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Coenzyme Q_{10} and endogenous antioxidants neuro-protect mice brain against deleterious effects of melarsoprol and *Trypanasoma brucei rhodesiense*

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Abstract

Melarsoprol (Mel B) is the only efficacious drug against late stage Human African Trypanosomiasis (HAT), but inadvertently is very toxic and induces Post Treatment Reactive Encephalopathy (PTRE) that is lethal in 5% of the patients. Investigations were conducted to establish the neuro-protective role of Coenzyme Q_{10} (CoQ_{10}) and other cellular antioxidants ((Manganese Superoxide dismutase (MnSOD), Glutathione Reductase (GR), Copper-Zinc Superoxide dismutase (SOD-1) and glutathione (GSH)) against Mel B toxicity, PTRE and putative resultant brain degeneration in a mouse model. Female Swiss-white mice were infected with *Trypanasoma brucei rhodesiense* parasite and manipulated to simulate all phases of PTRE and HAT. Expression profiles of the antioxidants in brain tissues were assessed using immunoblots, while GSH was measured spectrophotometrically. *Trypanosoma brucei rhodesiense* infection resulted in elevation of expression of endogenous antioxidants in the early stage of infection (21dpi), with significant expression (two fold) observed at the terminal stage of the disease (57dpi). CoQ10 assisted in boosting Levels of GSH upon induction of severe late stage of HAT. Similarly CoQ10 administration significantly augmented levels of SOD-1, GR and GSH in infected than in uninfected mice that were treated with Melarsoprol. The time dependent dynamics of antioxidant suppression due to Melarsoprol, and potential ameliorating effects of CoQ_{10} on the same, indicate putative mechanism underlying and antidote to the toxicity of the drug with potential application in formulation of novel Melarsoprol-based drugs and development of novel markers for staging the disease.
Key Words: *Trypanasoma brucei rhodesiense, endogenous antioxidants, late stage HAT; Coenzyme Q_{10}; Melarsoprol;*

Abbreviations: GSH, glutathione; CoQ10, Coenzyme Q10; MnSOD, Manganese Superoxide dismutase; GR, Glutathione Reductase; SOD-1, Copper-Zinc Superoxide dismutase; Mel B, melarsoprol; PTRE, Post treatment reactive encephalopathy; HAT, Human African Trypanoomiasis; HEPES, N-2 hydroxyethylpiperazine-N`-2 ethane sulfonic acid; ICDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; MnSOD, manganese superoxide dismutase; $NADP^+$, nicotinamide adenine dinucleotide phosphate, NO, nitric oxide; $ONOO^-$, peroxynitrite; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; TCA, tricarboxylic acid; DAB, diaminobenzidine; PBS, Phosphate buffered saline; dpi, days post infection.

1. INTRODUCTION

Human African Trypanosomiasis (HAT), or sleeping sickness, a neglected tropical disease that occurs in sub-Saharan Africa, is caused by *Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodesiense* parasite. The disease is classified into early and late stages.The late stage is accompanied by neurological dysfunction due to invasion of the brain by the parasites (Kennedy, 2004). HAT due to *T. b. rhodesiense* causes acute infection with CNS invasion occurring 21 days post infection (dpi) (Fevre *et al*. 2005). The invasion is associated with chronic meningitis that progresses to encephalitis and neuronal degeneration (Penetreath, 1995). Immunological factors accompanying the HAT progression have been well documented (Lejon *et al*. 2001).

However, the impact of the infection on antioxidant system is poorly understood despite established roles of endogenous antioxidants in ameliorating deleterious effects of pathogenic infections (Rubbo *et al*. 1994). The trivalent organic arsenical melarsoprol is the only effective drug for late-stage disease in both forms of HAT since the drug crosses the blood-brain barrier. However, the drug is potentially toxic to humans, resulting in mortality of up to 5% of the patients treated, due to post treatment reactive encephalopathy (PTRE) (Pepin and Milrod, 1994). Molecular processes that mediate the PTRE are poorly understood, and yet the process holds the key to counteracting the deleterious effects of PTRE and resultant mortality of patients. It is notable that despite efforts to develop newer drugs for CNS stage HAT, a drug as toxic as melarsoprol is still the only effective treatment for the late stage *rhodesience* infections. It is therefore prudent to dedicate resources to conduct research on strategies that would alleviate its toxicity and safe lives.

Hypotheses advanced to date suggest that PTRE is a product of toxicity of the arsenical moiety of melarsoprol (Hunter *et al*. 1992; Soignet *et al*. 1999). The toxicity is elicited by melarsen oxide, a metabolite of melarsoprol, which binds to vital metabolic enzymes resulting in oxidative stress (Fairlamb *et al*. 2003; Halliwell, 2001). The oxidative stress causes cellular damage and subsequently neurodegeneration. This is attributable to oxidation of vital cellular components by reactive oxygen species (ROS) and induction of nitric oxide synthase (iNOS) and synthesis of nitric oxide (NO) metabolites (Gorman et al. 1996; Keita *et al*. 2000). The NO facilitates 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 (Keita *et al*. 2000).

The brain counteracts the resultant oxidative stress through superoxide dismutase (SOD), glutathione peroxidase (GP_X), catalase (CAT) and glutathione (GSH) antioxidant defense systems that scavenge the radicals (Mataix *et al*. 1998). However, the ROS and NO metabolites can deplete the antioxidant defenses (Bolanos *et al*. 1995; Heals and Bolanos, 2002), increasing susceptibility of the neurons to oxidative stress, which in turn impairs their functions with putative resultant neurological dysfunction. Brain degeneration outcome in HAT is therefore partially a product of antioxidant factor suppression and resultant oxidative stress.

Coenzyme Q_{10} is a powerful antioxidant that is known to cross the blood brain barrier (Matthews *et al*. 1998). This has generated interest in the potential usefulness of CoQ_{10} to treat neurodegenerative diseases such as Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (Meredith *et al*. 2009). Importantly, CoQ_{10} protect against β -amyloid toxicity (McCarthy, 2004; Winkler-Stuck *et al*. 2004) and has been observed to effectively attenuate toxicity in transgenic mice following administration with 3-Nitropropionic acid (Matthews *et al*. 1998). In cultured cerebellar neurons, CoQ_{10} provide protection against glutamate toxicity (Favit *et al*. 1992). However, the potential role of CoQ_{10} in concert with endogenous antioxidant systems in protection or amelioration of Mel B toxicity to humans remains to be determined.

