PROTECTIVE ROLE OF ANTIOXIDANT IN AMELIORATING AFRICAN TRYPANOSOMIASIS - INDUCED BRAIN DEGENERATION IN A MOUSE MODEL

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EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

DECLARATION

I declare that this thesis is my original work and has not been submitted wholly or in part in thisform or any form for a degree in this or any other university.

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DEDICATION

I dedicate this thesis to Orwenyo family members for their support and encouragement and to my late father Dickson Nyanyuki Orwenyo who at all times provided unwavering courage and mentorship.

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ABSTRACT

Late stage Human African Trypanosomiasis (HAT) is manifested by brain degeneration following infection by *Trypanasoma brucei rhodensiense* and *Trypanasoma brucei gambiense* parasites. This stage can only be treated with melarsoprol (Mel B) which inadvertently induces Post Treatment Reactive Encephalopathy (PTRE) and a mortality of 5% among HAT infected patients. This is an unacceptable mortality for a modern human drug. Investigations were conducted to establish the protective role of Coenzyme Q10 (CoQ10) and endogenous antioxidants (Manganese Superoxide dismutase (MnSOD), Glutathione Reductase (GR), Copper-Zinc Superoxide dismutase (SOD-1) and reduced glutathione (GSH)) against PTRE and putative resultant brain degeneration in a mouse model. This study employed a well characterized mouse model in which female swiss white mice infected with *T. b. rhodesiense* were manipulated to simulate all phases of HAT, including PTRE. Expressions levels of the endogenous antioxidants in the brain tissues were assessed using imunoblot or spectrophotometric procedures. There were significantly higher expressions of MnSOD (P=0.0014), SOD-1 (P=0.0001), and GR (P=0.0083) in infected than uninfected mice 21 days post infection (dpi), which were two-folds lower than those observed at 57 dpi. Levels of GSH were significantly lower (P=0.0347) in infected 57dpi than uninfected mice 21dpi. Expressions of SOD (P=0.0429), GR (P=0.0001) and GSH (P=0.0001) were significantly higher in infected than in uninfected mice among Mel B treated mice. Mel B treated uninfected mice had significantly lower expressions of GR (P=0.0001) and GSH (P=0.0001) and MnSOD (P=0.0035), than untreated (uninfected) controls. Pre-treatment with CoQ10 significantly increased expression of GSH (P=0.0001) relative to the Mel B treatment alone. This is a significant finding that demonstrates that CoQ10 exposure prior to Mel B could boost GSH and neuroprotect in late stage therapy. The results indicate that the parasites and Mel B suppress the antioxidant system. The resultant time dependent dynamics of antioxidant suppression due to Mel B, and potential ameliorating effects of CoQ10 on the same, shed light on putative mechanism of toxicity of the drug during treatment of late stage HAT and a possible antidote of the toxicity. CoQ10 and endogenous antioxidants can find application in 1) formulation of novel Mel B based drugs against late stage HAT and 2) development of novel markers for staging the disease.

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LIST OF ABBREVIATIONS

BBB	Blood brain barrier
CAT	Catalase
CoQ10	Coenzyme Q10
CSF	Cerebral Spinal Fluid
DA	Diaminazene aceturate
DAB	3, 3-Diaminobenzidine
DPI	Days post infection
DTNB	5-(2-Aminoethyl)Dithio-2-Nitrobenzoic Acid
GP_X	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
HAT	Human African Trypanosomiasis
HEPES	N-2 hydroxyethylpiperazine-N`-2 ethane sulfonic acid
iNOS	Induction of nitric oxide synthase
Mel B	Melarsoprol
MnSOD	Manganase superoxide dismutase
NO	Nitric oxide
$ONOO^-$	Peroxynitrite
PAGE	Polyacrylamide gel electrophoresis
PTRE	Post treatment reactive encephalopathy
ROS	Reactive oxygen species
SDS	Sodium dodocyl sulphate
SOD	Superoxide dismutase
SOD-1	CuZn superoxide dismutase
T.b.r	*Trypanosoma brucei rhodesiense*

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CHAPTER ONE
INTRODUCTION

1.1 Background information

Human African Trypanosomiasis (HAT), or sleeping sickness, is a disease caused by infection with the protozoan *Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodesiense*. HAT is a neglected disease, affecting poor people in Africa (Trouiller *et al*., 2002). The neurological manifestations of HAT appear after the invasion of the central nervous system (CNS) by the parasites (Kennedy, 2004). Once parasites have established themselves within the CNS, a progressive breakdown of neurological function accompanies the disease, due to oxidative stress (Halliwel, 2001). It is evident that such oxidative stress results when cellular antioxidant capacity has been overwhelmed. A wide array of enzymatic and non-enzymatic antioxidant defenses exist in the brain that scavenges radicals, they include superoxide dismutase (SOD), glutathione peroxidase (GP_X), catalase (CAT) and glutathione (GSH) (Mataix *et al*., 1998). Alteration of this cellular antioxidant system can lead to neuronal degeneration.

Regulation of antioxidant enzymes in brain cells challenged with trypanosome infection or drugs (potentially toxic) such as melarsoprol has not been well studied in HAT. Inflammatory response in CNS pathology due to the parasites and post melarsoprol treatment has nevertheless been implicated, with microglia and astrocyte activation occurring during the CNS invasion process (Kennedy, 1999). Additionally, induction of nitric oxide synthase (iNOS) and synthesis of nitric oxide (NO) metabolites in the brain prior to neurological dysfunctions in HAT has been documented (Keita *et al*., 2000), with potential disruption of brain antioxidant system.

Whereas constitutive neuronal NOS-generated NO exerts a variety of normal neurological functions, continuous production of iNOS-generated NO induces toxic effects and contributes to pathological disorders (Clark and Cowden, 1999). Specifically, NO facilitate generation of lethal reactive metabolite species such as peroxynitrite ($ONOO^-$) in the brain, which nitrate vital proteins and/or enzymes in the brain, altering their structure and rendering them dysfunctional. Moreover, the metabolites depletes cellular antioxidant defenses such as glutathione (GSH) (Bolanos *et al*., 1997; Heales and Bolanos, 2002), increasing susceptibility of the neurons to oxidative stress, which in turn impairs their functions with putative resultant neurological dysfunction generally observed in the late stages of HAT infections. Other antioxidant enzymes (e.g. superoxide dismutase) and vital enzymes of the electron transport chain have been found to be targets of oxidant damage, further aggravating the biochemical

and neurological impact of the metabolites (Adams *et al*., 1986). Therefore, it is important to explain how the antioxidant system is controlled to maintain cellular integrity in conditions that may trigger oxidative stress, such as HAT.

It is possible that chronic astrocyte activation in trypanosomiasis can interfere with one of the major functions of the astrocytes in the brain i.e. neuroprotection and maintenance of a robust antioxidant system. It is vital to note that impaired astrocyte function could be highly deleterious to brain integrity in HAT. Although the neurons when cultured with NO-generating astrocytes show some minimal damage in their mitochondria (Nekhaeva *et al*., 2002), long-term exposure of the same generate neuronal death. This suggests that neurodegenerative disorders and pathological responses to trypanosome invasion of the brain may be associated with chronic iNOS induction, with decline in or impaired GSH trafficking (Arnaudo *et al*., 1991). This is evidenced in Parkinson's disease (PD), and Alzheimer's diseases (most debilitating neurodegenerative diseases) where induction of NO results in impairment of mitochondrial function (Jo *et al*., 2001), implicating astrocytic NO in neuronal demise.

One of the goals in this study was to determine if antioxidant coenzyme Q10 can attenuate Mel B toxicity and pathological lesions in the hippocampus. Therefore in this study we endeavored to determine if oral administration of coenzyme Q10 in the brain protected this pivotal organ from melarsoprol induced toxicity. Coenzyme Q10 is a powerful antioxidant that is known to cross the blood brain barrier. There is an increasing interest in the potential usefulness of co-enzyme Q10 to treat neurodegenerative diseases such as Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (Meredith *et al*., 2009). Importantly, CoQ10 has been shown to protect against β -amyloid toxicity (McCarthy *et al.,* 2004; Winkler-stuck *et al.,* 2004). Also Coenzyme Q10 has been reported to effectively attenuate toxicity in transgenic mice following administration with 3-Nitropropionic acid (Matthews *et al*., 1998). In cultured cerebellar neurons Coenzyme Q10 provided protection against glutamate toxicity (Favit *et al*., 1992). Hence there is need to full understand the potential neuroprotective role of CoQ10 in the brain, potential involvement of antioxidant capacity failure in HAT and Mel B toxicity.

In this study, we evaluated the effect of melarsoprol on antioxidant proteins, in particular MnSOD, SOD1, GSH and GR and the effect of CoQ10 on Mel B toxicity. It is vital to note that these antioxidants are regulatory protein that are highly sensitive to oxidative stress and thus there inactivation results in neurodegeneration (Pietrangelo, 2003; Hentze *et al*., 2004). Since arsenics are known to cause cell injury by oxidative stress, we hypothesis

that melarsoprol impairs these antioxidant enzymes and therefore impacts negatively on the performance of the brain to quench oxidative stress.

Melarsoprol induces post treatment reactive encephalopathy (PTRE) in patients after treatment resulting in death of up to 5% of patients (Pepin and Milord, 1994). This is a big challenge to the management of late stage HAT because melarsoprol is the only late stage drug that can be used to treat both *T. b. rhodesiense* and *T. b. gambiense*. The molecular processes that lead to PTRE are unclear however many hypotheses have been presented. They include immunological reactions to the parasite following therapy, immune complex deposition, and toxicity of the arsenical moiety of melarsoprol, autoimmune reactions or subcurative chemotherapy (Hunter *et al*., 1992).

In a clinical study involving leukemia patients, melarsoprol resulted in neurological seizures suggesting that melarsoprol itself is responsible for the reactive encephalopathy (Soignet *et al*., 1999). It has also been shown that melarsen oxide (metabolite of melarsoprol) could bind to vital metabolic enzymes and result in oxidative stress (Fairlamb *et al*., 2003). The importance of Melarsoprol in late stage HAT cannot be overemphasized, hence the need to fully understand molecular events that trigger PTRE.

1.2 Statement of the problem

Late stage HAT results in brain degeneration and death if untreated. Moreover, the treatment of late-stage HAT with melarsoprol is complicated by PTRE which augments the ongoing CNS pathology and can be fatal. After administration of this drug life threatening PTRE is induced with the overall mortality rate from melarsoprol therapy being 5%, which is unacceptably high. However, the underlying molecular events that contribute to the demise of brain nerve cells due to melarsoprol induced PTRE are not well characterized. Potential implication or involvement of the antioxidant system in PTRE could open new avenues that can be manipulated pharmacologically to reduce its occurrence and improve treatment outcome in HAT.

1.3 Objectives

1.3.1 General objective

To investigate the role of antioxidant defense capacity in late stage HAT infection in an experimental mouse model (Swiss white) of *T. b. rhodesiense*.

1.3.2 Specific objectives

1. To investigate the modulatory effects of CoQ10 on the trypanosome infection process and risk occurrence of melarsoprol induced PTRE.

2. To determine the impact of invasion of the brain by *T. b. rhodesiense* on antioxidant defense capacity.
3. To establish the role of impaired antioxidant defenses in melarsoprol- induced PTRE and toxicity.

1.4 Hypotheses

1. Coenzyme Q10 (CoQ1O) has no modulatory effect on the trypanosome infection process.
2. *T.b. rhodesiense* invasion of the brain have no effect on antioxidant defense capacity.
3. Melarsoprol-induced PTRE and toxicity do not impair antioxidant defenses.

1.5 Justification

Late stage HAT due to *T.b. rhodesiense* normally results in brain degeneration and the molecular processes that induce such brain damage have not been studied. Therefore understanding how brain cells die in HAT might help in development of strategies to protect the brain in late stage HAT.

Melarsoprol induction of PTRE is a big challenge to the management of late stage HAT because melarsoprol is the only late stage drug that can be used to treat *T. b. rhodesiense*. Since there are no treatment options available for late stage HAT due to *rhodesiense* apart from melarsoprol, it therefore means that understanding the role of antioxidant capacity may result in better management of HAT in patients. The antioxidant system therefore potentially presents critical pathways that can be targeted in development of tools for management of neurological manifestations due to HAT or related drugs that deserve further scrutiny. Such study will present alternative approaches e.g. antioxidant therapy to improve treatment outcome and reduce PTRE prevalence. Thus the information generated from this research will be critical in planning and execution of future treatment, may find application in facilitating use of non-drug interventions during therapy that might supplement the therapeutic effects of existing medications while enhancing treatment outcome. Such supportive interventions might include use of known effective antioxidant supplements.

CHAPTER TWO

LITERATURE REVIEW

2.1 Trypanosome invasion of the brain and neuropathogenesis in HAT

HAT due to *T.b. rhodesiense* causes acute infection with CNS invasion occurring as early as day seven post infection (Fevre *et al*., 2005). A number of successful attempts reveal that injection of *Trypanosoma brucei* into mice via the intraperitoneal route leads to a chronic infection in which the parasites are detectable in the CNS after 21 days. Additionally in other studies it has been shown that if the drug berenil (diminazene aceturate), which does not cross the blood-brain barrier is administered and clears the parasites from the extravascular compartment but not the CNS, is given 21–28 days after infection, the mice will develop a severe post-treatment meningoencephalitis, which persists after the parasitemic phase is over. This condition shows strong pathologic similarity to PTRE in humans. A consistent observation in the same studies shows that astrocytes are activated 14–21 days after infection and prior to the development of the inflammatory response (Nikolskaia *et al*., 2006), and that transcripts for several cytokines such as Tumor necrotic factor-α (TNF-α), Interleukin-1 (IL-1), IL-4, IL-6 and Interferon-γ (IFN-γ) can be detected in the brain at this time. Early astrocyte activation is therefore likely to be of central importance in generating the CNS inflammatory response. The pathologic lesions observed in late-stage sleeping sickness is a meningoencephalitis in which cellular proliferation occurs in the leptomeninges, and a diffuse perivascular white matter infiltration consisting of lymphocytes, plasma cells, and macrophages is prominent (Atouguia and Kennedy, 2000). Also, the perivascular cuffs and adjacent parenchyma contain markedly activated astrocytes and macrophages, and the white matter contains pathognomonic morular or Mott cells, which are thought to be modified plasma cells containing eosinophilic inclusions comprising Immunoglobin M (Lejon, 2002). PTRE shows an exacerbation of these pathologic features. Current understanding of the highly complex pathogenesis of sleeping sickness is based mainly on studies carried out either on patients' blood and CSF samples or in experimental animal models.

Several possible causes of PTRE have been suggested, including subcurative chemotherapy, abnormal immune responses to glial cell–attached antigens released from killed parasites following melarsoprol treatment, immune complex deposition, arsenical toxicity, and autoimmune mechanisms (Legros, 2002). Furthermore, PTRE has been studied in a reproducible mouse model that mirrors many of the pathologic features of the disease in humans (Maclean *et al*., 2001).

2.2 Role of microglia and Astrocyte in the CNS during HAT

Microglia and astrocytes represent highly reactive CNS cell populations, which respond to inflammatory and infectious stimuli by regulating molecules implicated in the control of the immune reaction (Curto *et al*., 2004). On the other hand, microglia cells play the main role in CNS immune vigilance. Some studies show that microglia is the CNS sensor of pathological events, as it is activated in the initial stages of responses to injuries, preceding reactions of other CNS cells (Kreutzeberg and Reinvich, 1997). Besides the classical response repertoire, with migration to the injury site, functional, morphological and immune phenotypic changes, the activated microglia can act as cytotoxic cells (Girard *et al*., 2003).

It has been determined experimentally that the, synergic effects of microglia and astrocytes are necessary for tissue repair after lesions, and they involve the control of the BBB, the entrance of blood cells, removal of proinflammatory cytokines and their regulation (Merril and Benveniste, 1996). Other experimental evidence suggests that in a response to invasion, the CNS may create its own defense system through the resident cells, the microglia and astrocytes, where by both cells modulate the function of hematogenous cells, establishing integration between the CNS resident cells and immune system cells (Gao *et al*., 2002).

Previous and current literature demonstrates the important role astrocytes play in neuroprotection and also as a source of reactive nitrogen species, which is a cell signaling molecule that can induce neuronal damage (Heals *et al*., 2004). This observation is important because a defect in normal astrocytic function will obviously render neurons more vulnerable to oxidative stress. Understanding the astrocytic/neuronal interactions that maintain normal brain function holds great promise in elucidating molecular mechanisms of neurological diseases.

2.3 Chemotherapy and treatment

The current treatment of HAT is based on four main drugs, namely suramin, pentamidine, melarsoprol, and eflornithine (difluoromethylornithine, or DFMO), with nifurtimox undergoing evaluation. Early-stage disease is treated with suramin in *rhodesiense* disease and with intramuscular pentamidine in *gambiense* disease according to established treatment protocols. Treatment is effective and prevents disease progression. The trivalent organic arsenical melarsoprol is the only effective drug for late-stage disease in both forms of HAT, as the drug crosses the blood-brain barrier (Atouguai and Kennedy, 2000). Therefore staging of HAT is of paramount importance for effective chemotherapeutic administration.

Studies conducted using immunosuppressant drug azathiaprine shows that this drug can prevent but not cure PTRE (Hunter *et al*.,1992), and the non-peptide Substance P (SP)

antagonist RP-67,580 has been shown to significantly ameliorate both the neuroinflammatory reaction and the level of astrocyte activation (Kennedy, 2008a; Kennedy, 2008b). Moreover, SP plays a role in generating the inflammatory response in this PTRE, recent evidence has shown that this is complex, since infected SP knockout mice show a novel phenotype in which the clinical and neuroinflammatory responses are dissociated with evidence of alternative tachykinin receptor usage (Kennedy, 2003).

The trivalent organic arsenical melarsoprol is the only effective drug for late-stage disease in both forms of HAT, as the drug crosses the blood-brain barrier. It is a combination of the trivalent organic arsenical melarsen oxide (Mel OX) with the heavy metal chelator BAL (British anti-lewisite, dimercaprol). Melarsoprol's metabolite- Mel OX is the active drug (Keiser *et al*., 2000). Mel OX inhibits the polyamine synthetic pathway in the trypanosome and its primary target is trypanothione (Fairlamb *et al.*, 2003).

Trypanothione the primary target of Mel OX is the major thiol-containing molecule in trypanosomes and is essential for maintaining an intracellular reduced environment by detoxifying oxygen radicals. As such, the binding of Mel OX to trypanothione leads to oxidative stress in the trypanosomes. Trypanothione functions like GSH. It is therefore possible that this could negatively affect human GSH. Through pharmacokinetic studies Melarsoprol, is usually given as two to four series of three daily intravenously injections, or a single daily injection for 10 days (Chappius, 2007). Since, it is insoluble in water then it must be dissolved in propylene glycol, and then given intravenously. For this reason, it is painful to administer and destroys veins after several applications. But the major problem with melarsoprol treatment is that it is followed by a severe post-treatment reactive encephalopathy (PTRE) in up to 10% of cases, with a fatality rate of about 50%. Thus the overall mortality rate from melarsoprol therapy is 5%, which is unacceptably high (Pepin, 1994). Although the mechanism of melarsoprol action has been extensively studied, it still remains unclear. Experimental studies show that once, the parasites is exposed to low (1–10 μM) levels of the drug rapidly lyses. Because the bloodstream forms are intensely glycolytic, any interruption of glycolysis or interference with redox metabolism should produce this effect. Thus a series of reports has detailed melarsoprol inhibition of trypanosome pyruvate kinase (Ki, 100 μM), phosphofructokinase (Ki, <1 μM), and fructose- 2, 6-bisphosphatase (Ki, 2 μM). It is likely that the rapid inhibition of fructose -2, 6,-bis-phosphate production is a key factor in halting glycolysis through downregulation of pyruvate kinase (Wang, 2004). Other studies indicate that melarsoprol and melarsen oxide form adducts with trypanothione (N1, N8-bisglutathionyl

spermidine), a metabolite unique to trypanosomes and believed to be responsible for the redox balance of the cell and detoxification of peroxides.

