter Parasitol*; 63:215-24.

Igbokwe IO. (1994). Mechanisms of cellular injury in African trypanosomiasis. *Veter Bull.* 64:611–20.

Igbokwe, I. O., & Mohammed, A. (1992). Some plasma biochemical changes in experimental Trypanosoma brucei infection of Sokoto Red goats. *Revue d'élevage et de médecine vétérinaire des pays tropicaux*, *45*(3-4), 287-290.

Igbokwe, I. O., Buratai, L. B., & Ubah, U. L. (2009). Serum and hepatic lipid levels in rats infected with Trypanosoma brucei. 191–195. https://doi.org/10.1007/s00580-008-0765-8

Imamura N, Fujimura K, Kuramoto A (1984) Ly m-phocyte subpopulations in pernicious anemia. N Engl J Med; 311: 56.

Ingram CF, Davidoff AN, Marais E (1997). Evalua-tion of DNA analysis for evidence of apoptosis in megaloblastic anemia. Br J Haematol; 96: 576–583.

Jamonneau V, Ilboudo H, Kaboré J, Kaba D, Koffi M, Solano P et al. (2012). Untreated infections by Trypanosoma brucei gambienseare not 100% fatal. *PLoS Negl Trop Dis*; 6:e1691.

Janeway C. A.. and Medzhitov R. (2002). Innate immune recognition. *Annual Review of Immunology*, vol. 20, pp. 197–216.

Jeffery, J., Millar, H., Mackenzie, P., Fahie-wilson, M., Hamilton, M., & Ayling, R. M. (2010). An IgG complexed form of vitamin B 12 is a common cause of elevated serum concentrations. *Clinical Biochemistry*, *43*(1–2), 82–88. https://doi.org/10.1016/j.clinbiochem.2009.08.022

Jessica A.S., Nynke E.H., Henk J.B., Sinead M.L., Coen D.A.S., Jan A.R., Paul A.J.K., Victor W.M.H., Hans S. (2012). Adenosylhomocysteine induces apoptosis and phosphatidylserine exposure in endothelial cells independent of homocysteine Atherosclerosis, 221 pp. 48-54

John Dacie, Barbara Bain, Bate Imelda and Mitchell Lewis, eds. (2001). Practical Haematology, 9 th edition. *London: C hurchill Livingstone*, pp. 34-35.

Julien B., Clotilde B., Bertrand C. (2015) Overview of the Diagnostic Methods Used in the Field for Human African Trypanosomiasis: What Could Change in the Next Years? Tropical Neuroepidemiology, Institute of Neuroepidemiology and Tropical Neurology, Université de Limoges, CNRS FR 3503 GEIST, 87000 Limoges, France

Kamidi, C. M., Auma, J., Mireji, P. O., Ndungu, K., Bateta, R., Kurgat, R., ... & Murilla, G. (2018). Differential virulence of camel Trypanosoma evansi isolates in mice. *Parasitology*, *145*(9), 1235-1242.

Karamshetty V., Acharya J.D., Ghaskadbi S., Goel P. (2016). Mathematical modeling of glutathione status in type 2 diabetics with vitamin B12 deficiency. *Front. Cell Dev. Biol*. 4:16. doi: 10.3389/fcell.2016.00016. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Kato CD, Nanteza A, Mugasa C, Edyelu A, Matovu E, Alibu VP. Clinical profiles, disease outcome and co-morbidities among T. b. rhodesiense sleeping sickness patients in Uganda. PLoS ONE 2015; 10:e0118370.

Kaushik RS, Uzonna JE, Zhang Y, Gordon JR, Tabel H. (2000). Innate resistance to experimental African trypanosomiasis: differences in cytokine (TNF-α, IL-6, IL-10 and IL-12) production by bone marrow-derived macrophages from resistant and susceptible mice. Cytokine. 12(7):1024–34.

Kennedy PG (2006). Diagnostic and neuropathogenesis issues in human African trypanosomiasis. Int J Parasitol. 36:505–12. 10.1016/j.ijpara.2006.01.012

Kennedy PG, Rodgers J, Jennings FW, Murray M, Leeman SE, Burke JM (1997). A substance P antagonist, RP-67,580, ameliorates a mouse meningoencephalitic response to Trypanosoma brucei brucei. Proc Natl Acad Sci USA; 94: 4167–70

Kennedy PGE. (2004). Human African trypanosomiasis of the CNS: current issues and challenges. J Clin Invest. 113:496–504. 10.1172/JCI200421052

Kennedy PGE. (2008). The continuing problem of Human African trypanosomiasis (sleeping sickness). *Ann Neurol*. 64:116–26. 10.1002/ana.21429 [PubMed] [CrossRef] [Google Scholar]

Kennedy PGE. (2013). Clinical features, diagnosis, and treatment of human African trypanosomiasis (sleeping sickness). Lancet Neurol. 12:186–94. 10.1016/S1474-4422(12)70296-X

Küpfer I, Hhary EP, Allan M, Edielu A, Burri C, Blum JA. (2011). Clinical presentation of T.b. rhodesiense sleeping sickness in second stage patients from Tanzania and Uganda. *PLoS Negl Trop Dis.* 5:e968.

Kushima Y, Hatanaka H. (1992). Interleukin-6 and leukemia inhibitory factor promote the survival of acetylcholinesterase-positive neurons in culture from embryonic rat spinal cord. *Neurosci Lett*. 143(1):110–4.

Lejon V, Lardon J, Kenis G, Pinoges L, Legros D, Bisser S, et al. (2002). Interleukin (IL)-6, IL-8 and IL-10 in serum and CSF of *Trypanosoma brucei gambiense* sleeping sickness patients before and after treatment. *Trans R Soc Trop Med Hyg*. 96(3):329–33.