This study was initiated to establish responses of neuronal endogenous antioxidant systems (MnSOD, SOD-1, GSH and GR) in a mouse model to Mel B and/or infection by *T. b. rhodesiense* in the absence or presence of Mel B and /or CoQ_{10}. Our data indicates that endo- or exogenous elevation of the antioxidant factors such as infusion of CoQ_{10} might potentially ameliorate the brain damage and concomitant clinical manifestations as a result of Mel B toxicity or *T. b. rhodesiense* parasites.

2. MATERIALS AND METHODS

2.1 Ethics Statement

This study was undertaken in adherence to experimental guidelines and procedures approved by the Institutional Animal Care and Use Committee (IACUC), the ethical review committee for the use of laboratory animals. A standard protocol was drawn up in accordance with the Good Laboratory Practice (GLP) regulation of the KARI-TRC.

2.2 Experimental Animals, Parasites and drugs

A colony of 60 eight-weeks-old female adult Swiss-white mice (20-30g) was obtained from Kabete Veterinary Investigation Laboratories in Nairobi, Kenya. The mice were maintained on mice pellets (Unga Feeds Ltd, Kenya) and water *ad libitum* at room temperature. Wood-chippings were provided as bedding material. All mice were treated with 0.02ml of invermectin (Ivermectin®, Anupco, Suffolk, England) injected subcutaneously to each mouse to rid the mice of ecto and endo parasites. The mice were allowed to acclimatize for two weeks before they were subjected to experimental procedures. *Trypanosoma brucei rhod*esiense parasites were sourced as cryo-preserved clone KETRI 2537 from Kenya Agricultural Reserach Institute- Trypanosomiasis Research Center (KARI-TRC), Muguga, Kenya). The parasite strain was previously isolated from a human host in Uganda in 1972. Melarsoprol (Special Paris Rsobal) was provided by WHO and Veriben® was acquired from Ceva Sante Animale La b Allastere 33501 Libourne Cedex , France.

2.3 Experimental design

The experiments were conducted in ten groups each consisting of six mice (Table 1). Mice in the first group served as healthy controls (un-infected). The second and third groups were infected with *T. b. rhodesiense*

for 21 and 57 days respectively. Mice in the sixth group were also infected with *T. b. rhodesiense* for 21 days and subsequently treated for four days with Mel B while those in the seventh group were similarly treated but had orally been treated with CoQ_{10} on every other day from seven days prior to infection by the parasite up to the termination of the group four (healthy controls/un-infected) experiments. Mice in the fifth group (uninfected mice) were treated in a similar manner to those in the sixth group but were uninfected. The fourth group were uninfected mice controls for the fifth, sixth and seventh groups. Mice in the eighth group were infected as the fourth group but treated with Diaminazine aceturate (to induce PTRE) (5mg/kg/day) instead of Mel B 21dpi for three days to induce/simulate PTRE. The mice were subsequently treated with Mel B when relapse of the infection was observed. Mice in group 9 were treated in a similar manner to those in group 8 but had orally been treated with CoQ_{10} on every other day from seven days prior to infection with the parasite up to the termination of the experiment. The relapse in group eight and nine occurred at 19-32 days while those in group 9 occurred at 30-44 dpi. The last group consisted of uninfected mice, and served as a control for groups eight and nine. Mel B and CoQ_{10} were administered at 3.6 mg/kg/day and 200mg/kg respectively. All mice in each group were euthanized with chloroform (trichloromethane) at the end of respective experimental setting.

Table1. Experimental groups and drug treatments

Mice group	Number of mice	Treatment
Group 1	6	Uninfected (control)
Group 2	6	*T. b. rhodesiense*–infected (sacrificed 21dpi)
Group 3	6	*T. b. rhodesiense*–infected (terminal end)
Group 4	6	Uninfected (control)
Group 5	6	Uninfected treated with Mel B
Group 6	6	*T. b. rhodesiense*–infected treated with Mel B treated
Group 7	6	*T. b. rhodesiense*–infected, CoQ_{10} treated with Mel B
Group 8	6	*T. b. rhodesiense*–infected PTRE induced and Mel B
Group 9	6	*T. b. rhodesiense*–infected, CoQ_{10}, PTRE induced and Mel B treated
Group 10	6	uninfected (control)

Key: dpi-days post infection, Mel B – melarsoprol, CoQ_{10} – Coenzyme Q_{10}, PTRE – Post treatment reactive encephalopathy.

2.4 Harvesting and processing of brain samples for analyses

The brain samples were harvested separately (21dpi, 25days after last dosage of Mel B, 57dpi and 65 days) from each of the euthanized and immediately washed in PBS at 4^0C. The brains were then snap frozen on dry ice in labeled cryotubes, before finally being stored in liquid nitrogen. The snap-frozen whole brains were homogenized on ice water (4°C) in 0.5 ml of homogenization buffer (0.25 M sucrose, 5 mM Hepes-Tris, pH 7.4, protease inhibitor cocktail (Sigma Aldrich) to make 10% (w/v) brain homogenate. The brain homogenates were later used for various biochemical analyses.

2.5 Assessment of GSH, MnSOD, SOD-1 and GR in the brain samples

The levels of GSH among the aliquots of the brain samples were separately determined spectrophotometrically (in relation to the time brain sample were harvested) based on Ellman's reagent (DTNB) using a multi-detection microplate reader (Bio-Tek Synergy HT). The expression profiles of MnSOD (SOD2), Cu/ZnSOD (SOD1) and GR were determined using Western blotting (SDS-PAGE). Proteins were separated on cast gradient gels (4–15% Tris–HCl, SDS Polyacrylamide) at 125 constant volts using the BioRad systems. After electrophoresis, the proteins were transferred and bound to a nitrocellulose membrane (Millipore) at 25 V for 150 min. Once the transfer was complete, the membranes were incubated in 5-10 (w/v) fat-free milk (blocking solution) for 4 hours at room temperature. The blocking solution was then discarded and the blots incubated in solutions containing, primary monoclonal antibodies (Santa Cruz Biotechnology, Santa Cruz CA) for MnSOD1, MnSOD2, 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, SOD-2 and GR were then determined using pixel density software UN-SCAN-IT (NIH, MD,). *β*-actin was used to confirm equal sample loading for all gel electrophoresis. All studies were carried out at least in triplicate

2.6 Statistical analyses

The 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).