It has been demonstrated that the melarsen–trypanothionine adducts (Mel T), inhibits trypanothione reductase, which has been attributed to the mode of action. However, melarsoprol and related arsenicals may also bind to other sulfhydrylcontaining agents in the cell, including dihydrolipoate and the closely adjacent cysteine residues of many proteins. Similar to pentamidine and diminazene, melarsoprol uptake into African trypanosomes has been attributed to the P2 purine nucleoside transporter; thus, significant levels can be concentrated in the cell from a low external (plasma) concentration (Fairlamb *et al*., 1992). Although most laboratory-generated melarsoprol-resistant strains have lost or modified the P2 transporter, clinical isolates appear to have retained uptake capacity (Koning, 2007).

2.4 Oxidative stress and effects of antioxidants

Molecules, such as hydrogen peroxide (H_2O_2) and peroxynitrate (ONOO), are not free radicals, but are generated through various chemical reactions. This free radicals and related molecules are often classified together as reactive oxygen species (ROS) to signify their ability to promote oxidative changes within the cell. Cells normally employ a number of defense mechanisms against damage induced by such free radicals (Sudha *et al*., 2001). For instance, body organs are liable to attack by this free radicals, but the brain is most susceptible to attack by the free radicals as it is rich in polyunsaturated fatty acids and highly oxygenated (Dusica and Vesna, 2002) and the damage so caused to the neurons by these free radicals cannot be repaired Moreover pathogenic lesions in brain have been reported in both sleeping sickness patients and *T.b. brucei* infected rat (Perry *et al*., 2002) that are brought about by the oxidative stress.

Problems normally occur when production of ROS exceeds their elimination by the natural antioxidant defense system, or when the latter is damaged. This imbalance between cellular production of ROS and the ability of cells to efficiently defend against them is called oxidative stress (OS). Oxidative stress can cause cellular damage and subsequent cell death mainly by apoptosis in neurodegeneration because ROS oxidizes vital cellular components such as lipids, proteins, and DNA (Gorman, 1996). Experimental studies have shown that iron may be essential, particularly during brain development, but its presence also means that injury to brain cells may release iron ions, which lead to oxidative stress via the iron-catalyzed formation of ROS (Andorn *et al*., 1990).

Nitric oxide (NO), is a key messenger implicated in a wide range of biological processes including cardiovascular and neuronal (Nathan, 1992) systems, and has been shown

to play a critical role in protection against parasitic infections as a regulatory molecule and cytotoxic mediator of the immune system. For example, macrophages from *T .b. brucei* infected mice have been shown to produce high levels of nitric oxide (Mabboth *et al*., 1995).

Although few physiological target molecules of NO have been clearly identified, its role in the protective mechanisms would occur through inactivation of critical enzymes and nitrosation of thiols and other nucleophilic residues (Stamler *et al*., 1992). Furthermore, NO can also react rapidly with a variety of radical species, like superoxide radical anion O^{2-}. While O^{2-} itself is not an efficient oxidizing agent, together with NO· it can produce the more powerful oxidizing peroxynitrite anion -ONOO**-** at an almost diffusion-limited rate (6.7 nM/s) (Huie and Padmaja, 1993), which depends on the concentrations of both radicals

A number of experiments shows that peroxynitrite and its proton-catalyzed decomposition products are capable of oxidizing a great diversity of biomolecules (Koppenol, 1998) including heme containing proteins such as hemoglobin and myeloperoxidase, seleno-proteins such as glutathione peroxidase, DNA or lipids within the cell, or nitrating and nitrosating phenolic compounds such as tyrosines of certain proteins like SODs, and can act as a source of toxic hydroxyl radicals. Consequently, peroxynitrite anion, like NO·, seems to play a major role in the protective mechanisms of the host against parasitic infections, for example, it has been shown that it's highly cytotoxic against *Trypanosoma cruzi* epimastigotes, the causal agent of Chagas disease, whereby it inactivates two key enzymes for their energetic metabolism, succinate dehydrogenase and NADH fumarate reductase (Rubbo *et al*., 1994), portraying the critical role NO play in the CNS. Why is this important? It demonstrates NO can be a source of toxic radicals during HAT in the brain.

Once oxidative stress results in, then appropriate defensive mechanisms that play a pivotal role has to be activated or stimulated to counteract the effect of ROS; such a role is played by the antioxidant defense mechanisms that enhances the process of superoxide removal, scavenging reactive oxygen/nitrogen species or their precursors, inhibition of ROS formation, binding of metal ions needed for the catalysis of ROS generation and up-regulation of endogenous antioxidant defenses. The protective efficacy of such antioxidants depends on the type of ROS that is generated, the place of generation (body barriers such as the blood brain barrier reduce the permeability of most antioxidants) and the severity of the damage (Halliwell, 1997).

The presence of a functional and versatile antioxidant system to remove the reactive nitrogen and oxygen species, and block their deleterious cellular effects is vital for normal cell function and survival. This antioxidant system is especially important in the brain due to its

high energy requirements and higher production of ROS. As part of the antioxidant system, SOD, GPx, and GSH are critical in this regard due to their ability to scavenge for reactive oxygen and nitrogen species thus, they metabolize toxic oxidative intermediates in the brain.

They require micronutrient as cofactors such as selenium, iron, copper, zinc, and manganese for optimum catalytic activity and effective antioxidant defense mechanisms (Halliwell, 2001). SOD, catalase, and glutathione peroxidase are three primary enzymes, involved in direct elimination of reactive oxygen species (hydroxyl radical, superoxide radical, hydrogen peroxide) whereas glutathione reductase, glucose-6-phosphate dehydrogenase, and cytosolic GST are secondary enzymes, which help in the detoxification of ROS by decreasing peroxide levels or maintaining a steady supply of metabolic intermediates like glutathione and NADPH necessary for optimum functioning of the primary antioxidant Enzymes (Singh *et al*., 2003).

2.4.1 Superoxide dismutase

The superoxide dismutase (SOD) are required for the initial dismutation of superoxide anion to hydrogen peroxide (H_2O_2), which, in turn, breaks down into oxygen and water by the action\of catalase and glutathione peroxidase (GPx). The respiratory chain in mitochondria is a major source of oxygen radicals. Manganase-SOD (MnSOD) is a nuclear-encoded primary antioxidant enzyme that functions to remove this superoxide radical (Homi *et al*., 2002).

Several studies have indicated the major importance of SODs and their regulation in protection of the brain from free radical injury. A study involving animal models of stroke shows that Copper Zinc SOD (CuZnSOD) is neuroprotective when over-expressed (Savvides *et al*., 2003), or given exogenously. It has also been hypothesized that neurons expressing nitric oxide synthase (NOS), the synthetic enzyme for nitric oxide (NO), are protected from peroxynitrite-related damage by their co-expression of MnSOD. Furthermore, Over expression of MnSOD has been observed to confer protection against both NO and N-methyl-D-aspartate toxicity in neuronal cell culture systems, thus emphasizing the critical role that they play. However, some studies show that, MnSOD, GPx and glutathione reductase are inactivated by $ONOO^-$ (Dukhande *et al*., 2006). Thus alteration or perturbation of the antioxidant system by $ONOO^-$ increases its cellular toxicity and that of other ROS. In other experimental systems investigating cerebral ischemia/ reperfusion, excitotoxicity, and/or cytokine toxicity, showed that all major cell types of the brain, including neurons, and/or astrocytes (Rohrdenz *et al*., 2001), respond by up regulation of MnSOD and CuZnSOD portraying the functional role of this dismutase.

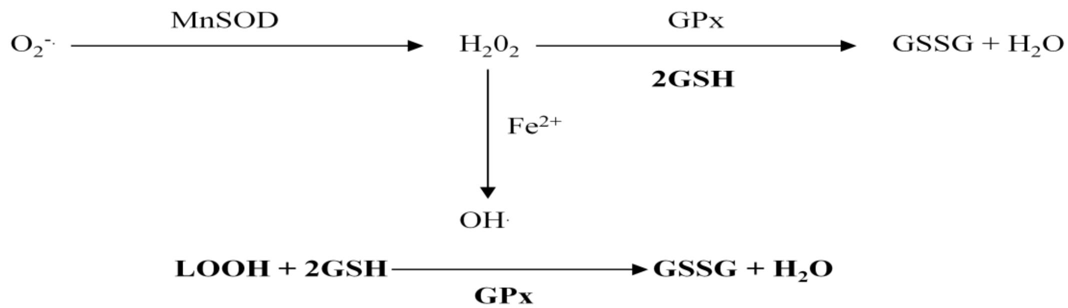

Fig.1. In the upper part dismutation reaction showing the major antioxidants in the brain while the lower part shows GSH-dependent reduction of fatty acid hydroperoxides (LOOH) to alcohols by GPx. Adapted from (Brigelius-Fohle, 1999).

2.4.2 Glutathione peroxidase

The selenium-containing peroxidases (glutathione peroxidase), catalyze the reduction of a variety of hydroperoxides (ROOH and H_2O_2) using GSH, thereby protecting mammalian cells against oxidative damage. It has also been observed that cytosolic and mitochondrial glutathione peroxidase ($_CGP_X$) reduces fatty acid hydroperoxides and H_2O_2 at the expense of glutathione. It's plausible to note that although, GP_X shares the substrate, H_2O_2, with catalase, and itself alone can react effectively with lipid and other organic hydroperoxides.

Studies involving animals cells, especially in human erythrocytes, shows that the principal antioxidant enzyme for the detoxification of H_2O_2 is considered to be GP_X, as catalase has much lower affinity for H_2O_2 than GP_X (Izawa *et al*., 1996). GP_X equally protects against the oxidation of dihydrorhodamine 123 (an indicator dye) by peroxynitrite, requiring GSH as reductant. Thus, there is also a function of GP_X and potentially of other selenoproteins containing selenocysteine or selenomethionine, in the GSH-dependent maintenance of a defense line against peroxynitritemediated oxidations, as a peroxynitrite reductase (Sies *et al*., 1997). Also it has been indicated elsewhere that Overexpression of GPx protects against experimentally-induced oxidative stress in stroke, equally GPx is a very effective and useful antioxidant enzyme because it is localized in both the cytosol and mitochondria (Hoehn *et al*., 2003).

2.4.3 Glutathione

Glutathione (GSH), L-γ-glutamyl L-cysteinyl L-glycine, is an important anti-oxidant. It accounts for about 90% of intracellular non-protein sulfur and is an essential reservoir for intracellular cysteine. Glutathione is very critical in maintaining the redox homeostasis of the cell and its depletion can induce oxidative stress, cell death, and ultimately death of the animal (Griffith, 1999). Reactive species and radicals can oxidize and damage proteins, lipids and

DNA. Therefore reactive oxygen species (ROS), reactive nitrogen and reactive sulfur can have deleterious impact on cells.

Reduced glutathione can neutralize this ROS by directly reacting with them. It repairs oxidized biomolecules and also can remove peroxide species with the help of the enzyme (GPx). NADPH acts as an indirectly anti-oxidant as it assists in the enzymatic conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) by glutathione reductase (GR). NADPH synthesizing enzymes such as NADP-linked isocitrate dehydrogenase (NADP-ICDH), Gluose-6-phosphate dehydrogenase G6PDH) and malic enzymes play an important role in oxidative stress by providing NADPH needed for this conversion.

Thus, activities of enzymes revolving around glutathione regeneration such as NADP-ICDH, G6PDH, GR and GPx are important markers for determining anti-oxidant status of the cell and the cellular damage induced by ROS. Other experimental studies, suggest that the female reproductive hormones may influence glutathione regeneration cycle and may render more protective effects on the female brain from oxidative stress compared to those on the male brain (Vikas *et al.*, 2009). Moreover, physiological processes such as myelination, lipid synthesis and critical growth spurt have been shown to control the activities of reduced GSH regenerating enzymes during development. Thus GSH regeneration cycle may play a key role in various brain disorders where oxidative stress is implicated (Vikas *et al.*, 2009).

In fact, the trypanosome parasite antioxidant trypanothione is synthesised from its mammalian equivalent, glutathione (Smith *et al.*, 1992). Such phenomenon implies that the trypanosomes protect themselves from oxidative stress at the expense of the mammalian host; and could deplete GSH needed to protect the human brain, resulting in oxidative stress. This forms the essence of determination of GSH state in our HAT mice model.

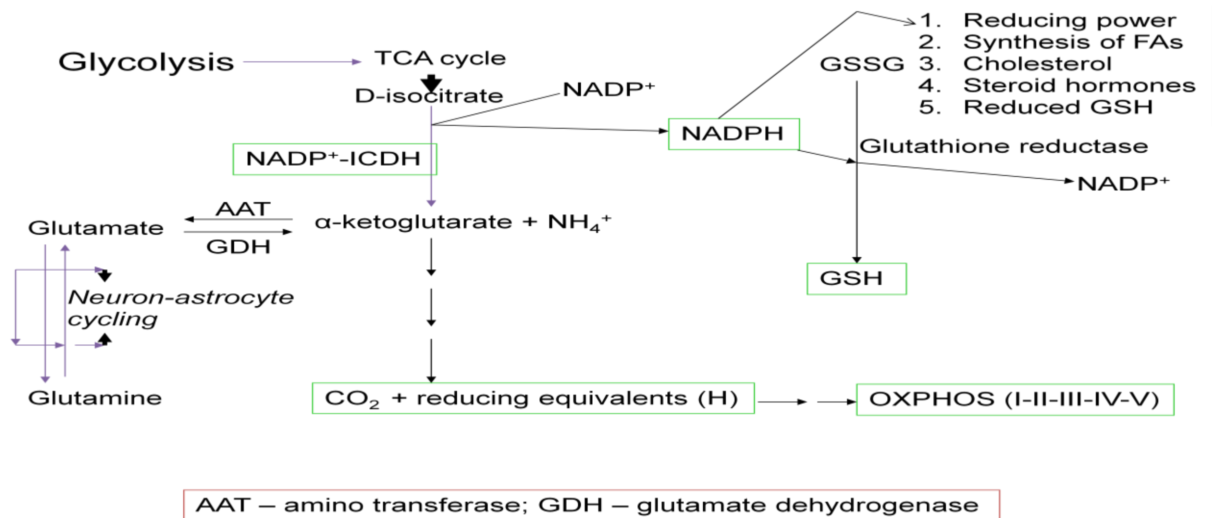

Fig.2. $NADP^+$-ICDH: TCA cycle enzyme & role as an antioxidant. Adapted from *Basic Neurochemistry 7th Edition Molecular, Cellular and medical aspects*

2.5 Brain degeneration

Many neurodegenerative disorders may be associated with chronic iNOS induction and decline in or impaired GSH trafficking (Torreilles *et al*., 1999; Estevez and Jordan, 2002; Heales *et al*., 2004). Similarly, increased NO formation and impaired mitochondrial function are also evident in Alzheimer's disease (AD) (Smith *et al*., 1997), implicating astrocytic NO in neuronal demise. In experimental models of AD, β-amyloid directly inhibits complex IV in brain mitochondria (Kish *et al*., 1992), hence the deleterious effects of β-amyloid are enhanced in the presence of NO (Casley *et al*., 2002). Moreover, in both PD and AD, astrocytic GSH status may dictate cellular susceptibility to reactive nitrogen species and have an effect on ROS generated from the mitochondrial electron transport chain (Vodovoltz *et al*., 1996).

It is important to note that in other encephalopathies like prions disease (mad cow disease; bovine spongiform encephalopathy), abnormal protein aggregation and folding is major contributory factor to neuronal death. Recent findings demonstrate that accumulation of iron might be critical in the formation and aggregation of the infectious prion protein (Singh *et al*., 2009a; Singh *et al*., 2009b). Since the invasion of the brain in late stage HAT interferes with the brain cellular antioxidant capacity, resulting in neurodegeneration and encephalopathy thus I further hypothesize that protein alteration and misfolding may contribute to neuronal death.

2.6 Co-Enzyme Q 10 and neuroprotection

Coenzyme Q10 (CoQ10) is an essential coenzyme in the mitochondrial electron transport chain that regulates cytoplasmic redox potential for oxidative phosphorylation. As an

obligatory coenzyme in the respiratory transport chain, it is essential for the generation of ATP; and hence is particularly important in cells with high metabolic demand such as the brain. CoQ10 is also potent endogenous antioxidants within the LDL molecule that scavenges ROS both in the blood circulation and CNS. The reduced form of CoQ10, ubiquinol, prevents the initiation and propagation of lipid peroxidation in plasma lipoproteins and membrane proteins; and is oxidized to ubiquinone in the process (Pepe *et al.,* 2007). Stocker *et al* (1991) showed that CoQ10 protected LDL-cholesterol more efficiently against lipid peroxidation than did Vitamin E. In addition, it has been shown that CoQ10 can also inhibit lipid peroxidation in mitochondria (Glinn *et al.* 1997), protein oxidation (Ernst *et al.,* 2004) and DNA oxidation (Tomasetti *et al.,* 1999). After its antioxidative action, ubiquinone can be recycled to its active, reduced ubiquinol form via the mitochondrial Q cycle. Furthermore, CoQ10 is a potent antioxidant capable of recycling and regenerating other antioxidants such as a-tocopherol and ascorbate (Sohal, 2004).

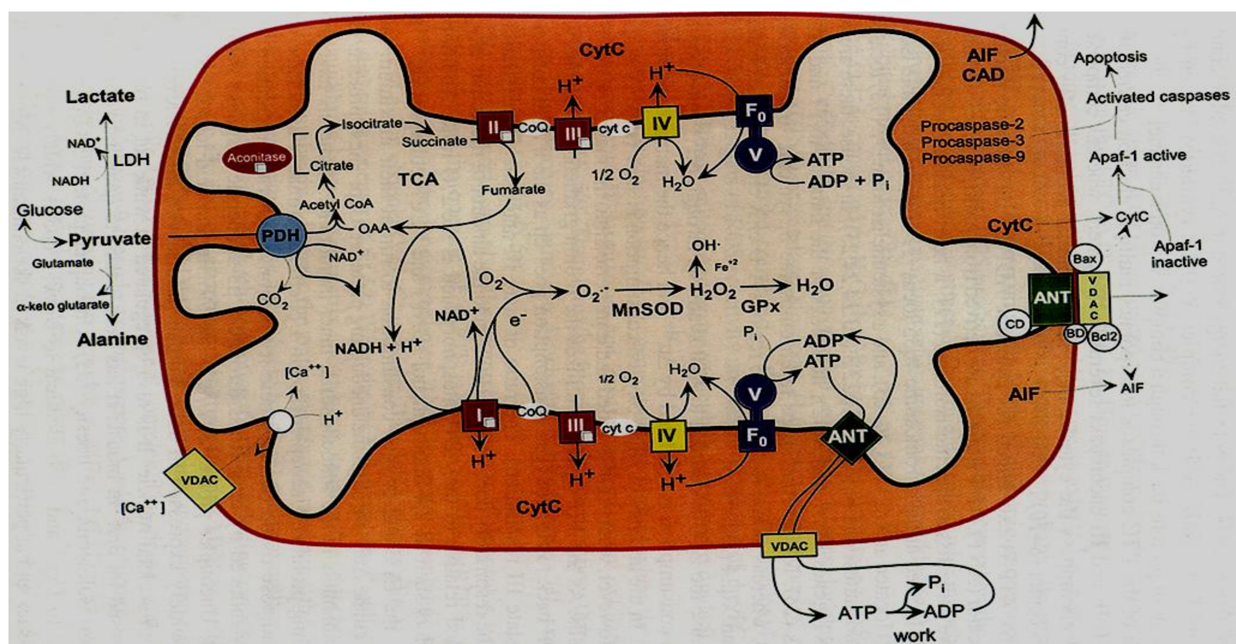

Fig.3. CoQ10 participates in electron transfer in the mitochondrial oxidative respiratory chain Upper part. When reduced; it is a powerful antioxidant that prevents oxidative damage by free radicals, including oxidation of lipids within the mitochondrial membrane lower part. Adapted from *William Copeland, Methods in Molecular Biology, Vol 197(2002)*

CHAPTER THREE

MATERIALS AND METHODS

3.1 Chemicals

Tris-HCl, SDS running buffer, Tris-Glycine transfer buffer, 4-15% SDS gradient acrylamide gels, SDS sample buffers, bovine serum albumin (BSA), Bromophenol blue, Coomassie Brilliant Blue-R250 and SDS protein standards were purchased from BioRad (Hercules, CA). Tween 20, sucrose, N-2 hydroxyethylpiperazine-N`-2 ethane sulfonic acid (HEPES), 3,3-Diaminobenzidine (DAB), L-Glutathione reduced standard (GSH), 5-(2-Aminoethyl)Dithio-2-Nitrobenzoic Acid (DTNB) were from Sigma (St. Louis, MO). Propylene glycol, Manganese chloride, acetic acid, hydrogen peroxide, Skimmed milk, methanol and sodium bicarbonate were obtained from Fischer Scientific (Fairlawn, NJ). Primary and secondary antibodies for MnSOD, GR, SOS-1 and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The protease inhibitor cocktail was from Roche Diagnostics (Mannheim, Germany) and the Lysis buffer was obtained from Active Motiff (Carlsbad, CA).