Lewicki, S., Lewicka, A., Kalicki, B., Kłos, A., Bertrandt, J., & Zdanowski, R. (2014). The influence of vitamin B 12 supplementation on the level of white blood cells and lymphocytes phenotype in rats fed a low-protein diet. *39*(4), 419–425. https://doi.org/10.5114/ceji.2014.47723

Loscalzo J. (1996). The oxidant stress of hyperhomocyst(e)inemia. *J. Clin. Investig*.98:5–7. doi: 10.1172/JCI118776. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Lu, W., Luo, Y., Chang, G., & Sun, X. (2011). Synthesis of functional SiO2-coated graphene oxide nanosheets decorated with Ag nanoparticles for H2O2 and glucose detection. *Biosensors and Bioelectronics*, *26*(12), 4791-4797.

Ludigs K, Parfenov V, Du Pasquier RA, Guarda G. (2012). Type I IFN-mediated regulation of IL-1 production in inflammatory disorders. *Cell Mol Life Sci*. 69(20):3395–418.

Mabbott N, Coulson P, Smythies L, Wilson R, Sternberg J. (1998). African trypanosome infections in mice that lack the interferon-gamma receptor gene: nitric oxide-dependent and-independent suppression of T-cell proliferative responses and the development of anemia. *Immunology*. 94(4):476.

Mabbott, N., & Sternberg, J. (1995). Bone marrow nitric oxide production and development of anemia in Trypanosoma brucei-infected mice. *infection and immunity*, *63*(4), 1563-1566.

Mackenzie PK, Boyt WP, Nesham VW and Pirie E. (1978). The aetiology and significance of phagocytosis of erythrocytes and leucocytes in sheep infected with Trypanosoma congolense. *Research in Veterinary Science*. 24: 4-7.

MacLean L, Odiit M, and Sternberg J. M. (2001). Nitric oxide and cytokine synthesis in human African trypanosomiasis. *Journal of Infectious Diseases*, vol. 184, no. 8, pp. 1086–1090.

MacLean LM, Chisi JE, Odiit M, Gibson WC, Ferris V, Picozzi K, et al. (2004). Severity of human African trypanosomiasis in East Africa is associated with geographic location, parasite genotype, and host inflammatory cytokine response profile. *Infect Immun*. 72(12):7040–4.

MacLean LM, Odiit M, Chisi JE, Kennedy PG, Sternberg JM. (2010). Focus-specific clinical profiles in human African trypanosomiasis caused by Trypanosoma brucei rhodesiense. *PLoS Negl Trop Dis* 4:e906.

MacLean LM, Odiit M, MacLeod A, Morrison L, Sweeney L, Cooper A, et al. (2007). Spatially and genetically distinct African Trypanosome virulence variants defined by host interferon-γ response. *J Infect Dis*. 196(11):1620–8.

MacLean LM, Reiber H, Kennedy PG, Sternberg JM. (2012). Stage progression and neurological symptoms in *Trypanosoma brucei rhodesiense* sleeping sickness: role of the CNS inflammatory response. *PLoS Negl Trop Dis*. 6(10):e1857.

Magez S, Geuskens M, Beschin A, Del Favero H, Verschueren H, Lucas R, et al. (1997). Specific uptake of tumor necrosis factor-α is involved in growth control of *Trypanosoma brucei*. *J Cell Biol.* 137(3):715–27.

Magez S, Radwanska M, Beschin A, Sekikawa K, De Baetselier P. (1999). Tumor necrosis factor alpha is a key mediator in the regulation of experimental *Trypanosoma brucei* infections. *Infect Immun.* 67(6):3128–32.

Magez S, Radwanska M, Drennan M, Fick L, Baral TN, Allie N, Jacobs M, Nedospasov S, Brombacher F, Ryffel B, De Baetselier P. (2007). Tumor necrosis factor (TNF) receptor-1 (TNFp55) signal transduction and macrophage-derived soluble TNF are crucial for nitric oxide-mediated Trypanosoma congolense parasite killing. J Infect Dis. Sep 15;196(6):954-62.

Magez S, Stijlemans B, Baral T, De Baetselier P. (2002). VSG-GPI anchors of African trypanosomes: their role in macrophage activation and induction of infection-associated immunopathology. *Microbes Infect.* 4(9):999–1006.

Magez S, Stijlemans B, Baral Toya and De Baetselier P. (2002). DNA from protozoan parasites *Babesia bovis*, *Trypanosoma cruzi*, and *T. brucei* is mitogenic for B lymphocytes and stimulates macrophage expression of interleukin-12, tumor necrosis factor-alpha, and nitric oxide. *Microbes Infect* 4: 999-1006

Magez S, Stijlemans B, Radwanska M, Pays E, Ferguson MA, De Baetselier P. (1998). The glycosyl-inositol-phosphate and dimyristoylglycerol moieties of the glycosylphosphatidylinositol anchor of the trypanosome variant-specific surface glycoprotein are distinct macrophage-activating factors. *J Immunol (Baltimore, Md: 1950).*160(4):1949–56.

Maloba, F. C., Ngotho, M., & Rajesh, K. (2012). Glial cells response during human african trypanosomiasis infection. *Sci Parasitol*, *13*(4), 145-152.

Manzanares W., Hardy G. (2010). Vitamin B12: The forgotten micronutrient for critical care. *Curr. Opin. Clin. Nutr. Metab. Care.*13:662–668. doi: 10.1097/MCO.0b013e32833dfaec. [PubMed] [CrossRef] [Google Scholar]

Manzanares, W., & Hardy, G. (2020). Vitamin B12 Pharmaconutrition for Covid-19 Farmaconutrición de la vitamina B12 para COVID-19.