3. RESULTS

3.1 Effects of *T. b. rhodesiense* infection, PTRE induction, Mel B and CoQ_{10} on synthesis of GSH in brain

Endogenous levels of glutathione were significantly higher (P = 0.0034) in *T. b. rhodesiense* infected mice sacrificed 21dpi than in control mice. Progression of the infection to the terminal stage beyond 21dpi reduced GSH levels (Fig.1a.). Treatment with Mel B depleted GSH in uninfected mice. Infected mice treated with Mel B had significantly lower (P = 0.0001) GSH levels than in those infected but orally administered with CoQ_{10} and treated with Mel B (Fig.1b.). Glutathione levels were significantly (P<0.05) higher in infected PTRE induced mice (Mel B treated) than in control mice. In infected PTRE mice treated with Mel B, levels of GSH were significantly higher ((P = 0.0017) than in respective infected mice (PTRE induced) that were given CoQ_{10} and treated with Mel B (Fig.1c).

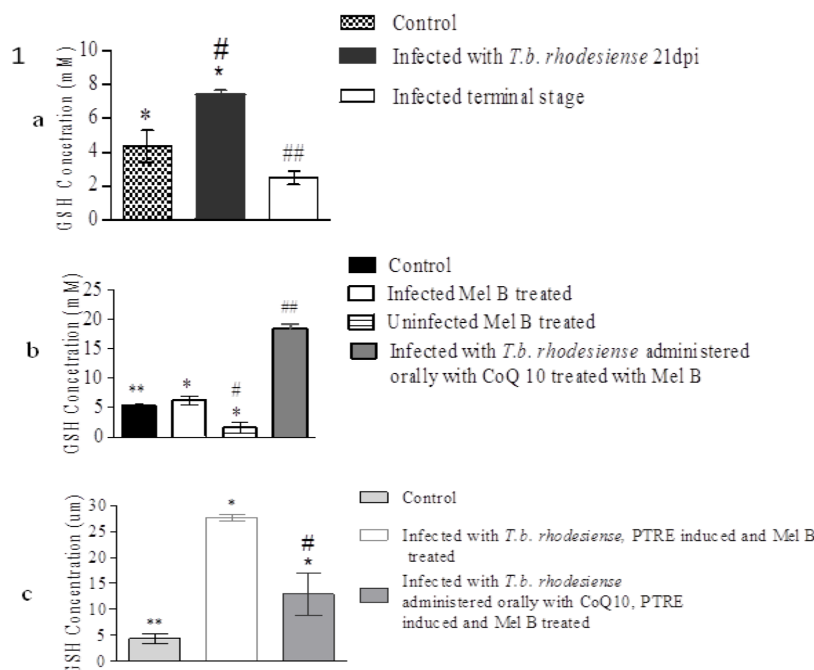

Figure 1 **(a)**. Mean reduced glutathione (GSH) concentration in brain homogenates of uninfected (control), *T. b. rhodesiense* infected mice sacrificed 21dpi and *T. b. rhodesiense* infected sacrificed at terminal stage (57dpi). **(b)**. Mean GSH concentration in brain homogenates of control, uninfected treated with Mel B, *T. b. rhodesiense* infected mice treated with Mel B and *T. b. rhodesiense* infected orally administered with CoQ_{10} and treated with Mel B. **(c)**. Mean GSH concentration in brain homogenates of uninfected (control), *T. b. rhodesiense* infected mice PTRE induced Mel B treated and *T. b. rhodesiense* infected orally administered with CoQ_{10} PTRE induced Mel B treated.

3.2 Effect of *T. b. rhodesiense*, Mel B, PTRE and CoQ_{10} on the expression of Glutathione reductase

Results of the expression of glutathione reductase (GR) in PTRE-induced Mel B and CoQ_{10} treated in infected mice are presented in Fig. 2 a, b, c. Statistical analyses revealed significantly higher GR expression in *T. b. rhodesiense* infected mice brain homogenates sacrificed 21dpi relative to the control mice (P=0.0001). On the other hand, GR expression was significantly elevated in brain homogenates of mice that were at the terminal stage than those at 21dpi (Fig.2b.) (P= 0.0006).Treatment with Mel B significantly lowered GR expression in uninfected mice relative to the control mice (P= 0.0001) (Fig.2a.). Infection of mice with *T. b. rhodesiense* and treatment with Mel B resulted in significantly higher expression of GR than in control mice (P=0.0001) (Fig.2a.).

Similarly, in infected mice treated with CoQ_{10} and Mel B, GR expression was significantly higher relative to the control mice (P=0.0001). The expression of GR in uninfected mice treated with Mel B was significantly lower relative to the *T. b. rhodesiense* infected mice treated with Mel B (P=0.0001). Similarly, Bonferroni's post hoc test comparison of the means revealed significantly higher expression of GR in brain homogenates of infected mice (PTRE induced) treated with Mel B than in control mice (P=0.0006). Infected mice in which PTRE had been induced and which were administered with CoQ_{10} and Mel B, GR expression was significantly lower relative to infected mice (PTRE induced) treated with Mel B, without CoQ_{10} (Fig.2c.).