3.2 Pathogenicity studies

3.2.1 Mice

All experimental procedures and protocols involving mice were reviewed and approved by Institutional Animal care and Use Committee (IACUC) of Trypanosomosis Research Centre (TRC) Muguga, Kenya. Eight weeks old Female adult Swiss White mice (weighing between 20-30g) from KABETE-VET LAB colony were maintained on mice pellets (Unga Feeds Ltd, Kenya) and water *ad libitum* at a temperature of 21-25°c. Wood-chippings were provided as bedding material. All mice were treated with 0.02ml of evermectin (Ivermectin®, Anupco, Suffolk, England) injected subcutaneously to each mouse and left to acclimatize for two weeks. This helped to eradicate both ecto and endo parasites which are known to interfere with the experiment by consuming a lot of blood. A total of 62 mice (60 experimental and 2 donors) were marked using picric acid to give them numbers for easy identification. Experimental mice were randomly selected and then divided into eight groups of six mice each. Two donor irradiated mice were obtained from ILRI for multiplication of *T. b. rhod*esiense clone, KETRI 2537.

3.2.2 Experimental design

Ten groups of mice were used in this study. Group 1 mice were not infected and were sacrificed 21 days post infection (DPI) of group 2. Group 2 were infected with *T. b. rhod*esiense clone, KETRI 2537 and sacrificed 21 DPI. Group 3 were infected and sacrificed

after reaching terminal end. Groups 4 were uninfected and sacrificed the same period as group 5, 6 and 7. Group 5 were uninfected and treated with 3.6mg/kg x 4 of Mel B 21 DPI of group 6 and 7, Group 6 were infected and treated with 3.6mg/kg x 4 of Mel B 21 DPI, sacrificed 24 hours after the last dosage (i.e 25 day). Group 7 were infected after they were orally administered with 200mg/kg CoQ10 every second day for one week and continued at the same dosage until sacrificed they were also treated with 3.6mg/kg x 4 of Mel B 21 DPI. Group 8 were infected and treated with 5mg/kg DA after relapse they were treated with 3.6mg/kg Mel B and then sacrificed 24 hours after the last dosage. Group 9 were infected after they were orally administered with 200mg/kg CoQ10 every second day for one week and continued at the same dosage until sacrificed at 21 DPI they were treated with 5mg/kg x 3 DA, after relapse they were treated with 3.6mg/kg x 4 Mel B and sacrificed 24 hours the last dosage. Group 10 was uninfected (control) sacrificed the same period as group 8 and 9. (Group 8. 9 and 10 were for severe late stage and they were treated with Mel B at the same period of time).

3.2.3 Infection of the mice and preparation of inoculum

Trypanosome stabilates stored in the trypanosome bank at -196°C liquid nitrogen in small capillary tubes of 20µl each, were brought in a cryovial and left to thaw allowing time for the parasites to adapt to the normal temperatures before infection. Viability of the parasites in the capillary tube were checked by putting a drop of its contents on a slide and covered with a cover slip and then viewed under a microscope. Thawed EDTA saline glucose (ESG) buffer was used to dilute the parasites. Viability was checked again, before injecting 0.2ml of the dilution to each donor mouse.

Follow up of the parasitaemia was done by taking a drop of blood from the tail and examining microscopically through a wet film. On the first peak of the parasitaemia (Kagira *et al.,* 2005), a few drops of blood were taken from the tail of one of the donor mouse and were mixed with 2 ml of ESG buffer in a bijou bottle. This blood solution was then diluted 10 times with ESG P^H 8.0 buffer using a leukopipette. The number of trypanosomes was estimated using an improved Neabauer chamber viewed under the microscope at 400x magnification. The 1st and the 2nd count of the parasites (C_1 and C_2 respectively) were made through all the 16 squares of the haemocytometer and the average count C_{av} calculated. Using the formulae below the number of trypanosomes for infection was quantified.

No. of trypanosomes = Average count x dilution factor x 10^4 trypanosomes/ml.

Serial dilution was done so as to get a solution of 5.0 x 10^4 trypanosomes/ml. To each experimental mouse, 0.2 ml of this dilution was injected intraperitoneally so that each mouse got 1.0 x 10^4 trypanosomes (Gichuki and Brun, 1999).

3.2.4 Parasitaemia, Body weight and Packed Cell Volume (PCV)

Mouse tail blood was collected daily for the first week and thereafter every two days. A drop of blood was placed in a clean slide, covered with a cover slip, the parasites counted using a light microscope. The pre-patent period was determined as the time it took for parasites to appear in the blood after which the parasitaemia levels were determined as the infection progressed. The matching technique of Herbert & Lumsden (1976) was used to assess parasitaemia level. The live body weight were determined every two days using the analytical electronic balance (Mettler PM34, DoltaRange®) during the period of infection of each individual mouse and those not infected. PCV was undertaken as outlined by Naessens *et al.* (2005). Infected and uninfected mice blood was collected from the tail vein using heparinized capillary tubes which were then sealed with plasticin at one end. The sealed capillaries were then centrifuged in a haematocrit centrifuge (Hawksley H England) at 10,000 revolutions per minute (RPM), for 5 minutes. PCV were read using the Micro- haematocrit reader and expressed as a percentage (%) of the total blood volume.

3.2.5 Treatment of mice

Oral administration of 200mg/kg of coenzyme Q10 was done after every second day to one group of mice, using a gavage needle was begun seven days prior to infection then continued thereafter. Infected mice administered with CoQ10, infected and uninfected mice; all were treated with melarsoprol 21 days post infection at a dosage of 3.6mg/kg x 4 days. After treatment mice were sacrificed 24 hours post the last dosage to obtain brain samples.

3.2.6 Severe late stage studies

To study severe late stage of HAT, three groups of mice were used; one group was infected with *T.b. rhodesiense* KETRI, 2537, another group of mice was orally administered with coenzyme Q10 200mg/kg that was continued up to the last treatment with melarsoprol following relapse and another group was used as control. At 21 days post infection these two groups of mice were treated sub-curatively with DA (Diaminazene aceturate) 5mg/kg x 3 days of the active ingredient, thereafter mice were monitored for relapse of parasitaemia. After relapse mice were treated with melarsoprol at a dosage of 3.6mg/kg x 4 days, mice were then sacrificed 24 hours post the last dosage to obtain brain samples. One group of infected mice but untreated was left to go to the terminal stage of the disease after which they were sacrificed at day fifty seven. Sensitivity and pathogenicity tests for *T.b .rhodesiense* strains are normally monitored for 60 days after which were sacrificed to obtain brain samples.

3.2.7 Brain harvesting and clinical changes

Each mice group, were sacrificed as per the experimental design (21 days post infection, 25 days post infection, terminal and severe late stage after relapse of trypanosomes). Briefly mice were euthanized with chloroform (trichloromethane). The brain samples were cut into two halves, one half for histopathology and the other half was snap frozen in dry ice before they were stored in liquid nitrogen. These brains were used for biochemical analyses in concert with the study objectives. The clinical picture, gross pathology and histopathology of mice were determined. The clinical status of the mice was determined as described by Gichuki & Brun (1999).

3.2.8 Determination of haematological values

Blood was collected directly from the heart during euthinization of the mice in a 1ml syringe flashed with 5% EDTA and transferred to Bijou bottles. Samples were analyzed using automated Bechman Coulter Counter (Coulter A´-T diff ™) and a complete blood haemogram of each experimental mouse, giving hemoglobin levels (HGB), haematocrit red cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and white blood cell count generated.

3.3 Sample preparation

Snap-frozen whole brains were homogenized on ice water (4°C) in 0.5 mls of 0.25 M sucrose, 5 mM Hepes-Tris, pH 7.4, with protease inhibitor cocktail to a final concentration of 10% (w/v). The homogenates were then aliquoted into 0.5 microfuge tubes (to avoid excessive freeze-thaw process) and stored in liquid nitrogen for analysis.

3.4 Glutathione (GSH) assay

Total GSH content was determined by employing the method of Griffith (1980) with slight modification. Briefly, the brain homogenates were mixed with a solution containing sulphosalicylic acid (4.31% (w/v)) and 0.25 mM EDTA. The GSH in the homogenates were determined chemically by reacting the GSH therein with Ellman's reagent (DTNB) and measuring the absorbance of the reaction product at 412 nm using a multi-detection microplate reader (Bio-Tek Synergy HT).

3.5 Coomassie Brilliant Blue staining for protein visualisation

An aliquot of each of the homogenized brain homogenate of different treatment groups stock were used. 40µl of each brain homogenate was mixed with 20µl of 3x sample buffer (500 mM Tris/HCl pH 6.8, 4.6% (w/v) SDS, 20% (v/v) glycerol, 10% (v/v) 2-mecarptoethanol and 0.004% bromophenol blue) and boiled for 5 min. The brain homogenate samples were then loaded onto an SDS-PAGE gel 1.5 mm thick, 5% stacking

and 10% linear gradient resolving gel (A. Electrophoresis was performed at a constant current of 20 mA (Laemmli, 1970). The resolving buffer (pH 8.3) used contained 25 mM Tris, 192 mM glycine, and 0.1% SDS. Gels were stained according to the protocol described by Johnstone and Thorpe (1982). Briefly, after electrophoresis, the gels were transferred into a clean glass plate container and 5 times gel volume of 0.025% Coomassie Brilliant Blue R-250 in 50% methanol and 10% acetic acid added. The container was covered and incubation done overnight at room temperature (RT) while shaking gently on an orbital shaker. The used stain was decanted, discarded and the gel quickly rinsed in distilled water. Destaining was thereafter done by successive incubation of the gel in several changes of the destain solution (5% methanol and 7.5% acetic acid) also at RT until proteins bands were conspicuously visualised against a clear background. The sensitivity of this protein stain is rated as 0.1-0.5 µg per protein band.

3.6 Western blotting

An aliquot of each of the homogenized brain homogenate of different treatment groups stock were used. Forty microlitre (40µl) of each brain homogenate was mixed with 20µl of 3x sample buffer (500 mM Tris/HCl pH 6.8, 4.6% (w/v) SDS, 20% (v/v) glycerol, 10% (v/v) 2-mecarptoethanol and 0.004% bromophenol blue) and boiled for 5 min. The brain homogenate samples were then loaded onto an SDS-PAGE gel 1.5 mm thick, 5% stacking and 10% linear gradient resolving gel. Electrophoresis was performed at a constant current of 20 mA (Laemmli 1970). The resolving buffer (pH 8.3) used contained 25 mM Tris, 192 mM glycine, and 0.1% SDS. The separated proteins loaded were then electro transferred from the gel onto 0.22 µm nitrocellulose blotting membrane following standard protocol (Towbin *et al*. 1979, Towbin and Gordon, 1984). After electrophoresis, the gel was placed onto transfer buffer presoaked 0.22 µm NCM (BA85, Schleicher and Schuell, Dassel, West Germany) laid on two sheets of Whatman 3MM filter paper which had previously been soaked in transfer buffer (20 % methanol, 192 mM glycine, 25 mM Tris–HCl, pH 8.3). Electrophoretic transfer of the antigens was carried out for 1.5 hr at a constant voltage of 100V at 4^0C. Following transfer of proteins from the gels to NCM but before probing the blots, the success of the transfer was determined by transiently directly adding 5µl of the aliquots to the membranes inform of immuno-dot blots spots after which they were blocked and probed together with other membranes. Once the transfer was complete, the membranes were incubated in 5% fat-free milk (blocking solution) for 1 hour at room temperature. The blocking solution was discarded and the blots incubated in solutions containing, primary antibodies (Santa Cruz Biotechnology, Santa Cruz CA) for SOD-1, MnSOD and GR

respectively followed by their respective peroxidase-conjugated secondary antibodies. The protein-antibody complexes were then visualized with the development system containing 0.10% 3, 3'-diaminobenzidine (DAB) in PBS (pH 7.4) containing 100 μl of 30% hydrogen peroxide The relative levels of SOD-1, MnSOD and GR were determined using pixel density analysis software (NIH, UN-SCAN-IT). *β*-actin was used to confirm equal sample loading for all gel electrophoresis.

3.7 Statistical analysis

The data was entered in Microsoft excel (version 2007). Student's t test was used to test the significant differences of PCV, haematological profile, mean liver and spleen tissue weight, prepatent and relapse period. Also statistical analysis was carried out using one-way ANOVA and post-hoc Bonferronis' test for multiple comparisons with significance level set at $P<0.05$. The statistical analyses was done using GraphPad Prism version 5 Software Inc., (San Diego CA).

CHAPTER FOUR
RESULTS

4.1 The modulatory effect of CoQ10 on pathogenesis of *T.b. rhodesiense* infection in mice

4.1.1 CoQ_{10} altered the time to relapse in mice infected with *T .b. rhodesiense*

The time to relapse in mice infected with *T. b. rhodesiense* and in which PTRE was induced with DA, and treated with CoQ_{10}, was 37 ± 3.512 days with a range of 30 – 44 days. In this group with CoQ_{10}, two mice did not relapse and were declared cured after 180 days, according to WHO guidelines. The relapse period in the PTRE mice that were given CoQ_{10} was longer than that of PTRE induced mice that did not receive CoQ_{10} as shown in table 1.

4.1.2 Packed Cell Volume, Parasitaemia development patterns and mean body weight

The changes in the PCV levels for the various treatment groups are presented in (Fig.4.). There was a gradual decline in the mean PCV levels following infection with *T. b. rhodesiense* 21 dpi which was significantly (P=0.0012) different from the control. Analysis with t-test revealed the means (± SEM) of 10.20 ± 2.083 with the 95% confidence interval 5.396 to 15.00). Significant improvement in the PCV levels in 200mg/kg orally administered *T. b. rhodesiense* infected mice was witnessed comparable to *T. b. rhodesiense* infected 21dpi CoQ_{10} un-supplemented group (Fig.4.). Similar, trend was observed in groups of mice where PTRE was induced to study severe late stage of HAT (Fig.4.). The rate of PCV fall was higher in *T. b. rhodesiense* infected, PTRE induced Mel B treated after relapse compared to mice that received similar treatment but were orally supplemented with CoQ_{10}. However, the healthy (controls) mice did not show any significant drop in the PCV during the period of study (P<0.05).

The daily mean number of trypanosomes in blood in the *T.b. rhodesiense* infected mice and *T.b. rhodesiense* infected CoQ10 administered mice up to 21 days post infection is shown in (Fig.6.). Although no significant (P>0.05) statistical difference in levels of second parasitaemic waves between the groups was observed, *T.b. rhodesiense* infected mice showed a higher second parasitaemic wave than *T.b. rhodesiense* infected CoQ10 group. Parasitaemia was characterized by two prominent parasitaemic waves a first observed on the fourth day of infection followed by a higher peak of wave on day eleven followed by a small decline in wave between day 12 and 15. In the *T.b. rhodesiense* infected mice initial wave occurred 3 to 4 days post infection with a peak at day 11. The *T.b. rhodesiense* infected CoQ10 administered mice had the initial wave between 3 to 4 days post infection with a peak at day 11 post infection of the prepatent period with a small wave at day 13 (Fig.6.). The maximum parasitaemia reached in the time course of the experiment was a mean of antilog 8.7

($5.01x10^8$) trypanosomes for *T.b rhodesiense* infected mice. With fluctuations in parasitaemia development in *T.b. rhodesiense* infected CoQ10 administered mice observed.

There is a high significant difference ($P<0.05$) in body weights in control mice (uninfected), *T.b. rhodesiense* infected mice and *T.b. rhodesiense* infected mice that went to terminal stage (Fig.5.). Also there is a high significant difference ($P<0.05$) in body weight among the group of mice for severe late stage; *T.b. rhodesiense* infected administered orally with CoQ10 treated with DA and Mel B and Infected with *T.b. rhodesiense* treated with DA and Mel B (Fig.5.)**.** The mean body weight varied with an increase in body weight of mice infected but minus CoQ10 drastically increasing between day 3 and 21 thereafter decreased sharply. After treatment with DA there was intermittent alternating in terms of increase and fall in body weight between 23 and 50 day even after relapse it normalized. While the group that was administered with CoQ10 showed different waves in body weight with increase in body weight up to 3 day post infection (25.5g). Then drastic decrease between 5 and 13 day (21g) thereafter increased again after treatment with DA on the 23 day was witnessed. After relapse there was a sharp decline in body weight between 55 and 59 day after which gain in body weight remained constant after treatment with Mel B. The mean live body weight of a second group of mice that were sacrificed on the 25 day showed a significant difference ($P<0.05$). With *T.b. rhodesiense* infected mice treated with Mel B having a higher mean body weight with a maximum of (29.5g). While *T.b. rhodesiense* infected CoQ10 administered mice treated with Mel B showered some fluctuations in terms of gain and losing body weight when compared to control (uninfected but treated with Mel B) (Fig.5.).

Table 1: Mean (± SME) Relapse period of *T.b. rhodesiense*, KETRI 2537 infected mice treated with DA and of *T.b. rhodesiense,* KETRI 2537 infected mice administered with CoQ10 treated with DA.

	Relapse period days	
Mice (n=4)	Mean	Range
Infected with *T.b. rhodesiense* + DA	27.25 ± 2.869	19 - 32
Infected with *T.b. rhodesiense* + CoQ 10 +DA	37.00 ± 3.512	30 - 44

Note: Results are mean ± SEM of 6 mice, no statistical significance ($P>0.05$) for relapse.

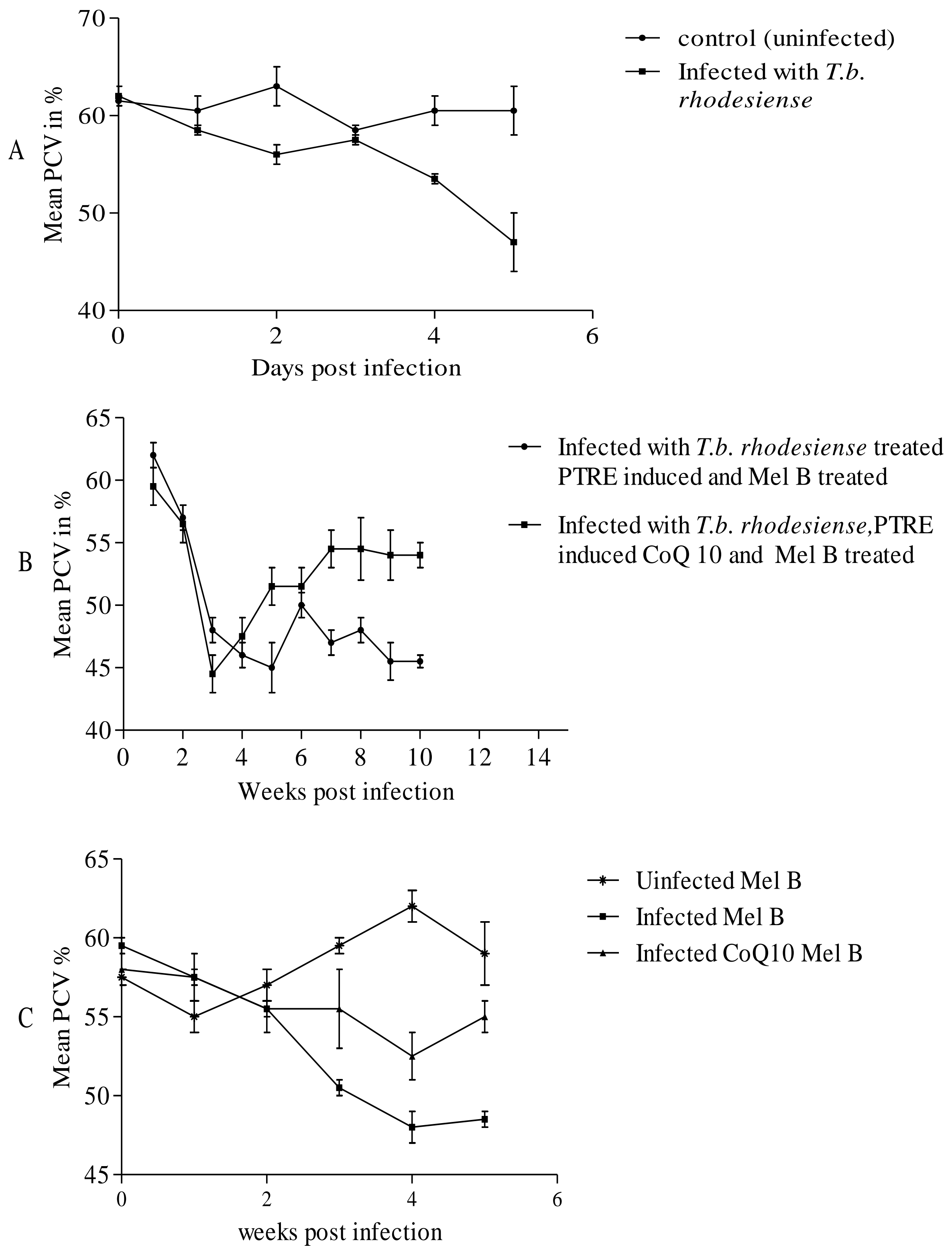

Fig.4. Mean packed cell volume changes in mice. The mice were either infected with *T.b. rhodesiense*, treated with Mel B/CoQ10 or PTRE induced.