Marcondes, M. C. G., Borelli, P., Yoshida, N., & Russo, M. (2000). Acute Trypanosoma cruzi infection is associated with , thrombocytopenia, leukopenia, and bone marrow hypoplasia: Reversal by nifurtimox treatment. *Microbes and Infection*, *2*(4), 347–352. https://doi.org/10.1016/S1286-4579(00)00333-6

Masocha W, Robertson B, Rottenberg ME, Mhlanga J, Sorokin L, Kristensson K. (2004). Cerebral vessel laminins and IFN-γ define *Trypanosoma brucei* brucei penetration of the blood-brain barrier. *J Clin Invest*.;114(5):689.

Mateo H, Marin C, Perez-Cordon G, Sanchez-Moreno M. (2008). Purification and biochemical characterization of four iron superoxide dismutases in Trypanosoma cruzi. *Memórias do Instituto Oswaldo Cruz*. 103(3):271-276

Mauel J. (1996). Intracellular survival of protozoan parasites with special reference to Leishmania spp., toxoplasma gondii and Trypanosoma cruzi. *Advances in Parasitology*. 38:1-51

Mbaya, A., Kumshe, H., & Nwosu, C. O. (2010). The Mechanisms of Anemia in Trypanosomosis : A Review.

McCaddon A. (2013). Vitamin B12 in neurology and ageing; clinical and genetic aspects. *Biochimie*.95:1066–1076. doi: 10.1016/j.biochi.2012.11.017. [PubMed] [CrossRef] [Google Scholar]

McCaddon A., Regland B., Hudson P., Davies G. (2002). Functional vitamin B 12 deficiency and Alzheimer disease. *Neurology*.58:1395–1399. doi: 10.1212/WNL.58.9.1395. [PubMed] [CrossRef] [Google Scholar]

Meshnick S R., Blobstein S. H., Grady R. W. and Cerami A. (1978). An approach to the development of new drugs for African trypanosomiasis. *Journal of Experimental Medicine* 22: 569-579.

Mesu V, Kalonji WM, Bardonneau C, Mordt OV, Blesson S, Simon F (2017). Oral fexinidazole for late-stage African Trypanosoma brucei gambiense trypanosomiasis: a pivotal multicentre, randomised, non-inferiority trial. Lancet 391:144–54. 10.1016/S0140-6736(17)32758-7

Mikkelsen, K., & Apostolopoulos, V. (2019). Vitamin B12, folic acid, and the immune system. *Nutrition and immunity*, 103-114.

Misra, U. K., Kalita, J., Singh, S. K., & Rahi, S. K. (2017). Oxidative Stress Markers in Vitamin B12 Deficiency. *Molecular Neurobiology*, *54*(2), 1278–1284. https://doi.org/10.1007/s12035-016-9736-2

Molina-Portela MP, Samanovic M, Raper J. (2008). Distinct roles of apolipoprotein components within the trypanosome lytic factor complex revealed in a novel transgenic mouse model. *J Exp Med.* 205(8):1721–8.

Molla, B., Worku, Y., Shewaye, A., & Mamo, A. (2015). Prevalence of strongyle infection and associated risk factors in equine in Menz Keya Gerbil District, North-Eastern Ethiopia. *Journal of Veterinary Medicine and Animal Health 7*(4), 117–121. https://doi.org/10.5897/JVMAH2014

Moreira E.S., Brasch N.E., Yun J. (2011). Vitamin B12 protects against superoxide-induced cell injury in human aortic endothelial cells. *Free Radic. Biol. Med*.;51:876–883. doi: 10.1016/*j.freeradbiomed*.05.034.

Morrison, L. J., Mclellan, S., Sweeney, L., Chan, C. N., Macleod, A., Tait, A., & Turner, C. M. R. (2010). Role for Parasite Genetic Diversity in Differential Host Responses to Trypanosoma brucei Infection □ ‡. *78*(3), 1096–1108. https://doi.org/10.1128/IAI.00943-09

Munoz-Fernandez MA, Fernandez MA, Fresno M. (1992). Activation of human macrophages for the killing of intracellular Trypanosoma cruzi by TNF-alpha and IFN-gamma through a nitric oxide-dependent mechanism. *Immunology Letters*.33(1):35-40

Murray, P. J., Allen, J. E., Biswas, S. K., Fisher, E. A., Gilroy, D. W., Goerdt, S., ... & Wynn, T. A. (2014). Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity*, *41*(1), 14-20.

Musaya, J., Matovu, E., Nyirenda, M., & Chisi, J. (2015). Role of cytokines in Trypanosoma brucei -induced anemia *: A review of the literature*. *27*(June), 45–50.

Naessens J, Kitani H, Nakamura Y, Yagi Y, Sekikawa K, Iraqi F. (2005). TNF-a mediates the development of anemia in a murine *Trypanosoma brucei rhodesiense* infection, but not the anemia associated with a murine *Trypanosoma congolense* infection. *Clin Exp Immunol*. 139(3):405–10.

Naessens J. (2006). Bovine trypanotolerance: a natural ability to prevent severe anemia and haemophagocytic syndrome? *International Journal for Parasitology*, vol. 36, no. 5, pp. 521–528.

Nakamura, S., Senoh, M., Nagahama, S. I., Iwasa, N., Yamada, T., Matsushita, T., ... & Chocho, K. (1998). High-power, long-lifetime InGaN/GaN/AlGaN-based laser diodes grown on pure GaN substrates. *Japanese journal of applied physics*, *37*(3B), L309.