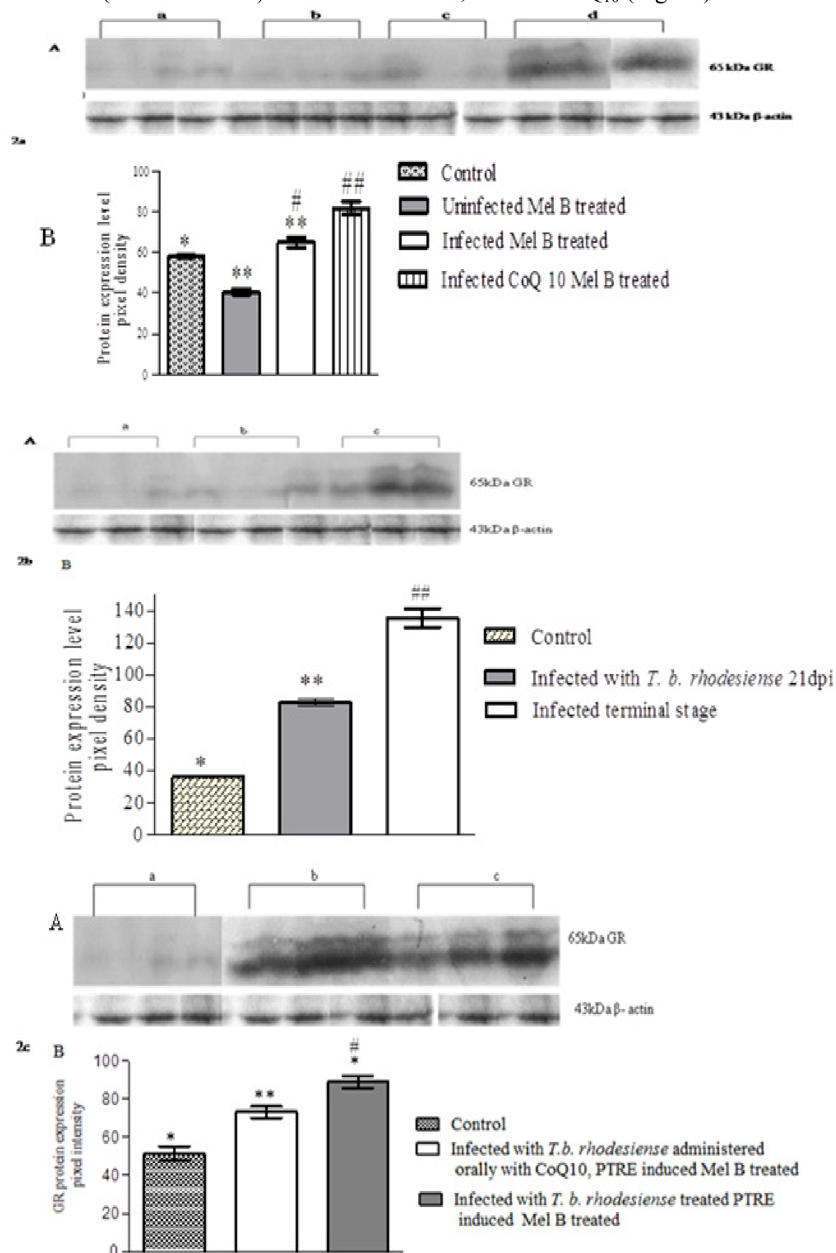

Figure 2 **(a)**. (A) Blot showing differences in the expression of Glutathione reductase protein in brain homogenate fractions from: (a) control, (b) uninfected mice treated with Mel B, (c) *T. b. rhodesiense* infected mice treated with Mel B 21dpi sacrificed 24hrs after the last dosage and (d) *T. b. rhodesiense* infected mice orally administered with 200mg/kg CoQ_{10} treated with Mel B 21dpi. A p value (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. **(b)**. 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 terminal stage brain homogenate. **(c)**. 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 treated and (c) infected orally administered with CoQ_{10} PTRE induced and Mel B treated brain homogenate. β-actin was used to show equal loading. (B)The histogram shows semi-quantitative determinations of GR protein expression, presented as percentage pixel intensity.

3.3 Effect of Mel B, PTRE induction, CoQ_{10} treatment and *Trypanasoma brucei rhodesiense* on SOD-1 expression at different stages of HAT

Results of the expression of SOD-1 in infected mice in which PTRE was induced and were administered with Mel B and CoQ_{10} are presented in Fig. 3a, b, c. The expression levels of SOD-1 in infected mice sacrificed 21dpi (P=0.0001) were higher than in the control mice;, with the expression of SOD-1 being significantly (P=0.0001) elevated at the terminal stage of *T .b. rhodesiense* infection than infected mice sacrificed 21dpi (Fig.3a.). When challenged with *T .b. rhodesiense* infection and upon treatment with Mel B, the brain homogenates of these mice showed a significant (P=0.0001) increase in expression of SOD-1 than control mice (Fig.3b.). Similarly, Bonferroni's post hoc test comparison of the means revealed significantly higher expression of SOD-1 in *T. b. rhodesiense* infected mice (PTRE induced orally administered with CoQ_{10}) treated with Mel B relative to the control mice (Fig.3c.).

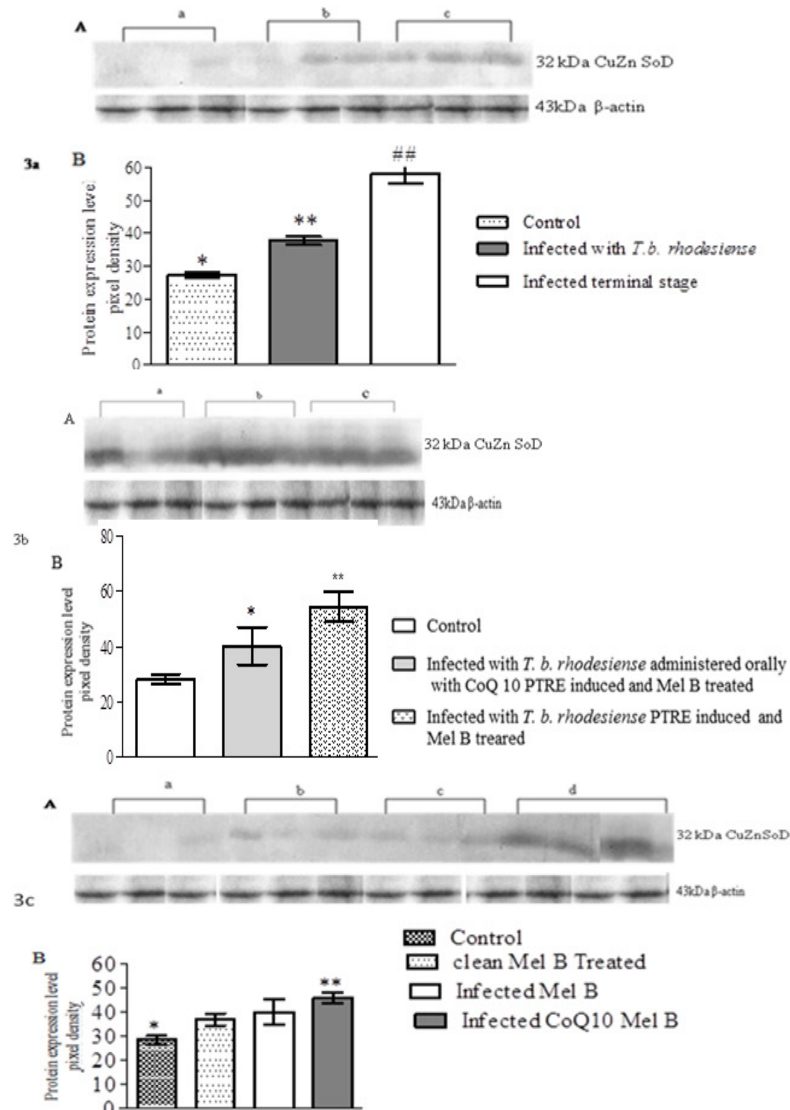

Figure 3 **(a)**. Increased expression of SOD-1 in *T. b. rhodesiense* infected brain homogenates. (A) Blot showing specific expression of SOD-1 which was determined in: (a) uninfected (control) (b) infected sacrificed 21dpi and (c) infected terminal stage brain homogenate. (B) The histogram shows semi-quantitative determinations of GR protein expression, presented as percentage pixel intensity. **(b)**. Increased expression of SOD-1 in *T. b. rhodesiense* infected brain homogenates of *T. b. rhodesiense* infected PTRE induced and Mel B treated. (A) Blot