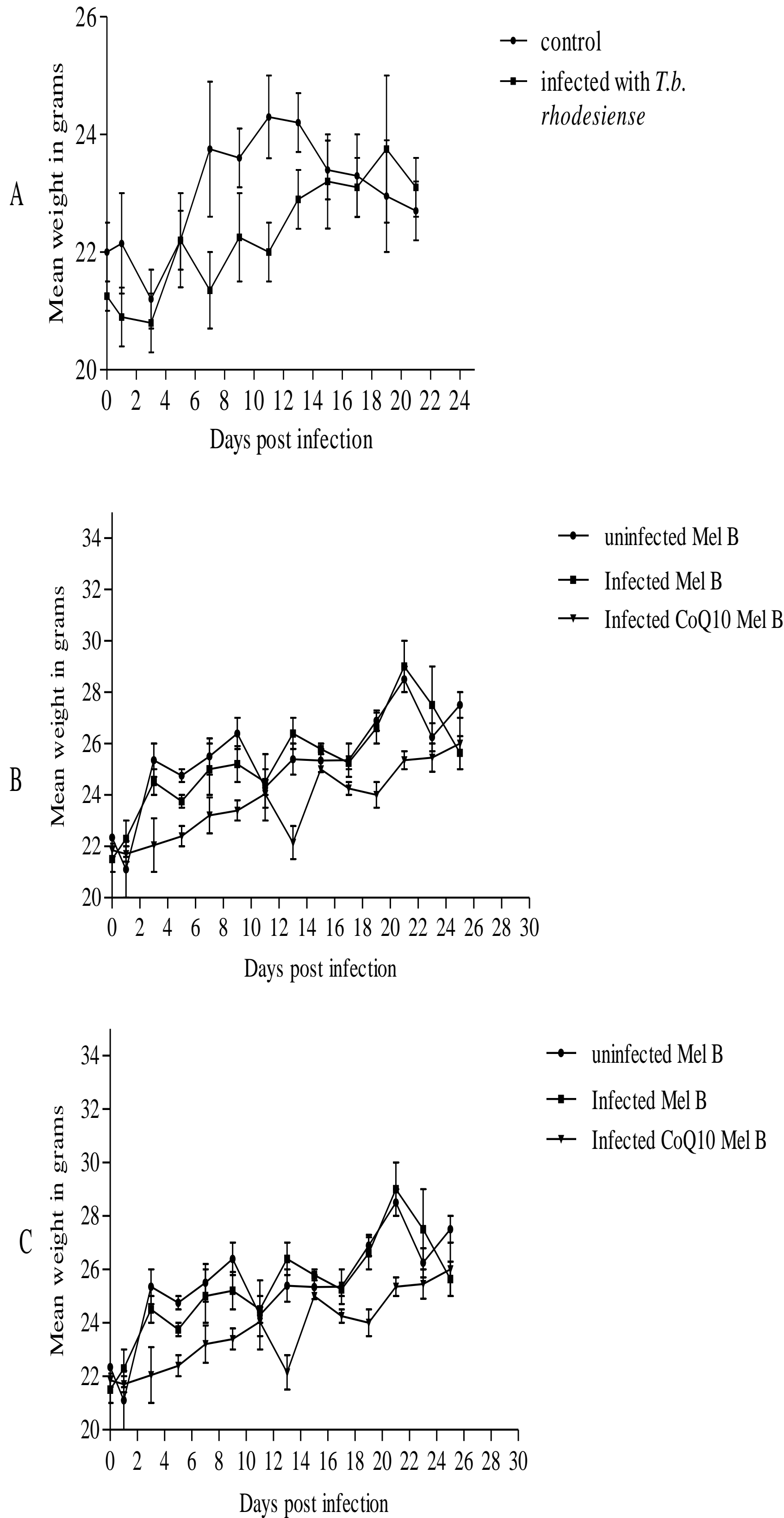

Fig.5. Changes in mean live weights in mice. The mice were either infected with *T.b. rhodesiense*, treated with 3.6mg/kg Mel B/ 200mg/kg CoQ10 or PTRE induced.

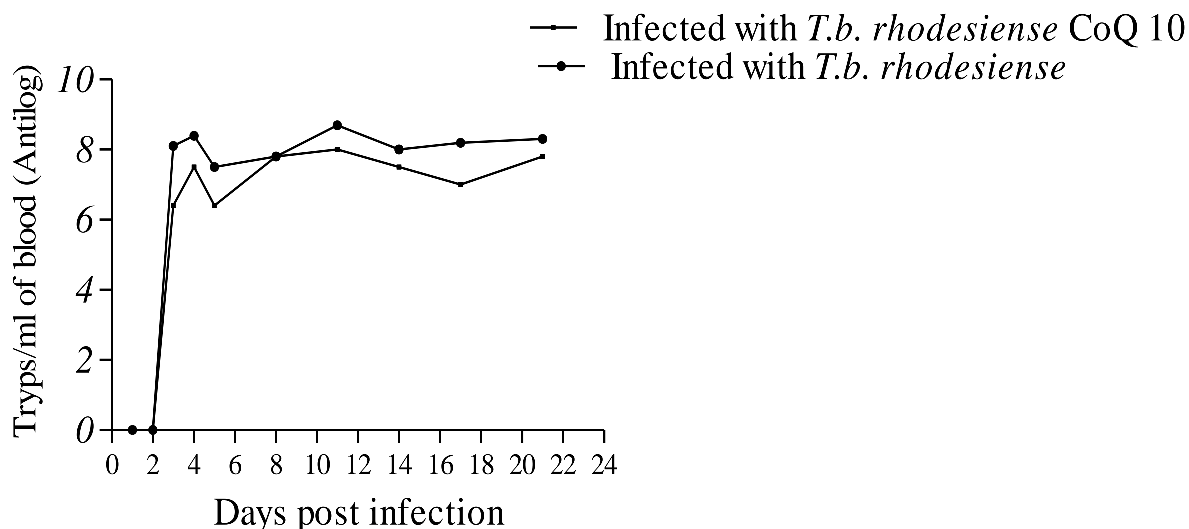

Fig.6. Mean parasitaemia progression in *T.b. rhodesiense* infected mice and *T.b. rhodesiense* infected CoQ10 administered mice sacrificed 24 hours last dosage of Mel B.

4.1.3 Effects of *T. b. rhodesiense*, CoQ_{10}, Mel B and PTRE induction on haematological profile (Hemoglobin, White Blood Cells and Red Blood Cells)

Results of the mean haematological profile levels for Haemogobin (Hg), Red blood cells (RBC) and White Blood Cells in (i) PTRE induced, (ii) Mel B and (iii) CoQ_{10} treated mice, intraperitoneally inoculated with *Trypanasoma brucei rhodesiense* mice are presented in Fig. (7-9.) One way ANOVA revealed that there was a significant difference in levels of Hg in *T. b. rhodesiense* infected minus treatment ($F_{2, 13}$ =11.12, P = 0.0015) or *T. b. rhodesiense* infection treated with Mel B and CoQ_{10} treatment ($F_{3, 20}$=5.145, P = 0.0085) or *T. b. rhodesiense* infection/PTRE induced/Mel B/ CoQ_{10} ($F_{2, 10}$ =7.370, P = 0.0108) treatment in responses to infection and treatment. Bonferroni's Multiple Comparison Test indicated significantly higher Hg levels in *T. b. rhodesiense* 21dpi relative to the infected mice that went to the terminal end (95% confidence 0.7218 to 6.895 P=0.0015) (Fig.7.). Infected mice treated with Mel B had significantly lower Hg levels compared to the Infected Mel B and CoQ_{10} treated mice (95% confidence -6.219 to -0.8806 P= 0.00085) (Fig.7.). Similarly, Bonferroni's post hoc test comparison of the means revealed significantly lower levels of Hg in infected mice (PTRE induced) treated with Mel B than in control mice (95% confidence -0.7217 to 7.093 P=0.0108). While in infected mice (PTRE induced) treated with Mel B alone, Hg was significantly lower relative to (PTRE induced), CoQ_{10} and Mel B treated mice, (95% confidence -7.856 to -0.3109, P=0.0108).(Fig.7.). Analysis of RBC using ANOVA revealed that there was a significant difference in levels of RBC in *T. b. rhodesiense* infected mice

(minus treatment) compared to the control ($F_{2, 13}$ =12.34, P = 0.0010). Bonferroni's Multiple Comparison Test indicated significantly higher RBC levels in *T. b. rhodesiense* 21dpi relative to the control (95% confidence 0.4641 to 4.549 P=0.0010) (Fig.9.). Infected mice treated with Mel B had significantly lower RBC levels compared to the control (95% confidence 2.729 to 6.887 P=0.0001). While infected mice treated with Mel B had significantly lower RBC levels relative to infected Mel B and CoQ_{10} treated mice (95% confidence -5.382 to -1.224 P= 0.0001) (Fig.9.). Similarly, Bonferroni's post hoc test comparison of the means revealed significantly lower levels of RBC in infected mice PTRE induced treated with Mel B than in control mice (95% confidence 3.561 to 7.997 P=0.0001). Infected mice (PTRE induced) treated with Mel B alone, RBC was significantly lower relative to (PTRE induced), CoQ_{10} and Mel B treated mice, (95% confidence -5.774 to -0.5248, P=0.0001). (Fig.9.).

Similarly, analysis of WBC using ANOVA revealed that there was a significant difference in levels of WBC in *T. b. rhodesiense* infected mice that were not treated ($F_{2, 13}$ =41.00, P =0.0001) or *T. b. rhodesiense* infection 21dpi/Mel B/ CoQ_{10} treatment ($F_{3, 20}$ =6.765, P = 0.0139) or *T. b. rhodesiense* infection/PTRE induced/Mel B/ CoQ_{10} ($F_{2, 20}$ =4.804, P = 0.0112) treatment in response to infection and treatment. Bonferroni's Multiple Comparison Test indicated significantly higher WBC levels in *T. b. rhodesiense* 21dpi relative to the mice that went to the terminal end (95% confidence -18.71 to -1.394 P=0.0139) (Fig.8.). Infected mice treated with Mel B had significantly higher WBC levels compared to the control (95% confidence -1787 to -169.3 P=0.0112). (Fig.8.).

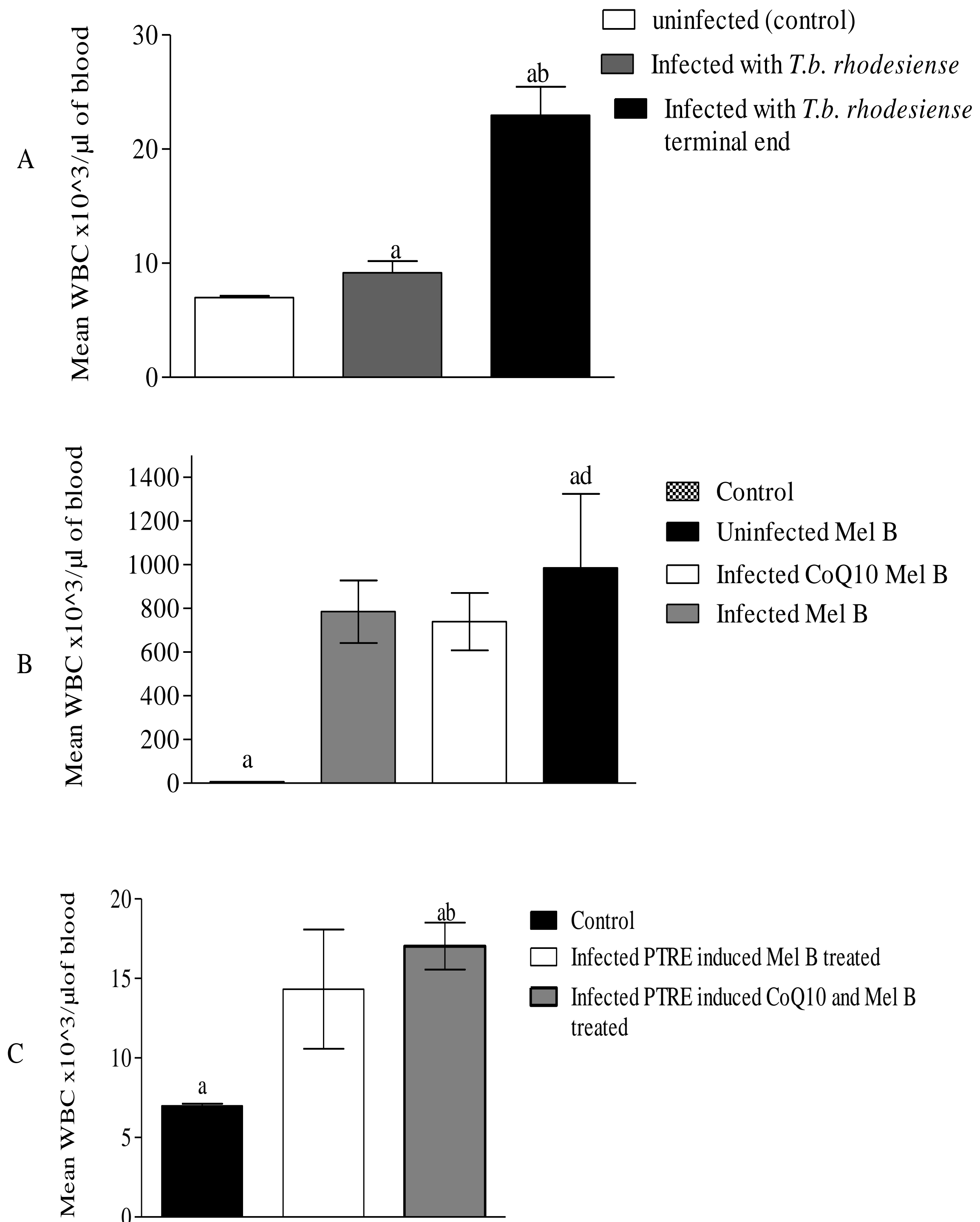

Fig.7. Effects of *T. b. rhodesiense* infection on WBC concentration in the blood. (A) There was (ab) significant ($P<0.05$) difference between (iii) and (ii). (B) ab indicates that WBC concentration is significantly different between control and Uninfected Mel B treated mice ($P<0.05$). The values for the control were very low and therefore the histogram for WBC fraction is too small to be clearly observed as indicated by "a". (C) ab represent significant difference ($P<0.05$) between (i) and (iii).

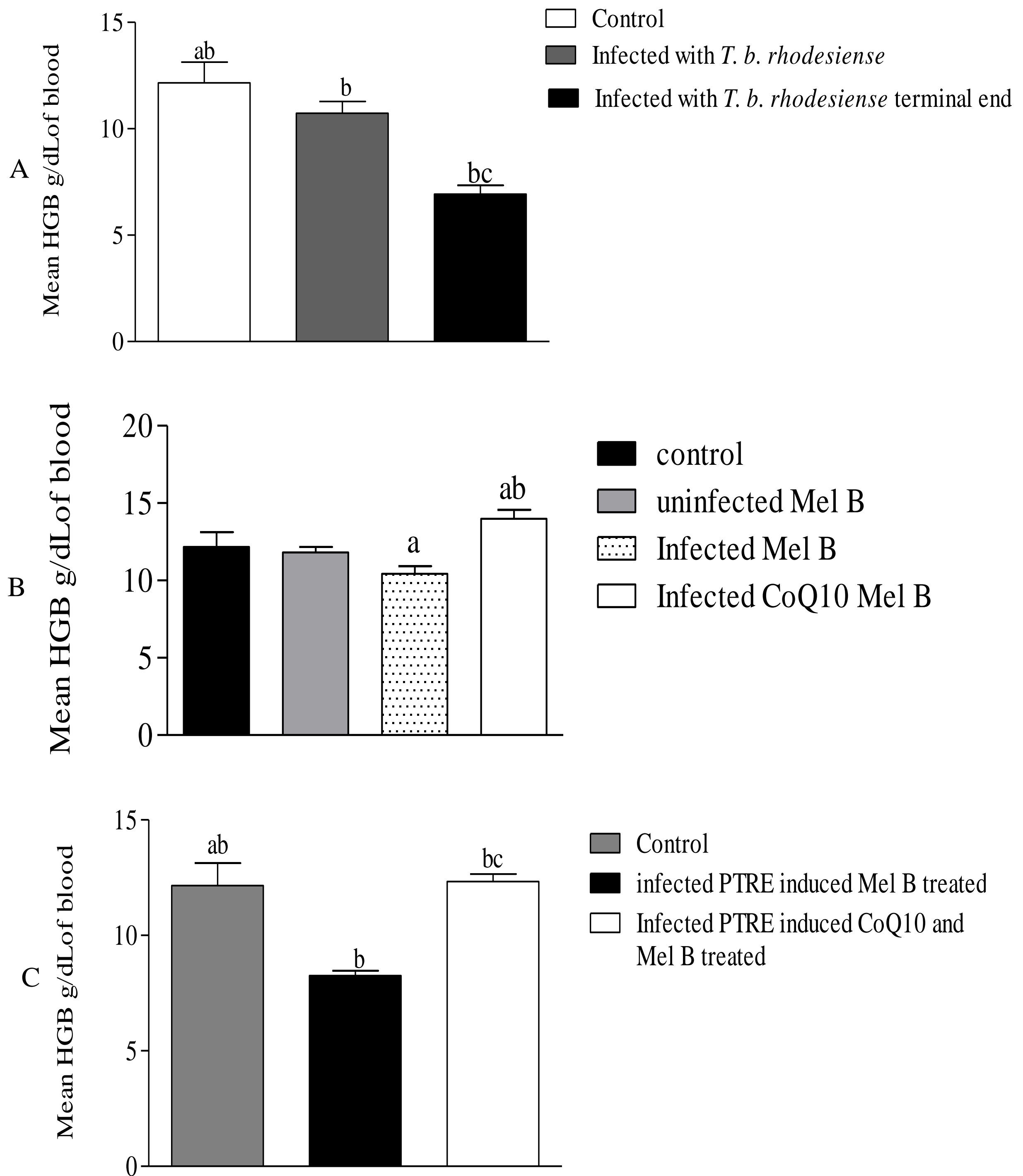

Fig.8. Effects of *T. b. rhodesiense* infection, PTRE, CoQ10 and Mel B treatment on haemoglobin (Hg) concentration measurement in blood (A). ab indicates significant difference (P<0.05) between (iii) and (iv). (B). ab represent significant difference (P<0.05) between ii and the control while bc represent significant difference (P<0.05) between (ii) and (iii). (C). ab represent significant difference (P<0.05) between (i) and (ii) while bc represent significant difference (P<0.05) between (ii) and (iii).