Namangala B, De Baetselier P, Beschin A. (2009). Both type-I and type-II responses contribute to murine trypanotolerance. *J Vet Med Sci*. 71(3):313–8.

Namangala B, Noël W, De Baetselier P, Brys L, Beschin A. (2001). Relative contribution of interferon-γ and interleukin-10 to resistance to murine African trypanosomosis. *J Infect Dis*. 183(12):1794–800.

Namangala, B., Hachaambwa, L., Kajino, K., Mweene, A. S., Hayashida, K., Simuunza, M., ... & Sugimoto, C. (2012). The use of Loop-mediated Isothermal Amplification (LAMP) to detect the re-emerging Human African Trypanosomiasis (HAT) in the Luangwa and Zambezi valleys. *Parasites & vectors*, *5*(1), 1-5.

Ndung'u K, Murilla GA, Thuita JK, Ngae GN, Auma JE, Gitonga PK, et al. (2020) Differential virulence of Trypanosoma brucei rhodesiense isolates does not influence the outcome of treatment with anti-trypanosomal drugs in the mouse model. PLoS ONE 15(11): e0229060.
https://doi.org/10.1371/journal.pone.0229060

Ngotho M, Kagira J, Jensen H, Karanja S, Farah I, Hau J. (2009). Immunospecific immunoglobulins and IL-10 as markers for *Trypanosoma brucei rhodesiense* late stage disease in experimentally infected vervet monkeys. *Trop Med Int Health*. 14(7):736–47.

Nwoha, R. I. O., & Omamegbe, J. O. (2015). Comparative Haematological Changes in Rats Experimentally Infected with Trypanosoma brucei brucei and Treated with Imidocarb Dipropionate and Diminazene Aceturate. *Research Journal of Veterinary Sciences*, *8*(2), 36–41. https://doi.org/10.3923/rjvs.2015.36.41

Nyariki et al (2014). Coenzyme Q10 prevented full blown splenomegaly and decreased melarsoprol-induced reactive encephalopathy in mice infected with Trypanosoma brucei rhodesiense

Nyariki, J. N., Thuita, J. K., Wambugu, A. M., Nyamweya, N. O., & Rashid, K. (2018). Coenzyme Q 10 and endogenous antioxidants neuro-protect mice brain against deleterious effects of melarsoprol and Trypanasoma brucei rhodesiense. *8*(4), 61–73.

Nyawira-Maranga D, Kagira JM, Kinyanjui CK, Muturi Karanja S, Wangari Maina N, Ngotho M. (2013). IL-6 is upregulated in late-stage disease in monkeys experimentally infected with *Trypanosoma brucei rhodesiense*. *Clin Dev Immunol*. 2013:320509.

Odiit, M., Kansiime, F., & Enyaru, J. C. (1997). Duration of symptoms and case fatality of sleeping sickness caused by Trypanosoma brucei rhodesiense in Tororo, Uganda. *East African medical journal*, *74*(12), 792-795.

Ogunsanmi AO, Taiwo VO. (2001). Pathobiochemical mechanisms involved in the control of the disease caused by Trypanosoma congolense in African grey duiker (Sylvicapra grimmia). *Veter Parasitol.* 96:51–63.

Oka T, Oka K, Hosoi M, Hori T. (1995). Intracerebroventricular injection of interleukin-6 induces thermal hyperalgesia in rats. *Brain Res*. 692(1):123–8.

Okomo M. C., Daulouede S., Lemesre J., N'Zila-Mouanda A, and Vincendeau P. (1995). Correlation of high serum levels of tumor necrosis factor-α with disease severity in human African trypanosomiasis. *American Journal of Tropical Medicine and Hygiene*, vol. 53, no. 5, pp. 539–543,

Okomo-assoumou MC, Daulouède S, Lemesre JL, N'zila-Mouanda A and Vincendeau P. 1995a. Correlation of high serum levels of tumor necrosis factor-a with disease severity in human African trypanosomiasis. Am J Trop Med Hyg 53: 539-543.

O'Leary F, Samman S. (2010). Vitamin B12 in health and disease. *Nutrients*. Mar;2(3):299-316. doi: 10.3390/nu2030299. Epub 2010 Mar 5.

Omer OH, Mousa HM, Al-Wabel N. (2007). Study on the antioxidant status of rats experimentally infected with Trypanosoma evansi. *Veter Parasitol*; 145:142–5.

Onyilagha, C., & Uzonna, J. E. (2019). Host immune responses and immune evasion strategies in African trypanosomiasis. *Frontiers in immunology*, *10*, 2738.

Oparah, Q. N., Sackey, A. B. K., Lawal, I. A., & Abdullahi, U. S. (2017). Haematological indices in trypanosoma brucei brucei (Federe isolate) infected nigerian donkeys (equus asinus) treated with homidium and isometamidium chloride. *Macedonian Veterinary Review*, *40*(1), 73–82. https://doi.org/10.1515/macvetrev-2017-0014

Paling RW, Moloo SK, Scott JR, McOdimba, FA, Logan-Henfrey LL, Murray M and William, DJ. (1991). Susceptibility of N'dama and Boran cattle to tsetse transmitted primary and rechallenge infections with homologous serodeme of T. congolense. *Parasite Immunology*. 13: 413-425.

Pays E. (2006). The variant surface glycoprotein as a tool for adaptation in African trypanosomes. *Microbes Infect.* 8(3):930–7.

Pedersen BK. (2009). The diseasome of physical inactivity—and the role of myokines in muscle—fat cross talk. J Physiol. 587(23):5559–68.