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showing specific expression of SOD-1 which was determined in: (a) uninfected (control), (b) infected orally administered with CoQ_{10}, PTRE induced and Mel B treated and (c) infected PTRE induced and Mel B treated following relapse brain homogenate. (B) The histogram shows semi-quantitative determinations of GR .protein expression, presented as percentage pixel intensity. **(c)**. 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 CoQ_{10} treated with Mel B and (c) infected treated with Mel B brain homogenate. (B) The histogram shows semi-quantitative determinations of SOD-1 protein expression, presented as percentage pixel intensity.

3.4 Effect of *T. b. rhodesiense* infection, PTRE, Mel B and CoQ_{10} treatment on the expression of MnSOD in mice brain homogenates

Results of the expression of MnSOD in PTRE-induced Mel B and CoQ_{10} treated mice (infected) are presented in Fig. 4a,b,c. Statistical analyses indicated significant differences in expression levels of MnSOD in *T .b. rhodesiense* infected mice at terminal stages (P=0.0004); much higher than mice sacrificed 21dpi. Expression of MnSOD in *T .b. rhodesiense* infected mice sacrificed at 21dpi was significantly higher (P=0.0004) than in control mice (Fig.4a.). Similarly, Bonferroni's post hoc test comparison of the means revealed significantly lower expression of MnSOD in uninfected Mel B treated mice relative to the control mice (P=0.0004). On the other hand, in infected mice treated with CoQ_{10} and Mel B showed a significantly higher expression of MnSOD than in infected Mel B treated (P=0.0004) (Fig.4b.) mice. Similarly, expression of MnSOD in brain homogenates of *T. b. rhodesiense* infected mice (PTRE induced) treated with Mel B was significantly (P=0.0004) lower than those administered with CoQ_{10}. *T .b. rhodesiense* infected mice (PTRE induced, Mel B treated) showed significantly (P=0.0004) lower expression of MnSOD than in control mice (Fig.4c.).

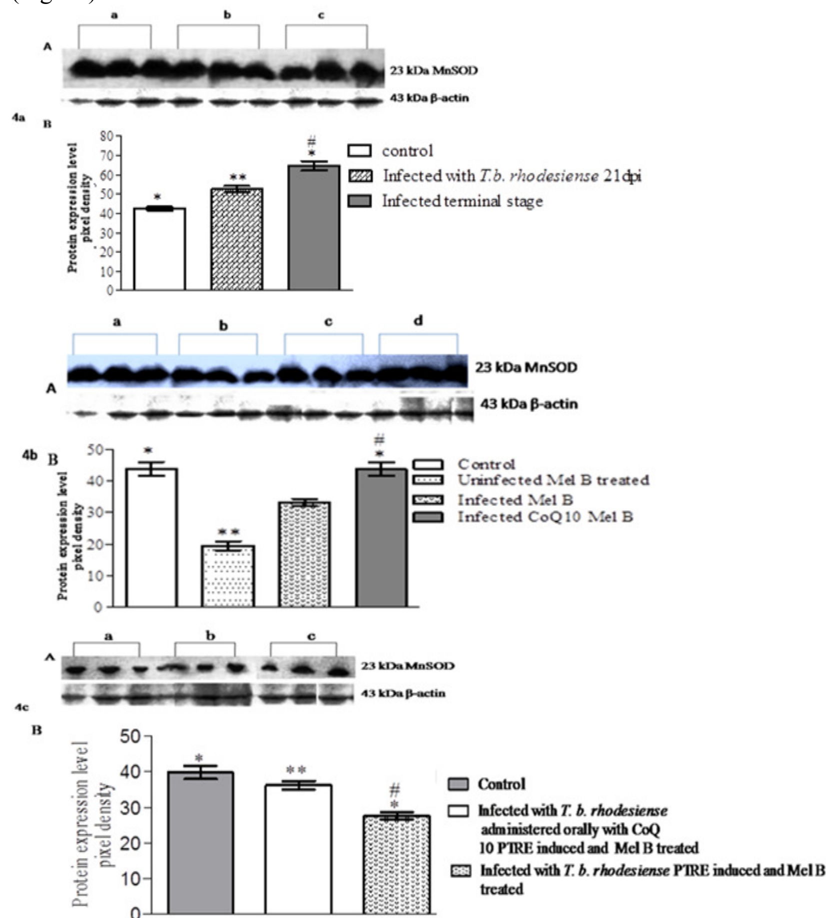

Figure 4 **(a)**. Semi-Quantitative determinations of MnSOD; (A) Blot showing MnSOD protein expression in: (a) control, (b) *T. b. rhodesiense* infected sacrificed 21dpi and (c) *T. b. rhodesiense* infected terminal stage. (B) The histogram shows semi-quantitative determinations of GR protein expression, presented as percentage

pixel intensity. **(b)**. 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 CoQ_{10} 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. **(c)**. Semi-Quantitative determinations of MnSOD, (A) Blot showing MnSOD protein expression in: (a) control, (b) infected orally administered with CoQ_{10} PTRE induced and Mel B treated and (c) infected PTRE induced and Mel B treated following relapse brain homogenates, which is expressed as percentage pixel.