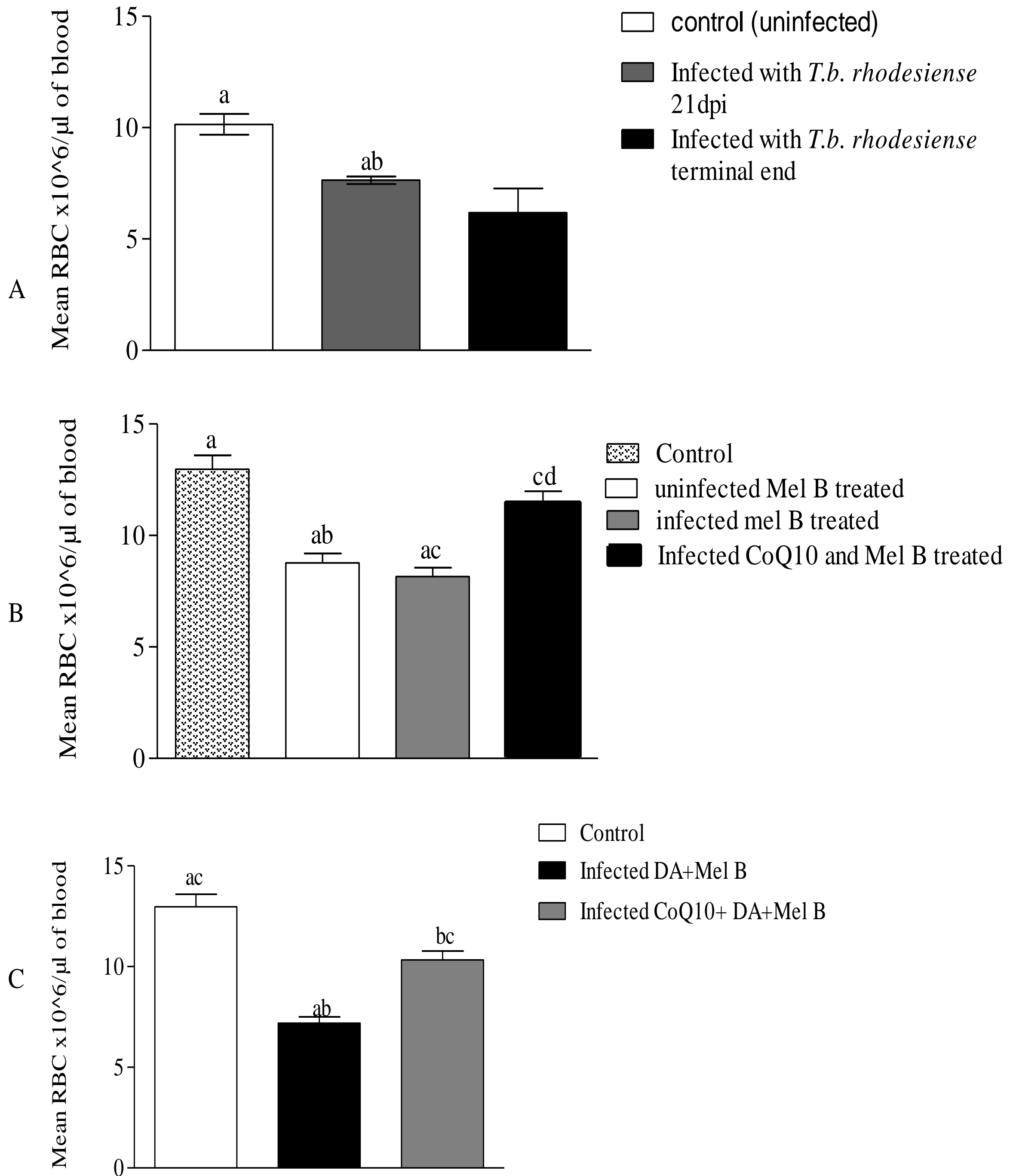

Fig.9. Effects of *T. b. rhodesiense* infection, PTRE, CoQ10 and Mel B treatment on RBC concentration measurement in blood: (A). ab represents significant ($P<0.05$) different between (i) and (ii). (B) ac represents significant ($P<0.05$) different between (iii) and (i) and cd represents significant ($P<0.05$) different between (iii) and (iv). (B).The letters (bc) indicates the RBC concentration is significantly ($P<0.05$) different between (ii) and (iii) and ab represents significant ($P<0.05$) different between (i) and (ii).

4.1.4 Mean weight of liver and spleen

Table 2: Mean weight of liver and spleen in *T.b. rhodesiense* infected and uninfected mice.

Mean tissue weight to body weight ratio (g/kg)		
	Liver	Spleen
Uninfected (Control) n=6	0.043	0.003[f]
Infected n=6	0.092[a]	0.044**
Infected Extremist n=5	0.095***	0.068[g]
Uninfected + Mel B n=6	0.044	0.003[h]
Infected + Mel B n=6	0.074[b]	0.034*
Infected + CoQ10 + Mel B n=6	0.054[c]	0.019
Infected + DA + Mel B n=4	0.085[d]	0.063[i]
Infected + CoQ10 + DA + Mel B n=4	0.063	0.032

Key: Significance difference between in liver sizes between uninfected control and infected mice sacrificed 21 days post infection is denoted by (a). Extremely significance difference between uninfected control and infected extremist is denoted by (***). Whereas that between uninfected + Mel B and infected + Mel B, infected mice + Mel B and Infected mice + CoQ10 + Mel B is denoted (b) and (c) respectively Sacrificed 24 hours the last dosage of Mel B. (d) denotes significance difference between infected mice + DA + Mel B and infected + CoQ10 + DA + Mel B for severe late stage. Very significance difference in spleen sizes between uninfected control and infected mice sacrificed 21 days post infection is denoted by (f). While that of uninfected control and infected extremist, between infected and infected extremist is denoted by (g) and (**) respectively. uninfected + Mel B and infected + Mel B, infected mice + Mel B and Infected mice + CoQ10 + Mel B is denoted (h) and (*) respectively Sacrificed 24 hours the last dosage of Mel B. (i) denotes significance difference between infected mice + DA + Mel B and infected + CoQ10 + DA + Mel B for severe late stage.

4.1.5 Haematoxylin and Eosin stained sections through the hippocampal brain region prepared from *T .b. rhodesiense* infected and uninfected mice, treated at different times

The neuropathological evidence shows an exacerbation of inflammation in the trypanosome infected mice in this study. As the infection progressed, there was an increase in development of perivascular cuffing (Fig.10.), inflammatory cellular infiltration, encephalitis, loss of distinct cellular structure and lining, reactive gliosis and proliferation of microglia cells as the infection progressed to the terminal end of the disease. These lesions were more pronounced and aggravated in the infected mice that were euthanized after reaching the terminal end compared to those that were euthanized at 21dpi (Fig.10.). Detachment of the choroid plexus (choroidosis) was observed only in the terminal group. A reactive encephalopathy was observed in uninfected mice treated with Mel B. Additionally, perivascular cuffing and cellular infiltration were evident but these lesions were more marked in infected mice treated with Mel B at 21dpi. The observed lesions were mild in infected mice that were given CoQ_{10} and administered with Mel B. For this group of mice, the hippocampus region showed distinctly normal layers. Treatment of infected mice with diminazene aceturate at 21dpi to induce and/or simulate PTRE and administration of Mel B following relapse resulted in 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. On the other hand, infected Mice orally administered with CoQ_{10} treated with diminazene aceturate and Mel B after relapse showed less of these lesions (Fig.10).

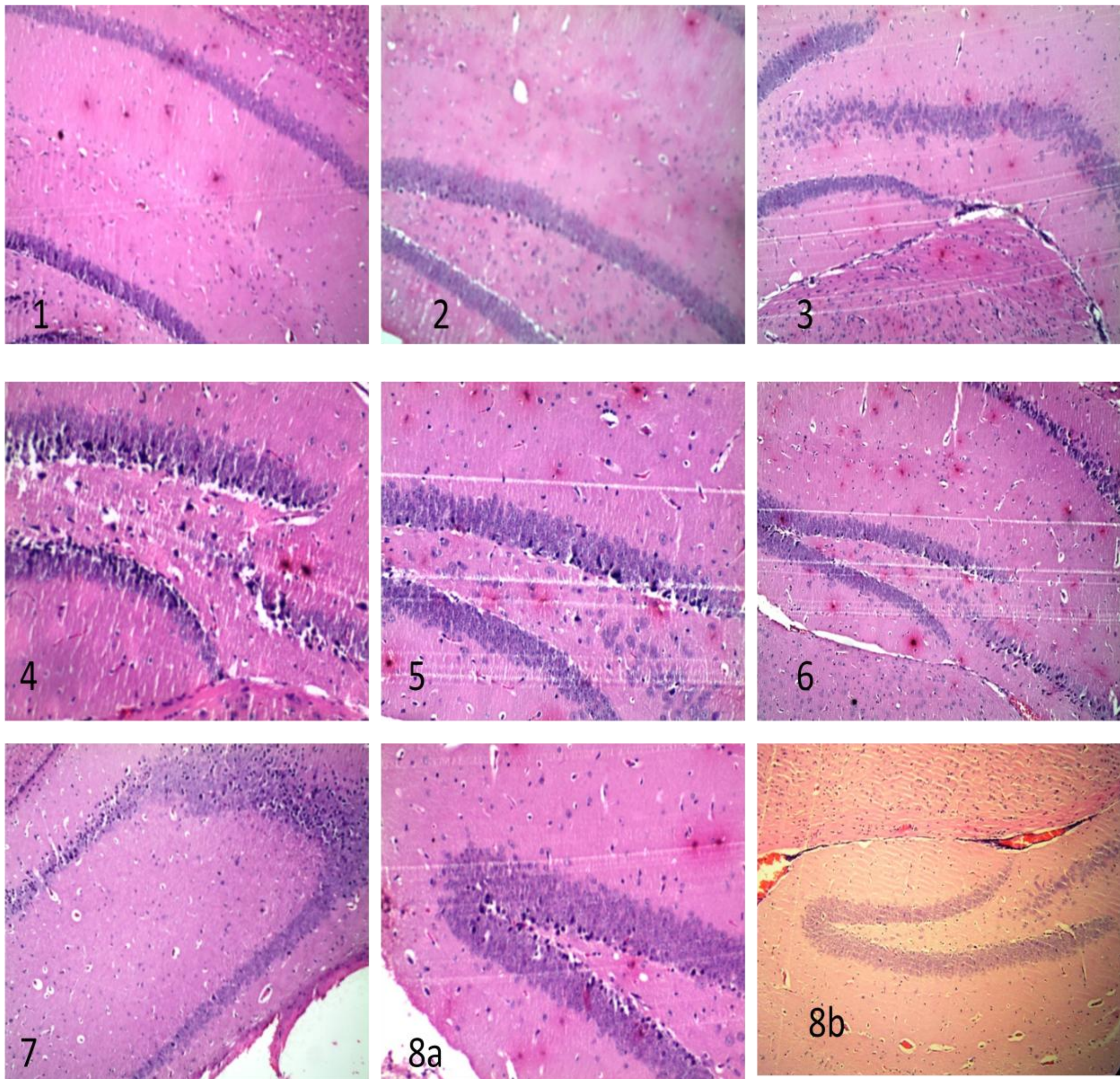

Fig.10. Haematoxylin and Eosin stained sections through the hippocampal brain region prepared from *T.b. rhodesiense* infected mice with different treatments. The sections show different neuroinflammation at different stage of disease course also with different treatments ; uninfected (control) (1), early-CNS stage (21dpi) (2), severe late-CNS stage (3), uninfected Mel B treated PTRE (4), infected Mel B treated 21dpi (5), infected CoQ10 Mel B treated 21dpi (6), infected CoQ10 DA and Mel B treated with less marked cellular infiltration and perivascular cuffing (7), and infected DA and Mel B treated the increasing development of the perivascular cuffs, cellular infiltration and encephalitis is demonstrated (8a & 8b). Original magnification ×100.

4.2 Effects of *T.b.rhodesiense* infection, PTRE induced, Mel B treatment and oral administration of CoQ10 on GSH synthesis in brain

Endogenous levels of glutathione were markedly increased in *T.b. rhodesiense* infected mice sacrificed 21dpi (6.4184mM) (Fig.11.). Progression of the infection to the terminal stage (extremist) induced a lowering of GSH levels (2.8209mM). Treatment with Mel B of uninfected mice depleted GSH (2.2693mM). While in infected mice treated with Mel B the levels of GSH was slightly higher (6.4184mM) when compared to the control but still lower when compared to infected orally administered CoQ10 treated with Mel B group which registered high levels of GSH (15.2225) (Fig.12.). Constitutive glutathione levels were prominently higher in infected orally administered with CoQ10 DA and Mel B group compared to control. In infected DA and Mel B group the levels of GSH were elevated compared to control but lower when compared to CoQ10 administered group (Fig.13.).

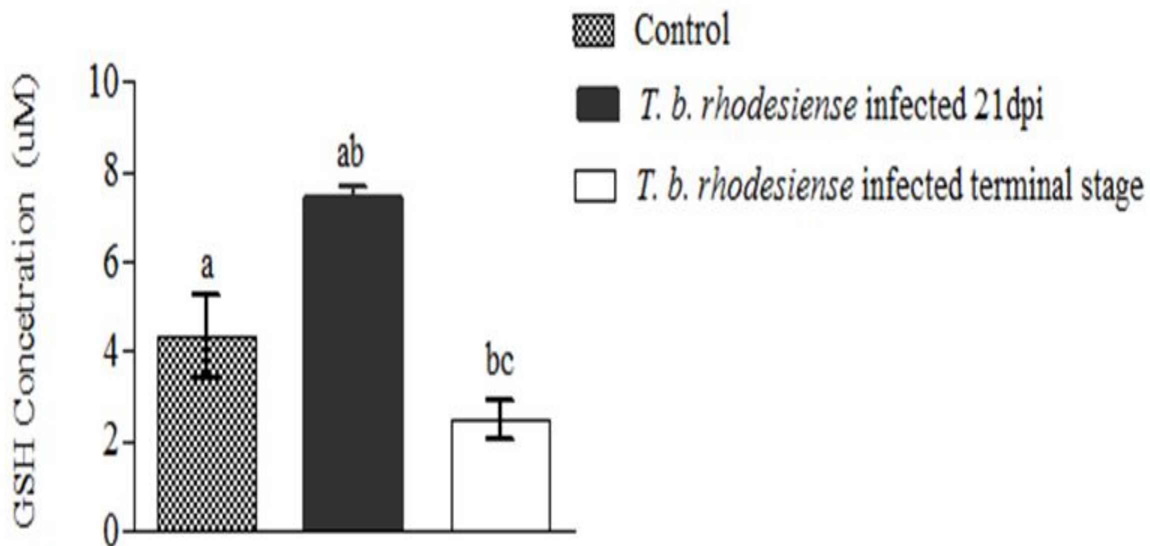

Fig.11. Mean reduced glutathione (GSH) concentration in brain homogenates of uninfected (control), *T.b. rhodesiense* infected mice (sacrificed 21 days post infection) and *T.b. rhodesiense* infected extremist mice (sacrificed after reaching terminal end). ab represent significant inter-group comparison (P<0.05) (control vs infected 21dpi). bc represent significant inter-group comparison (P<0.05) (infected extremist vs infected 21dpi)

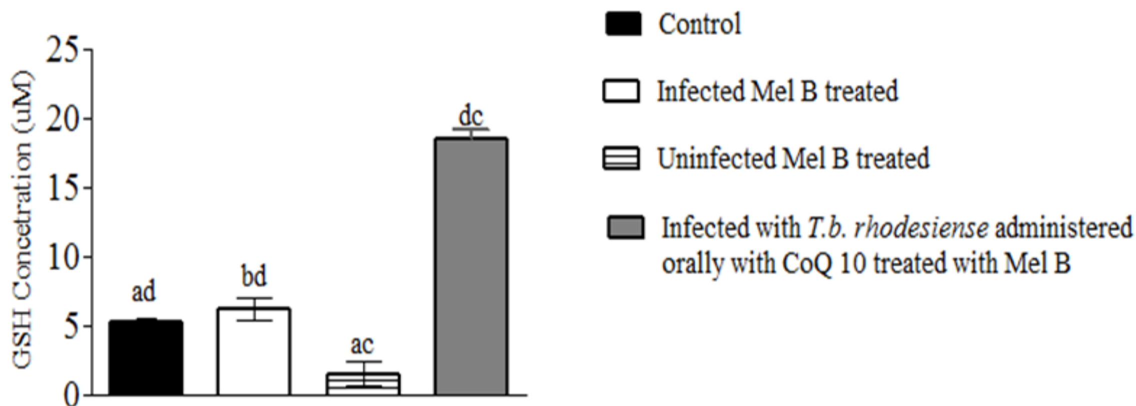

Fig.12. Mean GSH concentration in brain homogenates of uninfected (clean) treated with Mel B, *T.b. rhodesiense* infected mice treated with Mel B and *T.b. rhodesiense* infected orally administered with CoQ10 and treated with Mel B. ad represents significant inter-group comparison (P<0.05) (uninfected control vs infected CoQ10 Mel B treated). bd represent significant inter-group comparison (P<0.05) (uninfected Mel B treated vs infected CoQ10 Mel B) dc represents a significant intra-group comparison (infected Mel B treated vs Infected CoQ10 Mel B treated) (P<0.05).

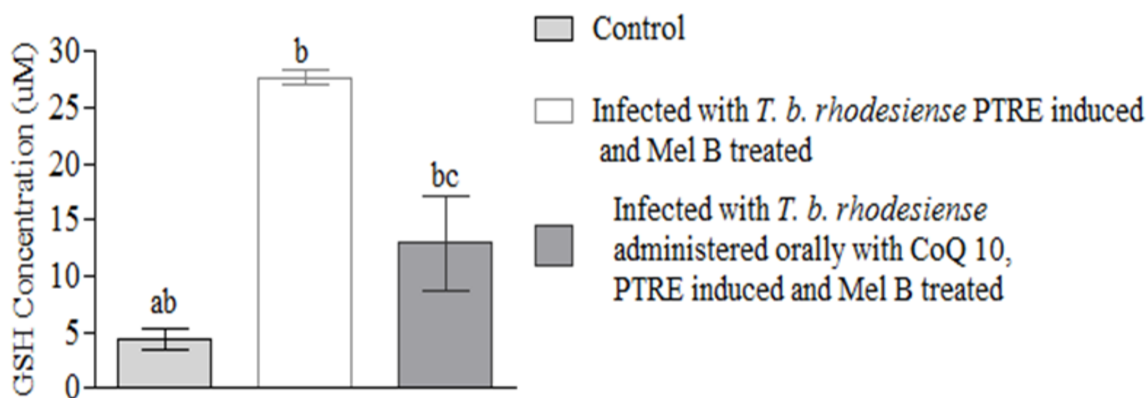

Fig.13. Mean GSH concentration in brain homogenates of uninfected (control), *T.b. rhodesiense* infected mice treated with DA and Mel B and *T.b. rhodesiense* infected orally administered with CoQ10 and treated with DA and Mel B. ab represents significant inter-group comparison (P=0.001) (control vs infected, DA and Mel B treated). bc represent significant inter-group comparison (P<0.05) (infected, DA and Mel B treated vs infected CoQ10, DA and Mel B treated).

4.3 Expression of Glutathione reductase in response to *T.b. rhodesiense* infected and different treatments

4.3.1 GR protein expression profile in Mel B treated and orally CoQ10 administered

GR expression in uninfected mice that were treated with Mel B decreased in comparison to the control. There was increase in expression of GR both in infected mice treated with Mel B and infected CoQ10 administered and treated with Mel B when compared to control (Fig.14.B.). This observation indicates that treatment with Mel B depletes GR which is consistent with the notion that Mel B is highly toxic and induces PTRE (Pepin, 1994). It appears to be expressed stably and at large amounts in infected CoQ10 Mel B treated than in Infected Mel B treated alone providing further compelling evidence that CoQ10 can protect GR by enhancing their expression while on the other hand it counteracts the deleterious effect of Mel B.