Perobelli, S. M., Galvani, R. G., Gonçalves-Silva, T., Xavier, C. R., Nóbrega, A., & Bonomo, A. (2015). Plasticity of neutrophils reveals modulatory capacity. *Brazilian Journal of Medical and Biological Research*, *48*, 665-675.

Piacenza L, Peluffo G, Alvarez MN, Kelly JM, Wilkinson SR, Radi R. (2008). Peroxiredoxins play a major role in protecting Trypanosoma cruzi against macrophage- and endogenously-derived peroxynitrite. *The Biochemical Journal*. 410(2):359-368. DOI: 10.1042/BJ20071138

Piacenza L, Peluffo G, Alvarez MN, Martinez A, Radi R. (2013). Trypanosoma cruzi antioxidant enzymes as virulence factors in Chagas disease. Antioxidants & Redox Signaling.19(7):723-734. DOI: 10.1089/ars.2012.4618

Pineyro MD, Pizarro JC, Lema F, Pritsch O, Cayota A, Bentley GA, et al. (2005). Crystal structure of the tryparedoxin peroxidase from the human parasite Trypanosoma cruzi. *Journal of Structural Biology.* 150(1):11-22. DOI: 10.1016/j.jsb.2004.12.005

Politis A., Olgiati P., Malitas P., Albani D., Signorini A., Polito L., De Mauro S., Zisaki A., Piperi C., Stamouli E., et al. (2010). Vitamin B12 levels in Alzheimer's disease: Association with clinical features and cytokine production. *J. Alzheimers Dis*. 19:481–488. doi: 10.3233/JAD-2010-1252. [PubMed] [CrossRef] [Google Scholar]

Puhl, H., Waeg, G., & Esterbauer, H. (1994). [46] Methods to determine oxidation of low-density lipoproteins. *Methods in enzymology*, *233*, 425-441

Qureshi, A. A., Guan, X. Q., Reis, J. C., Papasian, C. J., Jabre, S., Morrison, D. C., & Qureshi, N. (2012). Inhibition of nitric oxide and inflammatory cytokines in LPS-stimulated murine macrophages by resveratrol, a potent proteasome inhibitor. *Lipids in Health and Disease*, *11*, 1–17. https://doi.org/10.1186/1476-511X-11-76

Radi R. (2013). Peroxynitrite, a stealthy biological oxidant. *The Journal of Biological Chemistry*.; 288(37):26464-26472. DOI: 10.1074/jbc.R113.472936

Raes, J., Foerstner, K. U., & Bork, P. (2007). Get the most out of your metagenome: computational analysis of environmental sequence data. *Current opinion in microbiology*, *10*(5), 490-498.

Rath, M., Müller, I., Kropf, P., Closs, E. I., & Munder, M. (2014). Metabolism via arginase or nitric oxide synthase: two competing arginine pathways in macrophages. *Frontiers in immunology*, *5*, 532.

Reinitz DM, Mansfield JM. (1990). T-cell-independent and T-cell-dependent B-cell responses to exposed variant surface glycoprotein epitopes in trypanosome-infected mice. *Infect Immun.* 58(7):2337–42.

Rhind SG, Sabiston BH, Shek PN, Buguet A, Muanga G, Stanghellini A, et al. (1997). Effect of melarsoprol treatment on circulating IL-10 and TNF-α levels in human African trypanosomiasis. Clin Immunol Immunopathol. 83(2):185–9.

Road, D. (2001). Antigenic Variation in Trypanosomes : Enhanced Phenotypic Variation in a Eukaryotic Parasite.

Robins-Browne RM, Schneider J and Metz J. (1975). Thrombocytopenia in trypanosomiasis. *American Journal of Tropical Medicine and Hygiene*. 24: 226-231.

Rodgers, J., Steiner, I., & Kennedy, P. G. E. (2019). Generation of neuroinflammation in human African trypanosomiasis. *Neurology(R) neuroimmunology & neuroinflammation*, *6*(6), e610. https://doi.org/10.1212/NXI.0000000000000610

Rodgers, W. B., Gerber, E. J., & Patterson, J. (2011). Intraoperative and early postoperative complications in extreme lateral interbody fusion: an analysis of 600 cases. *Spine, 36*(1), 26-32.

Rousset F, Garcia E, Defrance T, Peronne C, Vezzio N, Hsu D-H, et al. (1992). Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc Natl Acad Sci USA.*;89 (5):1890–3.

Sabat R. (2010). IL-10 family of cytokines. Cytokine Growth Factor Rev. 21(5):315–24.

Saleh MA, Bassam MA, Sanousi SA. (2009). Oxidative stress in blood of camels (Camelus dromedaries) naturally infected with Trypanosoma evansi. *Veter Parasitol*; 162: 192–9.

Sarciron M, Gherardi A. (2000). Cytokines involved in Toxoplasmic encephalitis. *Scand J Immunol*. 52(6):534–43.

Schindler R, Mancilla J, Endres S, Ghorbani R, Clark SC, Dinarello CA. (1990). Correlations and interactions in the production of interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. *Blood*. 75(1):40–7.

Schleifer, K. W., & Mansfield, J. M. (1993). Suppressor macrophages in African trypanosomiasis inhibit T cell proliferative responses by nitric oxide and prostaglandins. *Journal of immunology (Baltimore, Md.: 1950), 151*(10), 5492-5503.

Shan XO, Aw TY, Jones DP. (1990). Glutathione dependent protection against oxidative injury. *Pharmacol Ther* 47:61-71.

Shi M, Wei G, Pan W, and Tabel H. (2004). Trypanosoma congolense infections: antibody-mediated phagocytosis by Kupffer cells. *Journal of Leukocyte Biology*, vol. 76, no. 2, pp. 399–405,.