4. DISCUSSION

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 a vital role of the cellular antioxidant system in the pathogenesis of HAT. Such a role creates an opportunity and target for scientists to explore as they strife to identify new targets for drug development or other interventions to improve treatment outcomes in HAT. Establishment of *T. b. rhodesiense* in CNS by 21dpi 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 antioxidant system as demonstrated by the low levels of GSH. 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 other studies in which cellular GSH content was depleted as a result of elevated oxidative stress (Dukhande *et al*. 2006). Similarly, GSH recycling is impaired due to NADPH and isocitrate dehydrogenase 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). Similarly, significant depletion of GSH was depicted in kidney, blood and liver in experimental infection of *T. congolense* (umar *et al*. 2010). 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. This scenario appears to suggest that with further investigations, coupling of anti-oxidant therapy with conventional treatment regimens might improve treatment outcome in HAT.

Reactive oxygen species can play an important role in health; however, overproduction of the same can do great damage in the brain and even result in neurodegeneration. Activation of astrcocytes due to parasite invasion of the brain is accompanied by nitric oxide (NO) production. Though beneficial, it has been postulated that NO can potentially harm brain cells due to overproduction. Specifically, NO facilitates generation of lethal reactive metabolite species such as peroxynitrite ($ONOO^-$) (Heals and Balanos, 2002) in the brain, which nitrate vital proteins and/or enzymes, altering their structure and rendering them dysfunctional. Moreover, the metabolites depletes cellular antioxidant defenses such as glutathione (GSH) and superoxide dismutases (SOD) (Bolanos *et al*. 1997), 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 also GSH depletion is strongly promoted by arsenic-induced apoptosis and enhanced arsenic-induced ROS formation (Del-Razo *et al*. 2001; Davison *et al*. 2003).

Oral administration of CoQ_{10} in infected mice that were treated with Mel B resulted in increased levels of GSH. Such a profound finding indicates that CoQ_{10} can support GSH status in the brain during Mel B treatment and reduce mortality due to Mel B toxicity. Hence these findings provide some evidence for a potential treatment regime for HAT incorporating CoQ10. Note that previous studies have reported that depletion of endogenous GSH antioxidant may be a significant factor in the pathogenesis of *T. congolense* infection. In this study, administration of exogenous vitamin C to infected animals, prevented disease-induced decreases in GSH and ascorbic acid (Umar *et al*. 2010). This assumption is supported by the fact that Coenzyme Q_{10} has been reported to effectively attenuate toxicity in transgenic mice following administration with 3-Nitropropionic acid (Matthews *et al*. 1998); and protection against glutamate toxicity in cultured cerebral neurons (Favit *et al*. 1992). Despite obvious shortcomings in our data in explaining the mechanistic details of cellular amount of Coenzyme Q_{10} penetrated and concentrated in the CNS, we propose that oral administration of CoQ_{10} could be used to block GSH depletion due Mel B toxicity and could attenuate PTRE. Additionally, we suggest that CoQ_{10} could be useful in maintenance of endogenous antioxidants in the brain during HAT therapy.

Note that oral administration of 200mg/kg of CoQ_{10} 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, the induction of GSH was more marked. Results from this study suggest that an increase in GSH, to a certain extent could alleviate the effect of oxidative stress induced by PTRE and virulent effect of *T. b. rhodesiense*, by boosting 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 CoQ_{10} orally administered, PTRE induced and Mel B treated mice could be attributed to the antioxidant capability of CoQ_{10} 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 CoQ_{10} 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 chain and oxidative phosphorylation which enhances an increased free radical leakage and thereby inducing oxidative stress, and subsequently stimulating MnSOD, SOD-1 and GR expression and 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 there protective role in brain cells threatened with *T. b. rhodesiense* infection or oxidative stress. In an earlier observation in cells challenged with energy failure by mitochondrial toxins and/or oxidative stress, there was induction of MnSOD and $NADP^+$-ICDH activity. 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 in our study. This 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 was noted 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 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. 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 *et el*. 1996). Mel B depletion of MnSOD in this study should not be a surprise because the mitochondrion is known to accumulate arsenic (Mazumder, 2005). This is an indication that possibly, Mel B toxicity targets vital mitochondrial processes in the brain. 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 (Chouchane and Snow, 2001). In this study, arsenic appeared to be an effective inhibitor of all of the proteins studied (Thomas *et al*. 2001). In light of this, MnSOD and GR depletion may be an important mechanism underlying Mel B induced neurotoxicity.

Additionally, treatment of infected mice with Mel B also showed depletion of MnSOD. As early 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.

CoQ_{10} 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 CoQ_{10} 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 CoQ_{10}. Consequently this study shows that CoQ_{10} can aid in quenching ROS. Apart from neutralizing the toxic effect of Mel B, exogenous CoQ_{10} can also can play an integral role in maintaining endogenous CoQ_{10} levels.

Importantly, CoQ_{10} has been shown to protect human neuroblastoma (SHSY-5Y) cells against paraquat-induced mitochondrial dysfunction as well as against β -amyloid toxicity (Winkler-Stuck *et al*. 2004). Thus, these findings suggest that other factors are required for the targeting of CoQ_{10} 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 mice on CoQ_{10} compared to the control and infected mice on Mel B alone. Supplementation with CoQ_{10} 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 CoQ_{10} treatment (Young *et al*. 2008). Similarly, in a transgenic mouse model of familial ALS that overexpresses SOD-1 with the G93 A mutation, treatment with CoQ_{10} 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 that could trigger neurodegeneration. Some of the crucial events like oral administration of CoQ_{10} may be “protective’’ in nature. Consequently, there is need to understand how CoQ_{10} interacts with Mel B to ameliorate its toxicity.

Glutathione reductase 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 CoQ_{10} infected mice on Mel B and infected mice on Mel B (without CoQ_{10}) indicate the important role it plays during oxidative stress.

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 stages of infection and at the terminal stage. There was an increased expression of GR in infected, PTRE induced and Mel B treated mice compared to the control. Note that treatment with diaminazine aceturate induces PTRE (Pepin, 1994). PTRE may be a major factor for increased production of ROS other than that induced by the parasites. Hence presence of PTRE and parasites can lead to a rise in SOD-1 and GR, when compared to CoQ_{10} supplemented mice. This study noted an increase in the expression of SOD-1 in CoQ_{10} orally administered, PTRE induced and Mel B treated mice suggesting that CoQ_{10} and SOD-1 act in concert in protecting brain cells against harmful effect of ROS. This is another indication that CoQ_{10} could alleviate PTRE and Mel B toxicity. Expression of both SOD-1 and GR is vital for maintenances of a robust antioxidant system that is functional. Other studies have revealed that overexpression of MnSOD and GPx protects neurons from the harmful effects of ROS in experimental stroke (Wallace, 2002; Hoen *et al*. 2004).