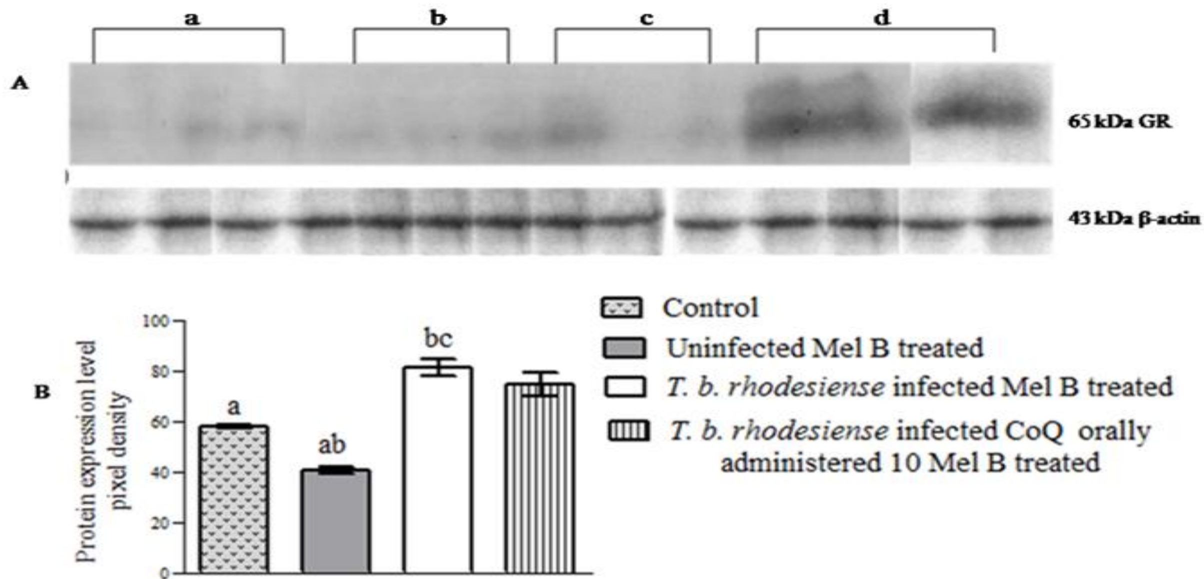

Fig.14. **(A)** Blot showing differences in the expression of Glutathione reductase protein in brain homogenate fractions from: **(a)** control, **(b)** uninfected mice treated with Mel B, **(c)** *T.b. rhodesiense* infected mice orally administered with 200mg/kg CoQ10 treated with Mel B 21DPI. ($P<0.05$) is considered significant for comparative studies and **(d)** *T.b. rhodesiense* infected mice treated with Mel B 21dpi sacrificed 24hrs after the last dosage and **(B)** The histogram shows semi-quantitative determinations of GR protein expression, presented as percentage pixel intensity compared to that in the control. ab represents significant inter-group comparison ($P=0.001$) (control vs uninfected Mel B treated); bc represent significant inter-group comparison ($P<0.05$) uninfected Mel B treated vs infected Mel B treated).

4.3.2 Expression GR protein profile in T.b. rhodesiense infected brain sacrificed 21DPI, infected extremist and uninfected

The effect of *T.b. rhodesiense* infection on glutathione reductase (GR) expression in brain homogenates of mice sacrificed 21dpi was markedly increased by compared to those in the controls. GR was more over expressed in brain homogenates of extremist (mice that went to the terminal end) than those in the corresponding control and mice sacrificed 21dpi (Fig.15.B.).

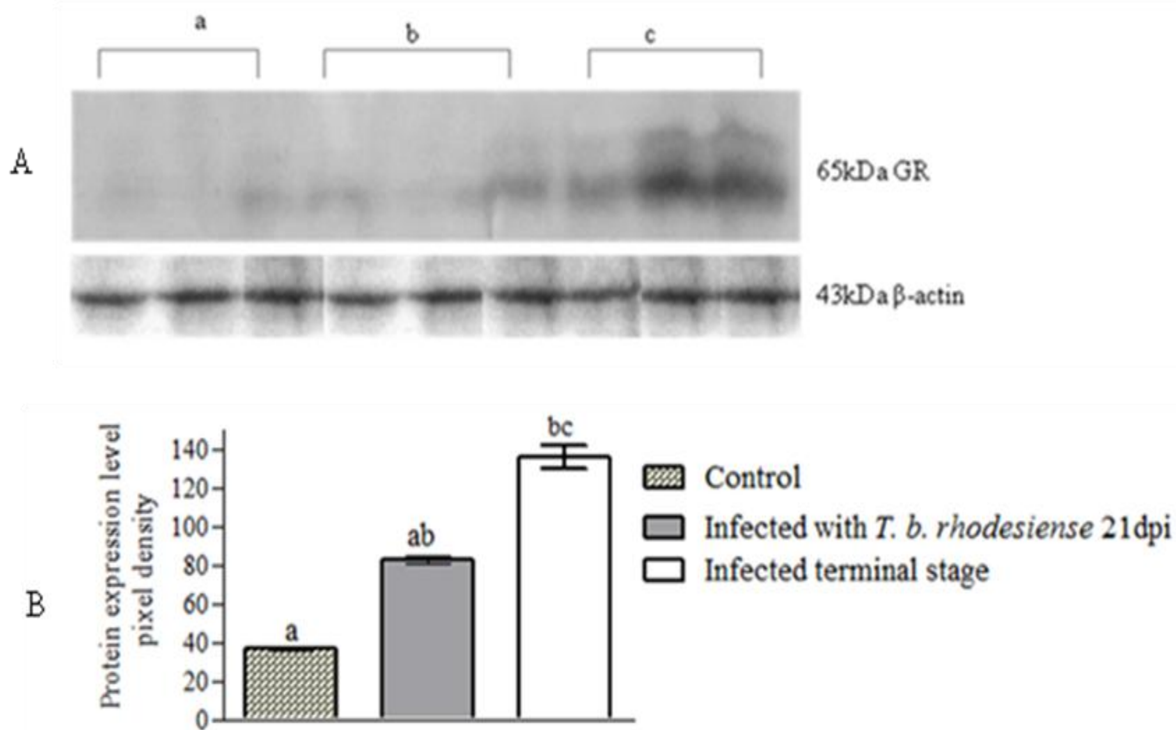

Fig.15. Determination of GR expression; **(A)** Immuno-blot of nitrocellulose membranes showing specific expression of GR protein that was determined in (**a**) uninfected (control), **(b)** infected sacrificed 21dpi and **(c)** infected extremist brain homogenate. P<0.05 is considered significant for comparative studies. **(B)**The histogram shows semi-quantitative determinations of GR protein expression, presented as percentage pixel intensity compared to that in the control. ab represent significant inter-group comparison (P<0.05) (control vs infected sacrificed 21dpi while bc represent significant inter-group comparison (P<0.05) (infected terminal stage vs infected sacrificed 21dpi.

4.3.3 Expression of GR on severe late stage HAT

Compared to the control, brain homogenates of mice that was infected and treated with DA and Mel B after relapse, GR expression was significantly elevated. Infected mice that were

administered with CoQ10 treated with DA and Mel B after relapse was higher when compared to the control (Fig.16.B).

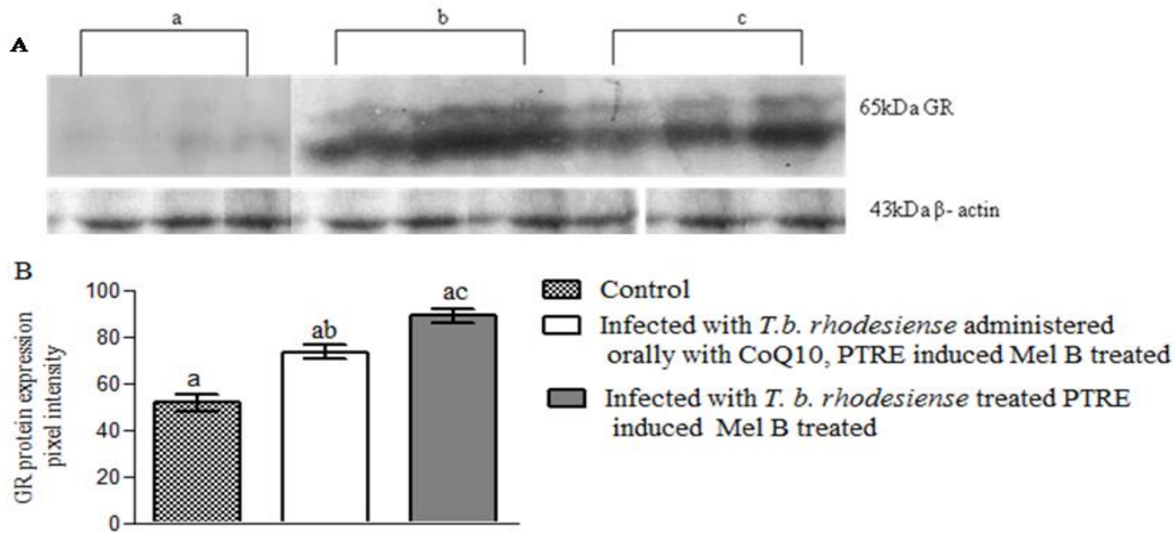

Fig.16. Determination of GR expression; **(A)** The blot shows specific expression of GR protein which was determined in **(a)** uninfected (control), **(b)** infected PTRE induced and Mel B and **(C)** infected orally administered with CoQ10 PTRE induced and Mel B brain homogenate β-actin was used to show equal loading. $P<0.05$ is considered significant for comparative studies. **(B)**The histogram shows semi-quantitative determinations of GR protein expression, presented as percentage pixel intensity compared to that in the control. ab represents significant inter-group comparison ($P<0.05$) (control vs *T.b. rhodesiense* infected mice administered with CoQ10 treated with DA and Mel B) and ac represents a significant inter-group comparison (Control vs *T.b. rhodesiense* infected mice PTRE induced and Mel B) ($P<0.05$).

4.4 Expression of SOD-1 protein in mice brain infected with *T.b. rhodesiense* at different time and treatment

4.4.1 SOD-1 protein expression in T.b. rhodesiense infected brain sacrificed at 21dpi, T.b. rhodesiense extremist and control

This experiment determined how *T.b. rhodesiense* infection, treatment with Mel B, DA and oral administration of CoQ10 affects SOD-1 expression. Compared to in uninfected (control) and infected sacrificed 21dpi, the percentage expression of SOD-1 was markedly increased in *T.b. rhodesiense* infected extremist (Fig.17.B), demonstrating dismutation activity against increased oxidative stress. Also there was elevation in percentage of SOD-1 expression in brain

homogenates of *T.b. rhodesiense* infected sacrificed 21dpi though not significantly different from the control (P>0.05) (Fig.17.B).

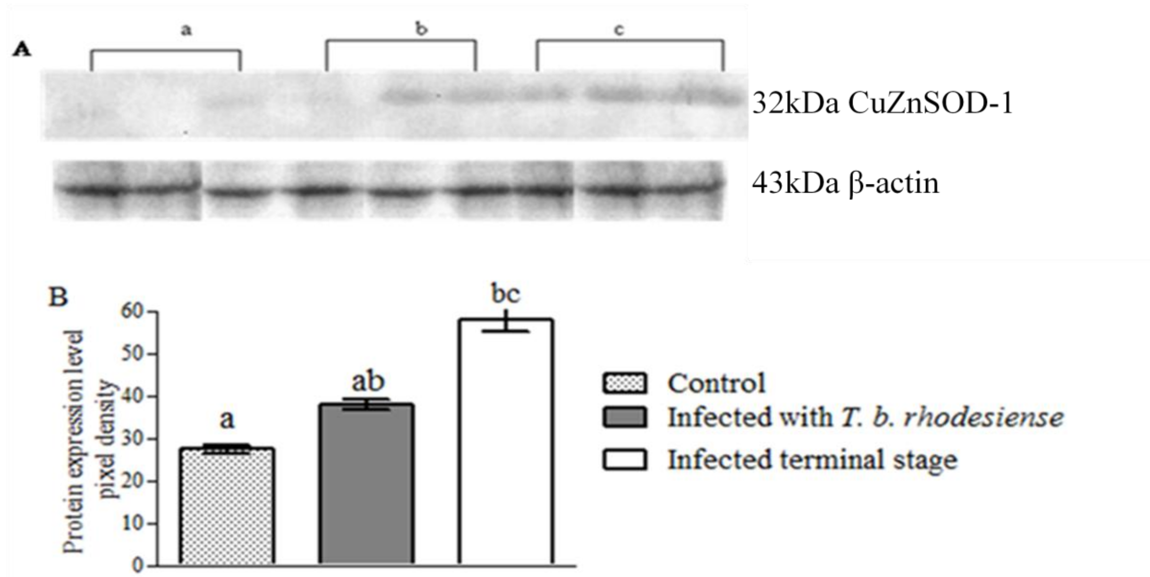

Fig.17. Increased expression of SOD-1 in *T.b. rhodesiense* infected brain homogenates. **(A)** Blot showing Specific expression of SOD-1 which was determined in: **(a)** uninfected (control) **(b)** infected sacrificed 21dpi and **(c)** infected extremist brain homogenate. (B) The histogram shows semi-quantitative determinations of GR protein expression, presented as percentage pixel intensity compared to that in the control. ab indicates significant (P<0.05) difference inter-group comparison (control vs *T.b. rhodesiense* infected 21dpi) while bc indicates significant (P<0.05) difference inter-group comparison (infected terminal stage vs *T.b. rhodesiense* infected 21dpi).

4.4.2 Expression of SOD-1 analysis during severe late stage

In analyzing the expression of SOD-1 in brain homogenates of mice infected with *T.b. rhodesiense* treated with DA to induce PTRE and then treated with Mel B following relapse and *T.b. rhodesiense* infected orally administered with CoQ10 treated with DA to induce PTRE and then treated with Mel B following relapse. The result shows that, *T.b. rhodesiense* infected treated with DA to induce PTRE and then treated with Mel B following relapse expressed SOD-1 protein more abundantly than the CoQ10 administered and control group (Fig.18.B.).

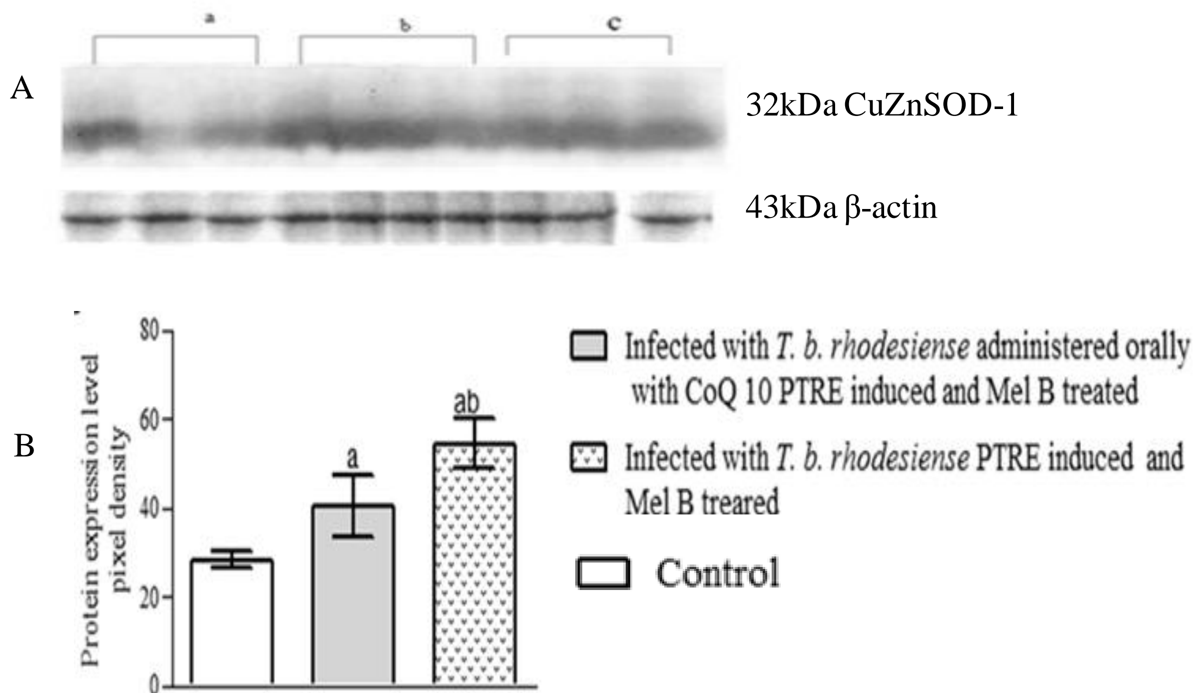

Fig.18. Increased expression of SOD-1 in *T.b. rhodesiense* infected brain homogenates of *T.b. rhodesiense* infected treated with DA and Mel B. **(A)** Blot showing specific expression of SOD-1 which was determined in: **(a)** uninfected (control), **(b)** infected orally administered with CoQ10 treated with DA and Mel B and **(c)** infected treated with DA and Mel B following relapse brain homogenate. **(B)** The histogram shows semi-quantitative determinations of GR .protein expression, presented as percentage pixel intensity compared to that in the control. ab represents significant inter-group comparison ($P<0.05$) (*T.b. rhodesiense* infected CoQ10 administered, treated with DA and Mel B mice vs *T.b. rhodesiense* infected treated with DA and Mel B mice).

4.4.3 Effect of Mel B treatment and oral administration of CoQ10 on infected and uninfected brain on expression of SOD-1

When challenged with *T.b. rhodesiense* infection and treated with Mel B, the brain homogenates of these mice showed a marked up-regulation in SOD-1 percentage expression in comparison to the control. Infected CoQ10 Mel B treated also uninfected treated with Mel B and infected CoQ10 treated with Mel B indicated increase in percentage expression of SOD-1 (Fig.19.B). Thus, this result indicates that expression of SOD-1 is a result of its exerting protective effect against increased oxidative stress and the deleterious effects of Mel B.

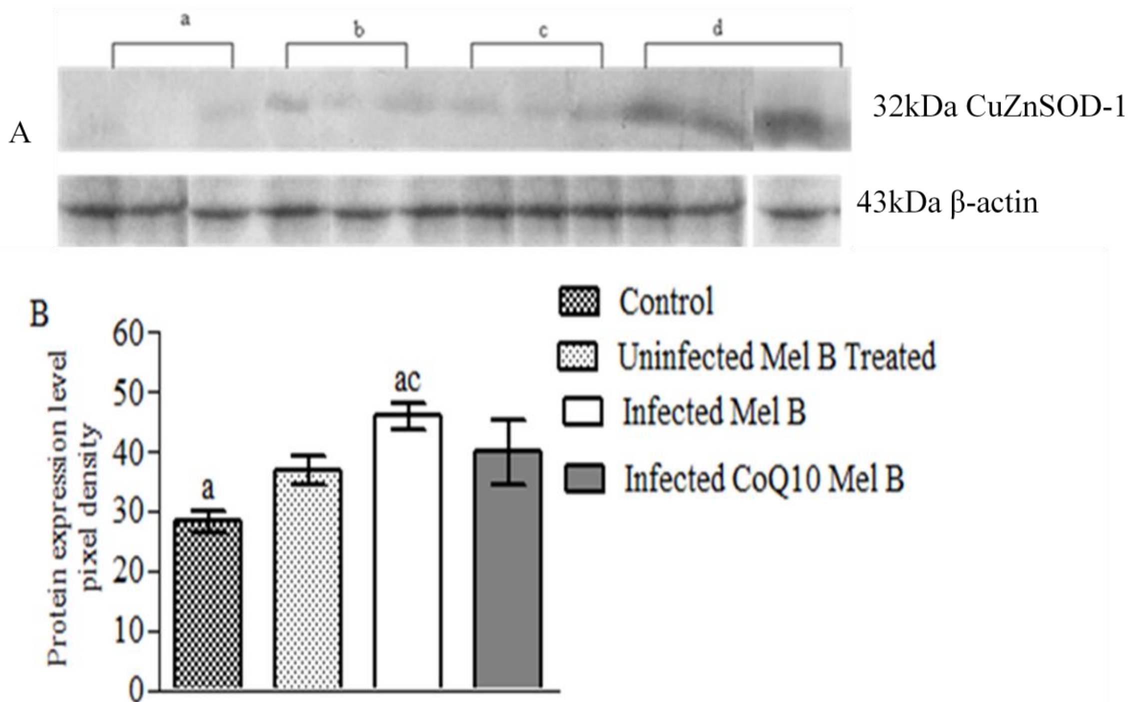

Fig.19. Increased expression of SOD-1 in *T.b. rhodesiense* infected brain homogenates of *T.b. rhodesiense* infected treated with Mel B. **(A)** Blots showing specific expression of SOD-1 which was determined in: **(a)** uninfected (control), **(b)** uninfected treated with Mel B, infected orally administered with CoQ10 treated with Mel B and **(c)** infected treated with Mel Be brain homogenate. **(B)** The histogram shows semi-quantitative determinations of SOD-1 protein expression, presented as percentage pixel intensity compared to that in the control. ac represents significant inter-group comparison ($P<0.05$) (control vs *T. b. rhodesiense* infected treated Mel B brain homogenates

4.5 Expression of MnSOD protein profile in *T.b. rhodesiense* infected brain, uninfected under different treatment

Effects of *T.b. rhodesiense* infected sacrificed at 21dpi, *T.b. rhodesiense* extremist, *T.b. rhodesiense* infected treated with Mel B, uninfected Mel B treated , *T.b. rhodesiense* infected Orally administered with CoQ10 treated with Mel B, *T.b. rhodesiense* infected Orally administered with CoQ10 treated with DA and Mel B and *T.b. rhodesiense* infected treated with DA and Mel B MnSoD proteins expression levels.