Shipton, M. J., & Thachil, J. (2015). Vitamin B 12 deficiency – A 21st century perspective. *15*(2), 145–150.

Shoda L. K.M, Kegerreis K. A, Suarez C. E. (2001). DNA from protozoan parasites *Babesia bovis, Trypanosoma cruzi,* and *T. brucei* is mitogenic for B lymphocytes and stimulates macrophage expression of interleukin-12, tumor necrosis factor alpha, and nitric oxide," *Infection and Immunity*, vol. 69, no. 4, pp. 2162–2171,.

Sileghem M, Darji A, and De Baetselier P (1991). In vitro simulation of immunosuppresion caused by *Trypanosoma brucei*," *Immunology*, vol. 73, no. 2, pp. 246–248.

Sileghem M, Flynn J. N, Logan-Henfrey L, and Ellis J. (1994). Tumour necrosis factor production by monocytes from cattle infected with *Trypanosoma (Duttonella) vivax* and

Trypanosoma (Nannomonas) congolense: possible association with severity of anemia associated with the disease," *Parasite Immunology*, vol. 16, no. 1, pp. 51–54,

Siqueira LDP, Fontes DAF, Aguilera CSB, Timoteo TRR, Angelos MA, et al. (2017) Schistosomiasis: Drugs used and treatment strategies. Acta Trop 176: 179-187.

Stephen Lorne. (1986). Trypanosomiasis. A Veterinary Perspective. New York: *Pergamon Press*, , pp 351-420.

Sternberg JM, Rodgers J, Bradley B, MacLean L, Murray M, Kennedy PG. (2005). Meningoencephalitic African trypanosomiasis: brain IL-10 and IL-6 are associated with protection from neuro-inflammatory pathology. *J Neuroimmunol*. 167(1):81–9.

Stijlemans B, De Baetselier P, Magez S, Van Ginderachter JA, De Trez C (2018) African trypanosomiasis-associated : the contribution of the interplay between parasites and the mononuclear phagocyte system. Front Immunol 9:218. https://doi.org/10.3389/fimmu.2018.00218

Stijlemans B, Vankrunkelsven A, Brys L, Magez S, and De Baetselier P (2008). Role of iron homeostasis in trypanosomiasis-associated , *Immunobiology*, vol. 213, no. 9-10, pp. 823–835.

Tabel, H., Wei, G., & Shi, M. (2008). T cells and immunopathogenesis of experimental African trypanosomiasis. *Immunological reviews*, *225*(1), 128-139.

Tachado DS and Schofield L. 1994. Glycosylphosphatidylinositol toxin of *Trypanosoma brucei*regulates Il-1a and TNF-a expression in macrophages by protein tyrosine kinase mediated signal transduction. Biochem Biophys Res Comm 205: 984-991.

Takeda K, Kaisho T, and Akira S. (2003). Toll-like receptors. *Annual Review of Immunology*, vol. 21, pp. 335–376.

Tamura J, Kubota K, Murakami H, et al: Im-munomodulation by vitamin B 12 : augmenta-tion of CD8+ T lymphocytes and natural killer (NK) cell activity in vitamin B 12 -defi-cient patients by methyl-B 12 treatment. Clin Exp Immunol 1999; 116: 28–34.

Taylor, K. A. (1998). Immune responses of cattle to African trypanosomes: protective or pathogenic?. *International journal for parasitology*, *28*(2), 219-240.

Thakkar K., Billa G. (2015). Treatment of vitamin B12 deficiency–Methylcobalamine? Cyancobalamine? Hydroxocobalamin?—Clearing the confusion. *Eur. J. Clin. Nutr*.69:1–2. doi: 10.1038/ejcn.2014.165. [PubMed] [CrossRef] [Google Scholar]

Thakkar, K., & Billa, G. (2015). Treatment of vitamin B12 deficiency–Methylcobalamine? Cyancobalamine? Hydroxocobalamin?—Clearing the confusion. *European journal of clinical nutrition*, *69*(1), 1-2.

Thompson CB and Jakubowski JA. (1988). The pathophysiology and clinical relevance of platelet heterogeneity. *Blood*. 72: 1-8.

Tomiotto-Pellissier F, Miranda-Sapla MM, Machado LF, Bortoleti B, Sahd CS, et al. (2017) Nanotechnology as a potential therapeutic alternative for schistosomiasis. Acta Trop 174: 64-71.

Tourkochristou, E., Triantos, C., & Mouzaki, A. (2021). The Influence of Nutritional Factors on Immunological Outcomes. *Frontiers in Immunology*, *12*(May), 1–17. https://doi.org/10.3389/fimmu.2021.665968

Trujillo M, Budde H, Pineyro MD, Stehr M, Robello C, Flohe L, et al. (2004). Trypanosoma brucei and Trypanosoma cruzi tryparedoxin peroxidases catalytically detoxify peroxynitrite via oxidation of fast reacting thiols. *The Journal of Biological Chemistry*. 279(33):34175-34182. DOI: 10.1074/jbc.M404317200

Trypanosomiasis, African (Sleeping Sickness). (2018). In Brunette, G. W. (Ed.), *CDC Yellow Book*. Centers for Disease Control and Prevention. https://relief.unboundmedicine.com/relief/view/cdc-yellow-book/204166/all/Trypanosomiasis_African_Sleeping_Sickness_

Tyagi N. (2005). Mechanisms of homocysteine-induced oxidative stress. *AJP Heart Circ. Physiol*.;289:H2649–H2656. doi: 10.1152/ajpheart.00548.2005. [PubMed] [CrossRef] [Google Scholar]

Umar IA, Ogenyi E, Okodaso D, Kimeng E, Stancheva GI, Omage JJ, Isah S, Ibrahim MA. (2007). Amelioration of anemia and organ damage by combined intraperitoneal administration of vitamins A and C to Trypanosoma brucei brucei-infected rats. *Afr J Biotechnol.* 6:2083–6.