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 mice treated with Mel B at relapse when compared to PTRE mice that received CoQ_{10} and Mel B, it possible that PTRE induction increased oxidative stress leading to depletion and inactivation of this important antioxidant. Peroxynitrite ($ONOO^-$) has been shown to inactivate 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 the role of vital antioxidant systems during active PTRE as a result of Mel B toxicity and oxidative stress 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.

Previous studies (Jo *et al*. 2002; Munich *et al*. 2003) and the findings from this study demonstrate the important role MnSOD, SOD-1, GSH and GR in combating oxidative stress in brain challenged with *T. b. rhodesiense*. The observed depletion of GSH in the terminal stage prompts to speculate that *T. b. rhodesiense* infection of the brain results into neurological dysfunction, which may impair NADPH generation and subsequently deplete intracellular GSH or its synthesis and intercellular cycling (Lee *et al*. 2003).

5. CONCLUSION

In conclusion, this study provides new information on how the brain challenged with *T. b. rhodesiense* infection, treatment with Mel B and oral administration of CoQ_{10} 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 CoQ_{10} on the same, indicate putative mechanisms with potential application in formulation of novel Mel B-based drugs that would be less toxic and safer to use.

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REFERENCES

1. Kennedy P.G. (2004). Human African trypanosomiasis of the CNS, current issues and challenges. J Clinl Investig 113: 496-504.
2. Fevre E.M., Picozzi K., Fyfe J., Waiswa C., Odiit M., Coleman P.G., Welburn S.C. (2005). A burgeoning epidemic of sleeping sickness in Uganda. *Lancet* 366: 745-747.
3. Pentreath V.W. (1995). Trypanosomiasis and the nervous system. Trans Roy Soc Trop Med Hyg *89:* 9-15.
4. Lejon V., Legros D., Rosengren L., Gastellu E.M., Büscher P. (2001). Biological data and clinical symptoms as predictors of astrogliosis and neurodegeneration in patients' second-stage *Trypanosoma brucei gambiense* sleeping sickness. Am J Trop Med Hyg 65: 931-935.
5. Rubbo H., Denicola A., Radi R. (1994). Peroxynitrite inactivates thiol-containing enzymes of *Trypanosoma cruzi* energetic metabolism and inhibits cell respiration. Arch Biochem Biophys 308: 96-102.
6. Pepin J., milrod F. (1994). The treatment of Human African Trypanosomiasis. Adv Parasitol 33: 1-47.
7. Hunter C.A., Jennings F.W., Adams J.H., Murray M., Kennedy P.G. (1992). Subcurative Chemotherapy may underlie fatal post-treatment reactive encephalopathies in human African trypanosomiasis. *Lancet* 339: 956-958
8. Soignet S.L., Tong W.P., Hirschfeld S., Warrell R.P. (1999). Clinical study of an organic arsenical, melarsoprol, in patients with advanced leukemia. Cancer Chemoth Pharm 44: 417-421.
9. Fairlamb A.H., Henderson G.B., Bacchi C.J., Cerami A. (2003). In vivo effects of difluoromethylornithine on trypanothione and polyamine levels in bloodstream forms of *Trypanosoma brucei*. Mol Biochemic Parasitol 24: 185-191.
10. Halliwell B. (2001). Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs Aging 18: 685-716.
11. Gorman A.M., McGowan A., O'Neil C., Cotter T. (1996). Oxidative stress and apoptosis in neurodegeneration population. JNeurolog Sci 139: 45-52.
12. Keita M., Vincendeau P., Buguet A., Cespuglio R., Vallat J.M., Dumas M., Bouteille B. (2000). Inducible nitric oxide synthase and nitrotyrsine in the CNS of mice chronically infected with *Trypanosoma brucei brucei*. Exp Parasitol 95: 19-27.
13. Mataix J.L., Quiles J.R., Huertas M., Battino N., Mañas M. (1998). Tissues specificinteractions of exercise, dietary fatty acids, and vitamin E in lipid peroxidation. Free Radical Biol Med 24: 511-521.
14. Bolanos J.P., Almeida A., Stewart V., Peachen S., Land J.M., Clark J.B., Heales S.J. (1997). Nitric oxide mediated mitochondrial damage in the brain: Mechanisms and implications for neurodegenerative diseases. J Neurochem 68: 2227-2240.
15. Heales S.J., Bolanos J.P. (2002). Impairment of brain mitochondrial function by reactive nitrogen species. The role of glutathione in dictating susceptibility. Int J Neurochem 40: 469-474.
16. Matthews R.T., Yang L., Browne S., Baik M., Beal M.F. (1998). Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects. Proc Natl Acad Sci USA 95: 8892-8897.
17. Meredith S., Beal F., Henchcliffe C. (2009). Coenzyme Q10 effects in neurodegenerative Disease. Neuropsychiatr Dis Treat 5: 597-610.
18. McCarthy S., Somayajulu M., Sikorska M., Borowy-Borowski H., Pandey S. (2004). Paraquat induces oxidative stress and neuronal cell death; neuroprotection by water-soluble Coenzyme Q10. Toxicol Appl Pharmacol 201: 21-31.
19. Winkler-Stuck K., Wiedemann F.R., Wallesch C.W., Kunz W.S. (2004). Effect of coenzyme Q10 on the mitochondrial function of skin fibroblasts from Parkinson patients. J Neurosci 220: 41-48.
20. Favit A., Nicoletti F., Scapagnini U., Canonico P.L. (1992). Neuroprotective agents and celebral ischaemia. J Cereb Blood Flow Metab 12: 638-645.