4.5.1 MnSOD expression profile in *T.b. rhodesiense* infected mice brain sacrificed 21dpi, infected extremist

Endogenous percentage expression levels of MnSOD in the *T.b. rhodesiense* infected that went to the terminal end were higher. Also in *T.b. rhodesiense* infected sacrificed at 21dpi brain homogenates the level of expression of MnSOD was markedly higher compared to the control (Fig.20.B.).

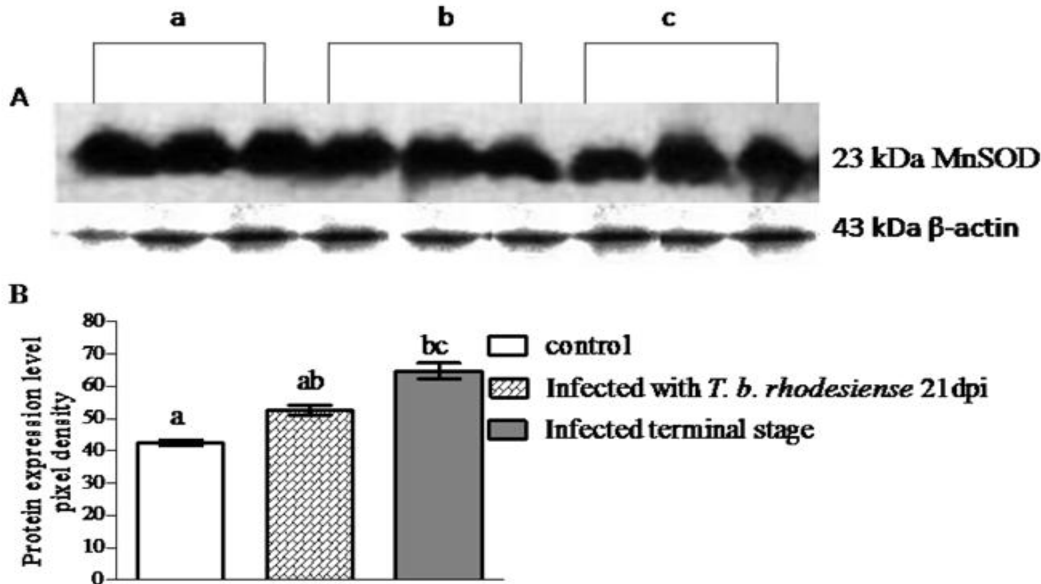

Fig.20. Semi-Quantitative determinations of MnSOD; **(A)** Blot showing MnSOD protein expression in: **(a)** control, **(b)** *T.b. rhodesiense* infected sacrificed 21dpi and **(c)** *T.b. rhodesiense* infected extremist. **(B)**The histogram shows semi-quantitative determinations of GR protein expression, presented as percentage pixel intensity compared to that in the control. ab indicates that the expression of MnSOD in *T.b. rhodesiense* infected sacrificed 21dpi is significantly different ($P<0.05$) from the respective control while bc indicates significant difference ($P<0.05$) between infected 21dpi Vs infected terminal stage.

4.5.2 Effect of Mel B treatment and oral administration of CoQ10 on infected and uninfected brain by MnSOD expression activity

Treatment with Mel B induced a lowering of expression of MnSOD in infected treated with Mel B brain homogenates. While brain homogenates that were orally administered with CoQ10 and treated with Mel B registered a slight decrease in MnSOD protein expression levels. Lowering of MnSOD protein expression was prominent in uninfected Mel B treated when all compared to the control (Fig.21.B.).

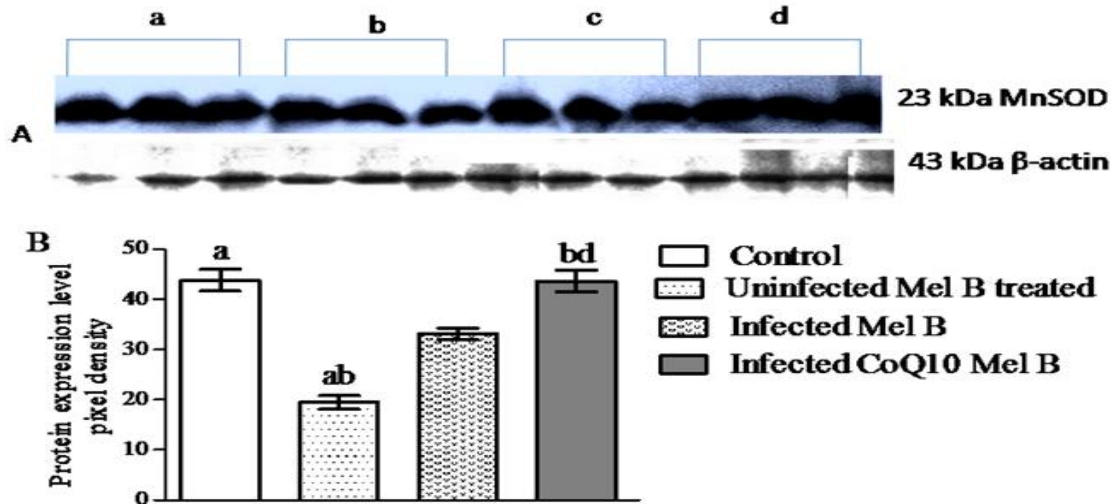

Fig.21. Semi-Quantitative determinations of MnSOD; **(A)** Blot showing MnSOD protein expression in: **(a)** uninfected (control), **(b)** uninfected treated with Mel B, **(c)** infected orally administered with CoQ10 treated with Mel B and **(d)** infected treated with Mel B brain. **(B)**The histogram shows semi-quantitative determinations of MnSOD protein expression, presented as percentage pixel intensity compared to that in the control. bd indicates that the expression of MnSOD in *T.b. rhodesiense* infected CoQ10 administered orally treated with Mel B is significantly different ($P<0.05$) from the respective uninfected treated with Mel B. ab represents significant inter-group comparison ($P<0.05$) (control vs uninfected treated with Mel B).

4.5.3 Expression of MnSOD-2 analysis during severe late stage

MnSOD protein expression in brain homogenates of *T.b. rhodesiense* infected treated with DA and Mel B and *T.b. rhodesiense* infected orally administered with CoQ10 treated with DA and Mel B of treatment induced decrease in expression of this protein and respectively when compared to the control (Fig.22.).

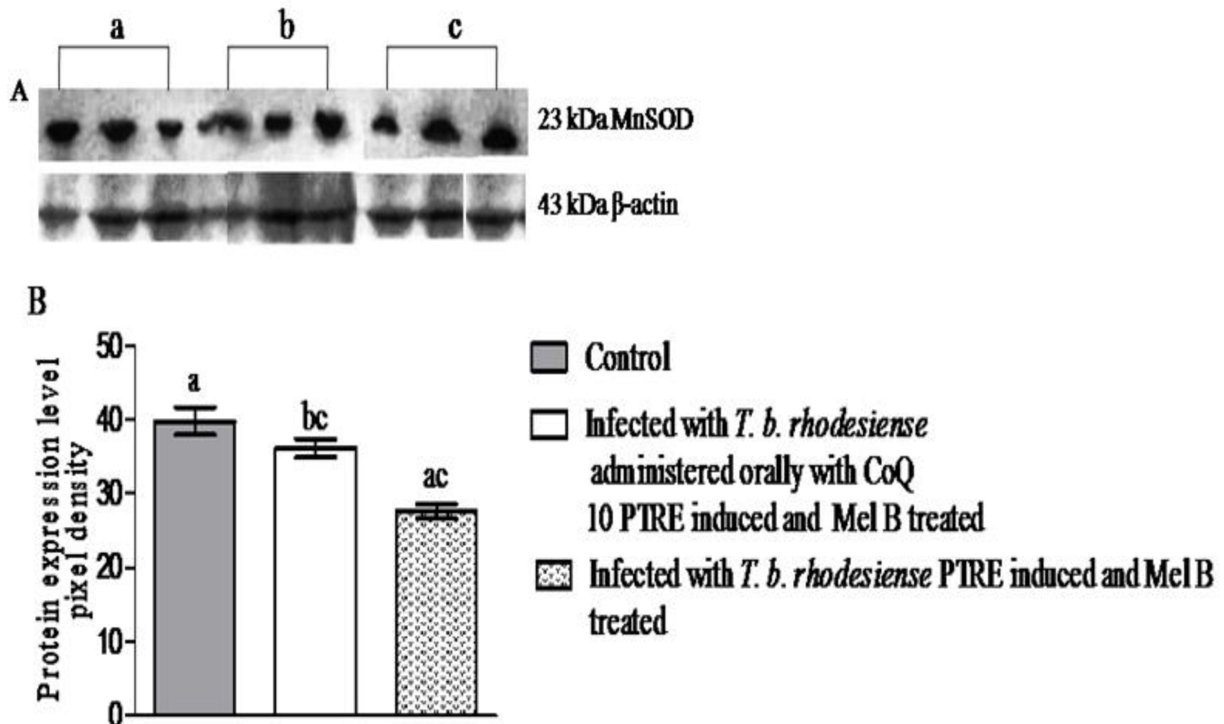

Fig.22. Semi-Quantitative determinations of MnSOD, **(A)** Blot showing MnSOD protein expression in: **(a)** control, **(b)** infected orally administered with CoQ10 treated with DA and Mel B and **(c)** infected treated with DA and Mel B following relapse brain homogenates, which is expressed as percentage pixel intensity compared to that in the control. ac indicates that the expression of MnSOD in *T.b. rhodesiense* infected treated with DA and Mel B following relapse is significantly different ($P<0.05$) from the control while bc indicates significant difference ($P<0.05$) between Infected CoQ10, DA and Mel B treated Vs Infected DA and Mel B treated.

CHAPTER FIVE

DISCUSSION

This study has shown that Coenzyme Q10 alters the pathogenesis of *T.b. rhodesiense* in mice. This is evidenced by the elevation of white blood cells, haemoglobin levels, red blood cells, platelets and prolonged relapse time following treatment with 5mg/kg DA. Significant extension of relapse in *T.b. rhodesiense* mice by oral CoQ10 administration in the present study is in agreement with the work by other researchers working with protozoan parasites, where Aflatoxicosis decreased morbidity and extended the survival time in mouse *P. berghei* model (Young *et al*., 1988; Hendrickse *et al*., 1986) and *P. falciparum* parasitaemia was significantly reduced in infected children (Hendrickse *et al.*, 1986).

The observed improved prolonged relapse of trypanosomes in mice orally administered with 200mg/kg CoQ10 in the study could be related to the protective role of the antioxidant activity of CoQ10. This antioxidant might have been well concentrated in the vital organs and extra vascular compartments hence exerting it is protective role. It's also possible that there might be interaction between CoQ10 and DA. However, this observation with no data presented needs further research. I therefore hypothesize that the ability of CoQ10 to delay relapse period could be attributed to its ability to aid total antioxidant defense capacity hence boosting cellular immunity. This has been shown to reduce oxidative stress by protecting the defense system against the damaging effects of ROS such as peroxyl radicals, singlet oxygen, peroxynitrite and nitric oxide.

It has been indicated that the reactive nitric oxide molecule is directly implicated in the pathogenesis of trypanosomiasis (Mabbort *et al*., 1995). On contrary there was no significance difference in the pre-patent period, even though in CoQ10 orally administered mice all mice became positive by day five, this does not suggest a transient protection of the host from initial infection by *T.b. rhodesiense.* The results in this study suggest a transient protection of the host by CoQ10 from *T.b. rhodesiense* infection by enhancing cellular immunity. This indicates that CoQ10 may most likely renders humans and animals less susceptible to reappearance of trypanosome infection following treatment or decrease disease transmission and infection incidence in the field. Remarkably, of the six mice in this group given CoQ10, two mice didn't relapse and they were declared cured after 180 days elapsed as per WHO guideline.

Elevation of WBC in *T.b. rhodesiense* infected CoQ10 oral administered mice that were treated with 3.6mg/kg Mel B 21dpi was unusual. This suggests that CoQ10 can be immunostimulatory rather than immunosuppressive, this is in consistence with Work by Bondy and Peskta (2000), using mycotoxin they also observed elevated white cell count in mice infected with *T.b. rhodesiense* thereby indicating that mycotoxin can be immunostimulatory rather than immunosuppressive, depending on the route of administration (Pier and Mcloughlin, 1985) and critical exposure window of dose and time .Reduced number of WBC at severe late stage could be due to severe suppression of lymphocyte proliferation in response to increased burden of *T.b. rhodesiense* infection.

Furthermore, platelet counts were within normal range but slightly elevated for severe late stage group that was orally administered with CoQ10 which was significantly different from the infected group but not orally administered that showed depletion of platelets. Petra *et al.* (2007) demonstrated that extracellular supply of CoQ10 through the circulation influences CoQ10 levels in blood cells. It is noteworthy that the platelets have mitochondrial and production of ROS which may be the contributory factor that resulted into low levels in infected group that were not administered CoQ10. This is due to the fact that ROS causes destruction of platelets and there was sufficient incorporation of CoQ10 thus quenching the oxidants resulting in the protection and maintenance of high levels of platelets.

African trypanosomes are extracellular parasites that survive in the blood stream and they are capable of generating reactive oxygen species (ROS). During oxidative metabolism ROS inflict damage on all classes of macromolecules and can ultimately lead to cell death. Indeed, ROS are implicated in a number of human neurodegenerative diseases (Sofic *et al*., 1992). One of the effects of *T.b. rhodesiense* is the reduction of red blood cells due to increase in the oxidative effect of RBC membranes. This stress is most acute in red blood cells because their membranes have a high content of polyunsaturated lipids and a rich oxygen supply making them vulnerable to lipid peroxidation (Taiwo *et al.*, 2003). Furthermore, lacking mitochondria, make them have no alternative means of generating reducing power (NADPH). The major role of NADPH in the red blood cells is to reduce the disulfide form of glutathione to the sulfhydryl form. Remarkably, in the present study all groups of mice that were orally administered with 200mg/kg of CoQ10 registered high levels of RBC compared to un-supplemented one, clearly indicating that CoQ10 protected RBC against lipid peroxidation due to its potent antioxidant

capability. The major mode of RBC elimination appears to be extra vascular destruction due to a massive eryrhrophagocytosis in the spleen and liver (Murray and Dexter, 1988).

Anemia observed in trypanosome infected un-supplemented mice in this study was expected since anemia in *T.b. rhodesiense* infection is well documented in various animal model including vervet monkeys (Thuita *et al*., 2008). In the present study oral administration of CoQ10 in *T.b. rhodesiense* infected mice shows that this antioxidant can prevent anemia as indicated by high levels of hemoglobin, haematocrit and RBC. Of interest Ognjanovic *et al* (2003) showed that pretreatment with Vitamin E decreased the toxic effects of Cadmium on the hematological values and has a protective role in anemia. Murray *et al* (1974) also showed that erythrophagocytosis an important mechanism for development of anemia in trypanosomiasis is blocked by immunosuppressants leading to attenuation of anemia. Sufficient evidence of significant CoQ10 induced antioxidant activity in *T.b rhodesiense* infected mice in this work explains similar effect on anemia observed in the two studies. This attenuation of anemia could also take place in humans. The mechanism by which CoQ10 attenuates pathogenesis of anemia in the present study could be multi- factorial perhaps involving up-regulation of erythroporetin activity or activation of essential co-factors. Reduced nephritis in *T.b rhodesiense* infected CoQ10 administered mice groups perhaps increased production of erythrogenin thus increasing erythroporetin activities.

Many studies have shown that decline in PCV is one of the key feature in the pathogenesis of HAT that contribute to morbidity and mortality thereby shortening the lifespan (Kagira *et al.,* 2006). Infected mice orally given CoQ10 in the present work registered higher levels in PCV that was significant compared to infected mice which can be related to resistance of RBC haemolysis. This finding provides compelling evidence to demonstrate that CoQ10 protects erythrocytes from haemolysis in vivo due to its antioxidant potency. ROS generated during infection leads to oxidative stress that can attack erythrocytes membrane causing its oxidation hence leading to haemolysis (Igbokwe *et al*., 1994). The antioxidant capability of CoQ10 led to the reduction in the susceptibility of erythrocytes to membrane oxidative damage thus protecting them.

Trypanosomes are covered by a glycoprotein coat that is encoded by many genes that are antigenically distinct thus making the parasite to evade host immunoprotective process called antigenic variation (Stuart *et al.,* 2008). Expression of several different waves of parasitaemia in

both orally administered CoQ10 infected mice and those unsupplemented is an indication of different variable antigen types (VATs) of trypanosomes to which immune response is elicited (Magez *et al*., 1994). It can be clearly stated that the antibodies raised against the *T.b. rhodesiense* infected mice were capable of responding to the various VATs expressed. Similar results have been observed in swiss white mice infected with bloodstream and cerebral spinal fluid forms of *T.b. rhodesiense* (Kariuki *et al.*, 2008).

Of interest was the increase of body weight in spite of the effects of trypanosomes in growth during the early stages of infection. This increase could be related due to the retention of body fluids in the form of edema that accompanies trypanosomiasis. However there was a drastic decline of body weight especially those mice that were infected but were never treated (terminal stage of the disease). Mice for severe late stage after treatment with 5mg/kg DA started gaining weight with those administered with CoQ10 having a slight higher gaining in body weight. But after relapse the body weight started declining in both groups of severe late stage with slight gaining in weight after treatment with Mel B the case occurred to mice that were sacrificed at 21 and 25 day with the uninfected groups indicating increase in body weight. Moreover, after treatment with Mel B among groups that was orally administered with CoQ10 showed gaining in body weight.

Cells and tissues that play a role in immune function are highly energy-dependent and therefore require an adequate supply of CoQ10 for optimal function. Several studies have demonstrated immune-enhancing effects of CoQ10 or its analogues (Bliznakov *et al.,* 1970; Meyer *et al.,* 1980; Saiki *et al.,* 1983). These effects included increased phagocytic activity of macrophages; increased proliferation of granulocytes in response to experimental infections. Prolonged survival in mice infected with *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* or *Candida albicans* has also been noted. Inoculation of animals with Friend leukemia virus reduced CoQ10 levels in the blood and spleen (Bliznakov *et al.,* 1975). Bliznakov (1973) showed that treatment of infected animals with CoQ10 increased the survival rate and decreased the severity of hepatomegaly and splenomegaly. This observation is in agreement with the present study in which infected mice orally administered with CoQ10 showed a greater decrease in severity of hapatomegaly and splenomegally (Table 2.).