Umar, M., Aziz, H. A., & Yusoff, M. S. (2010). Trends in the use of Fenton, electro-Fenton and photo-Fenton for the treatment of landfill leachate. *Waste management*, *30*(11), 2113-2121.

Urech K, Neumayr A, Blum J. (2011). Sleeping sickness in travelers - do they really sleep? *PLoS Negl Trop Dis.* 5:e1358. 10.1371/journal.pntd.0001358 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Van de Lagemaat, E. E., de Groot, L. C. P. G. M., & van den Heuvel, E. G. H. M. (2019). Vitamin B 12 in relation to oxidative stress: A systematic review. *Nutrients*, *11*(2). https://doi.org/10.3390/nu11020482

Vanhollebeke B, Truc P, Poelvoorde P, Pays A, Joshi PP, Katti R, et al. (2006). Human Trypanosoma evansi infection linked to a lack of apolipoprotein L-I. *N Engl J Med.* 355(26):2752–6.

Vellema P, Rutten VP, Hoek A, Moll L, Wen-tink GH: The effect of cobalt supplementa-tion on the immune response in vitamin B 12deficient Texel lambs. Vet Immunol Immu-nopathol 1996; 55: 151–161.

Vincendeau, P., & Bouteille, B. (2006). Immunology and immunopathology of African trypanosomiasis. *Anais da Academia Brasileira de Ciências*, *78*, 645-665.

Wahl SM, McCartney-Francis N, Mergenhagen SE. (1989). Inflammatory and immunomodulatory roles of TGF-β. *Immunol Today*. 10(8):258–61.

Wellde BT, Kovatch RM, Chunmo DA and Wykoff DE. (1978). Trypanosoma congolense: thrombocytopaenia in experimentally infected cattle. *Experimental Parasitology*. 45: 26-33.

Wheatley, C. (2006). A scarlet pimpernel for the resolution of inflammation? The role of supra-therapeutic doses of cobalamin, in the treatment of systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, and septic or traumatic shock. *Medical hypotheses*, *67*(1), 124-142.

Wijnands, K. A., Castermans, T. M., Hommen, M. P., Meesters, D. M., & Poeze, M. (2015). Arginine and citrulline and the immune response in sepsis. *Nutrients*, *7*(3), 1426-1463.

Wilkinson SR, Meyer DJ, Taylor MC, Bromley EV, Miles MA, Kelly JM. (2002). The Trypanosoma cruzi enzyme TcGPXI is a glycosomal peroxidase and can be linked to trypanothione reduction by glutathione or tryparedoxin. *The Journal of Biological Chemistry*. 277(19):17062-17071. DOI: 10.1074/jbc.M111126200

Woo, P.T.K. (1970) The Haematocrit Centrifuge Technique for the Diagnosis of African Trypanosomiasis. Acta Tropica, 27, 384-386.

World Health Organization (1998) Control and Surveillance of African Trypanosomiaisis. WHO Technical Report Series, Geneva 881:1–113.

World Health Organization (2021) "Control and surveillance of human African trypanosomiasis," *Report of a WHO Expert Committee*

World Health Organization. (2022). Report of the fourth WHO stakeholders meeting on gambiense and rhodesiense human African trypanosomiasis elimination: virtual meeting, 1-3 June 2021.

Young, M. F., Guo, J., Williams, A., Whitfield, K. C., Nasrin, S., Kancherla, V., Suchdev, P. S., Crider, K. S., Pfeiffer, C. M., & Serdula, M. (2020). Interpretation of vitamin B-12 and folate concentrations in population-based surveys does not require adjustment for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of (BRINDA) project. *American Journal of Clinical Nutrition*, *111*(4), 919–926. https://doi.org/10.1093/ajcn/nqz303

Zhang M, Han W, Hu S, Xu H. (2013). Methylcobalamin: a potential vitamin of pain killer. *Neural Plast*. 2013;2013:424651. doi: 10.1155/2013/424651. Epub Dec 26. [Article]

APPENDICES

APPENDIX 1: GSH PROTOCOL

To prepare 1M potassium phosphate buffer with 5 mM EDTA disodium salt, pH 7.5 (KPE) the following two solutions were prepared;

Solution A:

Prepared by dissolving 6.8 g KH_2PO_4 in 500 ml dH_2O the resulting solution was stored at 4 °C.

Solution B

Prepared by dissolving 8.5 g K_2HPO_4 in 500 ml dH_2O; also the resulting solution was Stored at 4 °C. 0.1M phosphate buffer was prepared by adding 16 ml of solution A to 84 ml of solution B to make a 100 ml after which 0.327g EDTA disodium salt was added (normally ETDA dissolves at a pH of 8.0). The pH was then adjusted to 7.5 by adding few drops of solution A. This buffer henceforth was known as KPE (potassium phosphate–EDTA) buffer, the buffer was stored at 4 °C.

5% w/v sulfosalicylic acid (SSA)

Was prepared by dissolving 1g of SSA in 19ml dH_2O thereby storing this solution at 4°C. Aliquotes of 50µl brain homogenates were then mixed with 50µl solution containing sulphosalicylic acid (5% w/v) and 0.25mM ethylenediaminetetraacetic acid (EDTA). The brain homogenate samples were then centrifuged at 8000 X *g* for 10 minutes at 4°C. The resulting supernatant was then transferred into new tubes for GSH assay.