21. Dukhande V.V., Malthankar-Phatak G.H., Hugus J.J., Daniels C.K., Lai J.C. (2006). Manganese-Induced Neurotoxicity is Differentially Enhanced by Glutathione: Depletion in strocytoma and Neuroblastoma Cells. Neurochem Res 31: 1349-1357.
22. Gora D., Sandhya M., Shiv G., Praveen S. (2006). Oxidative stress, α-Tochoperol, ascorbic acid and reduced glutathione status in schizophrenics. Indian J of Clin Biochem 21:34- 38.
23. Umar I.A., Igbalajobi F.I., Toh Z.A., Gidado A., Shugaba A., Buratai L.B. (2001). Effects of repeated daily doses of vitamin E. (alpha-tocopherol) on some biochemical indices of rats infected with T. bricei (Basa strain). Afr J Biol Sci 12: 1-7.
24. Smith K., Nadeau K., Walsh C.T., Fairlamb A.H. (1992). Purification of glutathionylspermidine and trypanothione synthetases from Crithidia Jasciculata. Prot Sci 1: 874-883.
25. Del-Razo L.M., Quintanilla-Vega B., Brambila-Colombres E., Calderon-Aranda E.S., Manno M.A., (2001). Stress proteins induced by arsenic. Toxicol Appl Pharmacol 177: 132-148.
26. Davison K., Cote S., Mader S., Miller W.H. (2003). Glutathione depletion overcomes resistance to arsenic trioxide in arsenic-resistant cell lines. Leukem 17: 931-940.
27. Umar I., Toma I., Akombum C., Nnadi C., Mahdi M., Gidado A., Igbokwe I., Buratai L. (2010). The role of intraperitoneally administered vitamin C during *Trypanosoma congolense* infection of Rabbits. Afr J Biotechnol 9: 5224-5228.
28. Tomasetti M., Littarru G.P., Stocker R., Alleva R. (1999). Coenzyme Q10 enrichment decreases oxidative DNA damage in human lymphocytes. Free Radical Biol Med 27:1027-1032.
29. Orina A.I., Vikas V.D., James C.K.L. (2007). Metabolic and antioxidant system alterations in an astrocytoma cell line challenged with mitochondrial DNA deletion. Neurochem Res 32:1906-1918.
30. Fairlamb A.H., Henderson G.B., Cerami A. (1989). "Trypanothione is the primary target for arsenical drugs against African trypanosomes. Proc Natl Acad Sci *USA* 86: 2607-2611.
31. Cunningham M.L., Zvelebil M.J., Fairlamb A.H. (1994). Mechanism of inhibition of trypanothione reductase and glutathione reductase by trivalent organic arsenicals. Eur J. Biochem 221: 285-295.
32. Larochette N., Decaudin D., Jacotot E., Brenner C., Marzo I., Susin S.A. (1999). Arsenite induces apoptosis via a direct effect on the mitochondrial permeability transition pore. Exp Cell Res 249: 413-421.
33. Li Y.M., Broome J.D. (1999). Arsenic targets tubulins to induce apoptosis in myeloid leukemia cells. Cancer Res 59: 776-780.
34. Cavigelli M., Li W.W., Lin A., Su B., Yoshioka K., Karin M. (1996). The tumor promoter stimulates AP-1 activity by inhibiting a JNK phosphatase. Eur Mol Biol Org 15: 6269-279.
35. Wang T., Kuo C., Jan K., Huang H. (1996). Arsenite induces apoptosis in Chinese hamster ovary cells by generation of reactive oxygen species. J *Cell* Physiol 169: 256-268.
36. Mazumder D.N. (2005). Effect of chronic intake of arsenic-contaminated water on liver. Toxicol Appl Pharmacol 206: 169-75.
37. Chouchane S., Snow E.T. (2001). In vitro effect of arsenical compounds on glutathione related enzymes. Chem Res Toxicol 14: 517-522.
38. Thomas D.J., Styblo M., Lin S. (2001).The cellular metabolism and systemic toxicity of arsenic. Toxicol Appl Pharmacol 176: 127-144.
39. Somayajulu M., McCarthy S., Hung M., Sikorska M., Borowy-Borowski H., Pandey S. (2005). Role of mitochondria in neuronal cell death induced by oxidative stress; neuroprotection by Coenzyme Q10. Neurobiol Dis 18: 618-627.
40. Yang X., Yang Y., Li G., Wang J., Yang E.S. (2008). Coenzyme Q10 attenuates beta-amyloid pathology in the aged transgenic mice with Alzheimer presenilin 1 mutation. J Mol Neurosci 34: 165-171.
41. Pepin J. (1994). Gambiense trypanosomiasis: frequency of, and risk factors for, failure of melarsoprol therapy. Trans Roy Soc Trop Med Hyg 8: 447-452.
42. Hoehn B., Yenari M.A., Sapolsky R.M., Steinberg G.K. (2003) Glutathione peroxidase overexpression inhibits cytochrome C release and proapoptotic mediators to protect neurons from experimental stroke. Stroke 34: 2489-2494.
43. Wallace D.C. (2002) Animal models for mitochondrial disease. In Copeland, WC (ed) Mitochondrial DNA: Methods & protocols. Humana Press, Totowa, NJ, Vol 197, pp.3-54
44. Hinerfeld D., Traini M.D., Weinberger R.P., Cochran B., Doctrow S.R., Harry J., Melov S. (2004). Endogenous mitochondrial oxidative stress: neurodegeneration, proteomic analysis, speficic respiratory chain defects, and efficacious antioxidant therapy in uperoxide dismutase 2 null mice. J Neurochem 88: 657-667.

45. MacMillan-Crow L.A., Crow J.P., Kerby J.D., Beckman J.S., Thompson J.A. (1996) Nitration and inactivation of manganese superoxide dismutase in chronic rejection of human renal allografts. Proc Natl Acad Sci 93: 11853-11858.
46. Savvides S.N., Scheiwein M., Bohme C.C., Arteel G.E., Karplus P.A., Becker K., Schirmer R.H. (2002). Crystal structure of the antioxidant enzyme glutathione reductase inactivated by peroxynitrite. J Neurochem 277: 2779-2784.
47. Jo S.H., Son M.K., Koh H.J., Lee S.M., Song I.H., Kim Y.O., Lee Y.S., Jeong K.S., Kim W.B., Park J.W., Song B.J., Huh T.L. (2001). Control of mitochondrial redox balance and cellular defense against oxidative damage by mitochondrial NADP+-dependent isocitrate dehydrogenase. J Biol Chem 276: 16168-16176.
48. Munich T., Yokota S., Dringen R. (2003). Cytosolic and mitochondrial isoforms of NADP+-dependent isocitrate dehydrogenases are expressed in cultured rat neurons, astrocytes, oligodenrocytes and microgial cells. J Neurochem 86: 605-614.
49. Lee H.J., Yang S.E., Park W.J. (2003) Inactivation of NADP+- dependent isocitrate. dehydrogenase by peroxynitrite. Implications for cytotoxicity and alcohol-induced liver injury. J Biol Chem 278: 51360-51371.
50. Griffith OW. Determination of Glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem 1980; 106: 207-212.