The neuropathological evidence shows an exacerbation of inflammation in the trypanosome infected mice in this study. As the infection progressed there was increase in

development of perivascular cuffing, inflammatory cellular infiltration, encephalitis, loss of distinct cellular structure and lining, reactive gliosis. Proliferation of micriglia cells was aggravated as the infection progressed to the extreme this lesions were more pronouncement in the infected mice that were euthanized after reaching the terminal stage of the disease compared to those that were euthanized at 21dpi. Detachment of the choroid plexus (choroidosis) was observed only in the extremist group, this could be due the lesions becoming more severe, the edema and the increased numbers of plasma cells caused wide expansion of the plexus (Moulton, 1986). The reported lesions have been reported previously with activation of the astrocytes and microglia (Adams *et al*., 1986). The severe inflammatory cell infiltration in the infected extreme mice could have also resulted from the increased number of necrotic cells (Anderson, 1985). During the terminal phase of CNS infection, histological evidence of meningoencephalitis is evident (Kennedy, 2006).

A reactive encephalopathy was observed in uninfected mice treated with Mel B, perivascular cuffing, cellular infiltration was also evident but this lesions were more marked in infected mice treated with Mel B 21dpi; the recognized neurotoxicity of melarsoprol a reactive encephalopathy is well investigated (Pepin and Milord, 1994).The observed lesions were mild in infected CoQ10 administered Mel B treated mice in which the hippocampus region showed distinct layers. In this study it is evident that CoQ10 can to certain extent reduce neurotoxicity of melarsoprol a reactive encephalopathy while at the same time reduces the inflammation due to the trypanosomes.

Treatment of infected mice with diminazene aceturate (DA) at days 21dpi and Mel B following relapse resulted in a severe meningitis, prominent perivascular cuffing by lymphocytes and macrophages, reactive gliosis, hemorrhage (RBC present in the parenchyma), encephalitis, marked increase in the cellularity infiltration. When the drug DA is administered, which is known to clear the parasites from the extra vascular compartments but not the CNS since this drug does not cross the BBB. This leads to the production of an experimental PTRE that closely mimics the disease following melarsoprol in humans and persists after relapse of the parasitaemia (Kennedy, 1999). Treatment with Mel B following relapse resulted in the inflammatory processes being exacerbated highly. In contrast, infected Mice orally administered with CoQ10 treated with diminazene aceturate and Mel B after relapse showed less encephalitis, minimal perivascular cuffing and meningitis, very few inflammatory cells in the choroid tissue,

cellular infiltration was less and a marked reduction in loss of distinct structures. This results demonstrates that CoQ10 can significantly ameliorates the CNS inflammatory responses and reduce PTRE. Similarly, the drug azathiaprine prevents but does not ameliorate the PTRE (Hunter *et al*., 1992), eflornithine (DFMO), prevents and ameliorates the PTRE and reduces the extent of astrocyte activation (Jennings *et al*., 1983). Non-peptide Substance P (SP) - receptor antagonist RP also has been shown to ameliorate the CNS inflammatory response and reduces the level of astrocyte activation (Kennedy *et al*., 1997).

Our results show that invasion of the brain by *T.b rhodesiense* induces elevation of GSH levels, while Mel B and progression of the infection to terminal stage significantly depletes GSH. The levels of brain GSH at 21dpi tends to be elevated than those in the corresponding control and infected mice that went to the terminal end, suggesting that oxidative stress may be one of the major contributing factors in modulating these differences. Establishment of *T.b. rhodesiense* in CNS by day 21 normally results in production of free radicals, which in turn stimulates total GSH to combat the deleterious effects of ROS. As the infection progress towards the terminal end, oxidative stress tends to overwhelm the GSH as evident by the low levels of this antioxidant. Given the robust antioxidant role played by GSH in the brain, its depletion would make neurons and other cells in the brain vulnerable. This observation is in agreement with work by Dukhande *et al* (2006) where they showed that cellular GSH content can be depleted as a result of elevated oxidative stress. Similarly, GSH recycling is impaired due to $NADPH^{+}$ ICDH inactivation as a result of generation of ROS (Dukhande *et al*., 2006). In another study, increased levels of free radicals depleted GSH in chronic schizophrenia (Gora *et al*., 2006). Other studies have revealed depletion of GSH and ascorbic acid in the blood, liver and kidney by *T. congolense* infection, agreeing with several earlier reports in which *T. brucei* (Umar *et al*., 2001), *T. evansi* (Nyden, 1948) and *T. gambiense* (Ameh, 1984) have been used for experimental infection of various species of animals. In fact, the trypanosome parasite antioxidant trypanothione is synthesized from its mammalian equivalent, glutathione (Smith *et al.*, 1992). This phenomenon implies that the trypanosomes protect themselves from oxidative stress at the expense of the mammalian host; and could deplete GSH needed to protect the human brain, resulting in oxidative stress. The implication of this scenario is that coupling of anti-oxidant therapy with conventional treatment regimens might improve treatment outcome in HAT.

Reactive species can do great damage in the brain and even result in neurodegeneration. Activated astrcocytes due to parasite invasion of the brain is accompanied by NO production. Though beneficial, NO can potentially harm brain cells. Specifically, NO facilitate generation of lethal reactive metabolite species such as peroxynitrite ($ONOO^-$) (Bolanos *et al*., 1997; Heales and Bolanos, 2002) in the brain, which nitrate vital proteins and/or enzymes in the brain, altering their structure and rendering them dysfunctional. Moreover, the metabolites depletes cellular antioxidant defenses such as glutathione (GSH) (Bolanos *et al*, 1997; Heales and Bolanos, 2002), increasing susceptibility of the neurons to oxidative stress, which in turn impairs their functions with putative resultant neurological dysfunction generally observed in the late stages of HAT infections.

Furthermore, our study clearly showed that treatment of uninfected mice with Mel B, resulted in glutathione depletion in the brain. It is possible that melarsen oxide, a toxic metabolite of Mel B impairs critical proteins/enzymes necessary for GSH restoration in the CNS. Mel B is arsenic-based drug. Arsenic has the ability to complex with sulphydryl groups depleting cellular reduced glutathione (GSH) levels (Del-razo *et al*., 2001). Davison *et al* (2003) found out that GSH depletion was strongly promoted by arsenic-induced apoptosis and enhanced arsenic-induced ROS formation in NB4.

Oral administration of CoQ10 in infected mice that were treated with Mel B resulted in increased levels of GSH. Such a profound finding indicates that CoQ10 can support GSH status in the brain during Mel B treatment. Thus, these results provide further compelling evidence that Coenzyme Q10 can effectively attenuate Mel B toxicity and protect GSH depletion and might be useful in the combination treatment of HAT as an alternative antioxidant therapy. Similarly it has been reported that depletion of endogenous GSH antioxidant may be a significant factor in the pathogenesis of *T. congolense* infection but upon administration of exogenous vitamin C to infected animals prevented these disease-induced decreases in GSH and ascorbic acid (Umar *et al*., 2010). This assumption is supported by the fact that Coenzyme Q10 has been reported to effectively attenuate toxicity in transgenic mice following administration with 3-Nitropropionic acid (Matthews *et al*., 1998); as well as protection against glutamate toxicity in cultured cerebral neurons (Favit *et al*., 1992). The possible contributory factor may be the antioxidant action of CoQ10 to quench the toxicity and also keeping the level of free radicals in the brain low, thereby preventing oxidative damage. Despite obvious shortcomings in our data in

explaining the mechanistic details of cellular amount of Coenzyme Q10 penetrated and concentrated in the CNS, we propose that oral administration of CoQ10 could be used to reduce GSH depletion due Mel B toxicity and could attenuate PTRE. Additionally, we suggest that CoQ10 could be useful in maintenance of endogenous antioxidants in the brain during HAT therapy.

On the other hand, oral administration of 200mg/kg of CoQ10 in infected mice in which PTRE had been induced at 21dpi and treated with Mel B following relapse resulted in elevated GSH. Moreover, in un-supplemented mice induced a more marked increase in cellular GSH level. Thus, our results suggest that increase in GSH was to combat the effect of oxidative stress induced by PTRE and virulent effect of *T.b. rhodesiense* to aid antioxidant activity.

Since treatment with DA induces PTRE and clears the parasite from the haemolymphatic system and not the CNS, it is plausible that both PTRE and parasites in the CNS could be the major contributing factors of ROS that led to the increase in levels of GSH. Following relapse and treatment with Mel B the GSH levels decreased than the expected values due to Mel B toxicity. The observed low levels of GSH in the CoQ10 orally administered, PTRE induced and Mel B, will be attributed to the antioxidant capability of CoQ10 not only to attenuate the toxicity of Mel B, but also to provide protection against generated oxidative stress. Consistent with this possibility, is an earlier observation that in vitro supplementation with CoQ10 provided DNA protection against hydrogen peroxide induced oxidative stress (Tomasetti *et al*., 1999). Clearly further studies are required to test this intriguing possibility in primates.

Our results further show a robust antioxidant enzyme response from MnSOD, Cu/ZnSOD and GR in the initial stages of infection (mice sacrificed 21dpi) with *T.b. rhodesiense* to the terminal end. The notion that by 21dpi the parasite have established within the CNS is well documented, thus by this period the impairment of major components of the CNS was on course. This includes components of the electron transport and oxidative phosphorylation which enhances an increased free radical leakage and thereby inducing oxidative stress, and subsequently stimulating MnSOD, SOD-1 and GR expression activity. The expressions of these important proteins that constitute the first line of defense for ROS removal were markedly increased as the infection progress towards the terminal end.

Robust overexpression of MnSOD, Cu/ZnSOD-1 and GR demonstrates their protective role in brain cells threatened with *T.b. rhodesiense* infection or oxidative stress. Consistent with

this possibility is the earlier observation in cells threatened with energy failure by mitochondrial toxins and/or oxidative stress that resulted in an increased induction of MnSOD and $NADP^{+}$-ICDH activity (Orina *et al.,*2007). Consequently, the observed progression of infection towards terminal stage in CNS resulting into elevated oxidative stress was accompanied by acute increase in MnSOD, SOD-1 and GR protein expression. This finding suggests that the three proteins act in concert and are critical in the control of oxidative stress. The implication is that presence of ROS brought about by *T.b. rhodesiense* infection may exert stimulatory or inductive effect on MnSOD, SOD-1 and GR expression.

It is important to note that the level of expression of MnSOD and GR decreased in uninfected mice treated with Mel B. These findings clearly demonstrate the toxic effect of Mel B (which is a known arsenic compound) that resulted in depletion of MnSOD and GR. Melarsen oxide, a metabolite of melarsoprol has been shown to interact with thiols such as glutathione and thioredoxin forming a stable adduct (Fairlamb *et al*., 1989; Cunningham *et al*., 1994). The stable adduct formed is a competitive inhibitor of the respective flavoproteins namely glutathione reductase and thioredoxin reductase, whose responsibility is to maintain intracellular thioredoxin and glutathione in the reduced form (Cunningham *et al*., 1994). Melarsen oxide also potently inhibits the flavoproteins glutathione reductase and thioredoxin reductase directly by interacting with catalytically active sulphydryl groups present in both enzymes (Cunningham *et al*., 1994). Other studies reveal that in addition to causing mitochondrial toxicity (Larochette *et al*., 1999) impairing microtubule polymerization, (Li and Broome, 1999) and deregulating a number of proteins and enzymes through sulfhydryl binding,(Cavigelli *et al*., 1996), the arsenic induces oxidative stress and generation of hydrogen peroxide and other ROS (Wang, 1996). Mel B depletion of MnSOD is not a surprise because the mitochondrion is known to accumulate arsenic. Other studies have shown evidence that arsenic molecules suppress MnSOD (Mazumder, 2005).

Once in the mitochondria, the arsenic is known to inhibit succinic dehydrogenase activity and can uncouple oxidative phosphorylation. The resulting fall in ATP levels affects a number of cellular functions including expression of MnSOD protein synthesis. Consistent with this observation is an in vitro study that was done to determine the effect of arsenical compounds on GSH-related enzymes glutathione-reductase, peroxidase and transferase. In this study arsenic appeared to be an effective inhibitor of all of the proteins studied (Chouchane and Snow, 2001;

Thomas *et al.,* 2001). In light of this, MnSOD and GR depletion may be an important mechanism underlying Mel B induced neurotoxicity.

On the other hand, treatment of infected mice with Mel B also shows depletion of MnSOD. As earlier noted during progression of infection, there is elevation of MnSOD. Thus it can be conclusively argued that before treatment, expression of MnSOD is induced by ROS; triggered by *T.b. rhodesiense* infection, but treatment with Mel B impairs expression of MnSOD, perhaps due to Melarsoprol toxicity and PTRE.

CoQ10 has been studied in multiple in vitro models of neuronal toxicity, with results that overall have supported a neuroprotective effect. In neuronal cell models of oxidative stress, pre-treatment with CoQ10 preserves mitochondrial membrane potential and reduces generation of reactive oxygen species (Somayajulu *et al*., 2005). Since *T.b. rhodesiense* is known to cause acute infection, production of ROS as a result of this parasite is extremely robust thus expression of MnSOD was important despite administration of CoQ10. Consequently this study shows that CoQ10 can aid in quenching ROS. Apart from neutralizing the toxic effect of Mel B, exogenous CoQ10 can also can play an integral role in maintaining endogenous CoQ10 levels.

Importantly, CoQ10 has been shown to protect human neuroblastoma (SHSY-5Y) cells against paraquat-induced mitochondrial dysfunction as well as against β -amyloid toxicity (McCarthy *et al.,*2004; Winkler-stuck *et al.,* 2004). Thus, these findings suggest that other factors are required for the targeting of CoQ10 to the mitochondria and sub cellular distribution in order to enhance its maximal antioxidant protective effect against MnSOD depletion either by oxidative stress or Mel B toxicity. However, in the current study SOD-1 was highly expressed in the CoQ10 orally administered compared to the control and infected Mel B treated alone. Supplementation with CoQ10 has a profound effect on protecting SOD-1 against oxidative stress and toxic effects of Mel B and PTRE. It is noteworthy that increased levels of malondialdehyde and down-regulation of SOD-1, both markers of oxidative stress observed in the transgenic mice, were ameliorated by CoQ10 treatment (Yang *et al*., 2008). Similarly, in a transgenic mouse model of familial ALS that over-expresses SOD-1 with the G93 A mutation, treatment with CoQ10 at 200 mg/kg/day, significantly increased mean life span (Matthews *et al.,* 1998). Moreover, the current studies pinpoint putative crucial events that may precede or supersede depletion of the antioxidants by Mel B and elevation of oxidative stress will be geared to

neurodegeneration. Some of the crucial events like oral administration of CoQ10 may be “protective’’ in nature. Consequently, there is need to understand how CoQ10 interacts with Mel B to ameliorate its toxicity.

GR protein normally acts to maintain high levels of reduced glutathione in the cytosol, with the concomitant oxidation of NADPH. GR transforms oxidized glutathione to the reduced form, thus elevation of this important protein in both CoQ10 infected Mel B and infected Mel B alone indicates the important role it plays during oxidative stress. However, GR was highly elevated in infected Mel B treated than in CoQ10 orally administered; this observation is as a result of increased production of oxidative stress that further stimulates expression of GR. CoQ10 acts directly to scavenge the ROS hence reducing oxidative stress. In such a scenario, levels of GR expression would be low due to reduced need for antioxidant power. Significant increase in SOD-1 protein expression and GR activity in mice infected with *T.b. rhodesiense* suggest that the two antioxidant proteins may be up-regulated and down-regulated when the CNS is challenged with toxicity and PTRE as a result of treatment with Mel B and elevation of ROS resulting from brain degeneration. Therefore, apart from CoQ10 administration, other strategies that can increase the expression of the two proteins simultaneously may protect cells from oxidative stress insult.

The rise in expression of SOD-1 and GR protein activity during severe late stage was similar to the increase observed for both of them and MnSOD during initial stage of infection and at the terminal stage. There was an increased expression of GR in infected, PTRE induced and Mel B treated compared to the control. Note that treatment with DA induces PTRE (Pepin, 1994). PTRE may be also a major stimulating factor for production of ROS apart from the ones inducted by the parasites alone, it is as a result of both PTRE induced and parasites that the rise of SOD-1 and GR was witnessed when compared to CoQ10 supplemented. More profoundly was an increase in the expression of SOD-1 in CoQ10 orally administered, PTRE induced,suggesting that CoQ10 and SOD-1 act in concert in protecting brain cells against harmful effect of ROS, DA induced PTRE and Mel B treatment. Expression of both SOD-1 and GR is vital for maintenances of a robust antioxidant system that is functional. Consistent with this conclusion is the experimental studies that revealed that over-expression of MnSOD and GPx protects neurons from the harmful effects of ROS in experimental stroke (Hoehn *et al*., 2003; Wallace, 2002).

Normally brain cells have very high energy requirements and therefore exhibit a high rate

of ROS production from the mitochondrial electron transport activities coupled to oxidative phosphorylation (Hinerfeld *et al.,* 2004). In light of the finding of a significant decrease in expression of MnSOD in PTRE, it's possible that the DA induced PTRE, increased oxidative stress leading to depletion and inactivation of this important antioxidant. For example, $ONOO^-$ has been shown to inactivates MnSOD, GPx and GR expression (MacMillan-Crow *et al*., 1996; Savvides *et al.,* 2002), and cellular GSH content is generally depleted as a result of induction of oxidative stress (Dukhande *et al*., 2006). Understanding how MnSOD, SOD-1 GR and GSH regulate antioxidant systems and their vulnerabilities to DA induced PTRE, toxicity and possibility of Mel B PTRE and ROS as a result of *T.b. rhodesiense* infection could be important for development of pharmacological and or nutritional strategies to counter oxidative stress in the brain challenged with *T.b. rhodesiense* infection. Nevertheless, this antioxidant system will be insufficient to completely prevent oxidative damage under most physiological conditions (Hoehn *et al*., 2003).

In view of the discussion above, these results suggest that invasion of the brain by *T.b. rhodesiense* leads to production of ROS which in turn triggers the expression of MnSOD, GR, and SOD-1 metabolism. This proposed mechanism, however, requires further investigation. Nevertheless, previous studies (Jo *et al*., 2001; Munich, 2003; Lee *et al*., 2003) and the findings from this study demonstrate important role MnSOD, SOD-1, GSH and GR play in combating oxidative stress in brain challenged with *T.b. rhodesiense*.). Furthermore depletion of MnSOD in Mel B treatment and DA casts doubts on what exact mechanism leads to depletion of this important antioxidant apart from its toxicity, oxidative stress and PTRE even though superoxide dismutases and vital enzymes of the electron transport chain have been found to be targets of oxidant damage, further aggravating the biochemical and neurological impact of the metabolites (Kennedy, 1999). Enzyme dysfunction and production of oxidative stress are implicated in neurodegenerative diseases, and certainly merits further investigation.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Results from this study has demonstrates that CoQ_{10} can significantly ameliorate the CNS inflammatory responses, reduce PTRE and prevents full brown splenomegaly. This demonstrates that CoQ_{10} deficiency may be common following HAT outbreak and that supplementation with CoQ_{10} may improve immune function and reduce the severity of haemolypmhatic and neurological pathogenesis of the disease. Consequently, in the near future, it will be critical to determine endogenous CoQ_{10} levels in HAT and following treatment with Mel B.

Also this study provides new information on how the brain challenged with *T. b. rhodesiense* infection, treatment with Mel B and oral administration of CoQ10 alters the antioxidant system. Our results suggest that antioxidant system regulated by MnSOD, SOD-1, GR and GSH protects brain cells during HAT disease process and from Mel B toxicity. The time-dependent dynamics of antioxidant suppression due to Mel B, and potential ameliorating effects of CoQ10 on the same, indicate putative mechanisms and a potential antidote to the toxicity of the drug with potential application in formulation of novel Mel B based drugs and development of novel markers for staging the disease. However, there is need to understand how CoQ10 interacts with DA and Mel B to ameliorate its toxicity; hence the following recommendations will help shed more light on the same.

i. Further investigation to determine endogenous CoQ_{10} levels in HAT and following Mel B treatment.
ii. Need to understand how CoQ10 interacts with Mel B to ameliorate its toxicity i.e to carry out Mel B neurotoxicity tests.
iii. Further research to investigate the putative role of CoQ_{10} to alter *T. b. rhodesiense* pathogenesis using primates.
iv. Further interrogations to determine precise pharmacological dynamics of CoQ_{10} underlying the expression of endogenous antioxidants and amelioration of PTRE.
v. Further investigation to counter check whether the anti-mouse primary antibodies cross reacts with those in the parasite.

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