APPENDIX 2: GSH ASSAY STANDARD SOLUTION

Was prepared first by making 0.5% SSA (0.1g of SSA was dissolved in 19 ml of dH_2O), GSH stock solution was then prepared by dissolving 1mg of the standard powder in 19 ml of 0.5% SSA to constitute 200 µM GSH standard solution.100 µl of 200 µM GSH standard solution was diluted in serial dilution with 100 µl of 0.5% SSA in 1.5 ml eppendorf tubes, the following GSH standard solutions were made 100mM, 50mM, 25mM, 12.5mM, 6.25mM, 3.13mM, 1.56mM and 0.78mM.

DTNB solution

Was prepared by dissolving 0.06g/100 ml in KPE buffer this solution was stored in 4°C for use within the same day.

GSH concentration measurement

Was determined by adding 20 µl both standards and the brain supernatant separately into a 96 well plate in triplicates followed by addition of 100µl of DNTB, the plate was incubated at 37°C for 10 minutes, then the absorbance was read at 405 nm using a micro plate reader at an interval of 30 sec i,e 30sec, 60sec, 90sec and 120sec. The time interval that gave the best GSH standard graph was chosen for determining the GSH in the brain samples.

APPENDIX 3: MALONDIALDEHYDE (MDA) - PROTOCOL

To determine lipid peroxidation levels in murine brains during ECM, malondialdehyde levels were measured by assays of thiobarbituric acid reactive species (TBARS) (Draper and Hadley, 1990) and the formation of dieneconjugated species (Puhl et al., 1994).

Homogenize brains from mice at day 6 of ECM in cold phosphate buffer, pH 7.4 with butylated hydroxytoulene (BHT) (final concentration 0.2%).

Mix the brain homogenate samples (0.5 ml) with equal volume of thiobarbituric acid 0.67%

Heat at 92-96^{o}C for 30 min.

Quantify the Thiobarbituric acid reactive species production at 535nm using a spectrometer

Evaluate the formation of diene-conjugate:

Extract lipids by a panel of chloroform: methanol (2:1, v:v)

Quantify the resulting organic phase at 234 nm.

Express the results were as malondialdehyde and diene equivalents per milligram of protein

APPENDIX 4: NITRIC OXIDE PROTOCOL

Griess reaction

Prepare griess reagent (10 µl per microplate well): mix together equal volumes of components A and B in an Eppi.

Mix together in wells, 10 µl of Griess reagent + 75 µl of samples (duplicate or triplicate) + 65 µl of A. dest. Incubate for 30 min.

Prepare blank (row H_{1-3}): mix together 10 µl of Griess reagent + 140 µl of A. dest

Prepare the standard: dilute the sdt at 1/10 (eg: 50 µl sdt in 450 µl A. dest) in order to get a concentration of 100 µM in row A_{1-3}. Make a serial dilution from row B-G_{1-3} (50-1 µM). Get in the sdt wells a total volume of 75. Therefore put first in rows B-G_{1-3} 75 µl of A_{1-3}. dest and 150 µl of 100 µM sdt in row A and then perform the serial dilution. Add to the sdt wells 10 µl of Griess reagent +65 µl of A. dest.

Measure at 548 nm

Note: for the right template, open SoftMax Pro, go to protocol->ELISA Endpoint->HRP and TMB. For setting enter 548nm.

APPENDIX 5: ETHICCAL APPROVAL FORM

NATIONAL MUSEUMS OF KENYA

Institute of Primate Research

Address: P. O. Box 24481-00502 Karen Nairobi Kenya I Tel: +254 02 2606235 | Fax: +254 02 260623 1
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INSTITUTIONAL REVIEW COMMITTEE (IRC)

FINAL PROPOSAL APPROVAL FORM

Our ref: ISERC/08/2017

Dear Dr. Alfred Orina Isaac,

It is my pleasure to inform you that your proposal entitled "**putative effects of cyanocobalamin in the regulation of immune responses during severe stage of Human African Trypanosomiasis**" has been reviewed by the Institutional Review Committee (IRC) at a meeting of 20" February 2018. The proposal was reviewed on the scientific merit and ethical considerations on the use of animals for research purposes.

This proposal was approved with the following recommendation;

The committee is guided by the Institutional guidelines as well as International regulations. Including those of WHO, NIH, PVEN and Helsinki Convention on the humane treatment of animals for scientific purposes and GLP.

This proposal has been approved and you are bound by the IPR Intellectual Property Policy.

Signed...K.E.Dhola... For Chairman IRC: Dr Kelly Olhola.

INSTITUTE OF PRIMATE RESEARCH
INSTITUTIONAL REVIEW COMMITTEE
P. O. Box 24481-00502 KAREN
NAIROBI - KENYA
APPROVED

Signed.....Secretary IRC: Dr. MERCY AKINYI

Date 05/03/2018

APPENDIX 6: PUBLICATION

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Vitamin B12 blocked *Trypanosoma brucei rhodesiense*-driven disruption of the blood brain barrier, and normalized nitric oxide and malondialdehyde levels in a mouse model

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APPENDIX 7: PLAGIARISM REPORT

James Ochieng Oula

ORIGINALITY REPORT

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PRIMARY SOURCES

1	Lynn Kitwan, Celestine Makobe, Raymond Mdachi, Dawn Nyawira Maranga, Alfred Orina Isaac, James Nyabuga Nyariki. "Coenzyme Q10 prevented Trypanosoma brucei rhodesiense-mediated breach of the blood brain barrier, inflammation and organ damage in late stage of Human AfricanTrypanosomiasis", Journal of Parasitic Diseases, 2022 Publication	2%
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