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Correlations of Reduced Glutathione and Glutathione Peroxidase Activities with Biochemical Markers of Liver and Kidney Damage in *Trypanosoma brucei brucei* Infected Rats

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Abstract
The correlations of reduced glutathione (GSH) and glutathione peroxidase (GPx) levels with biochemical markers of liver and kidney damage in *Trypanosoma brucei* infection were studied in rats. Forty adult male rats divided into 2 groups of control and infected were used. Infected rats were inoculated intraperitoneally with 1.0 ml of blood at concentration of 1 x 10^6 trypanosomes per ml. Serum and tissue samples were collected on days 0, 3, 5 and 7 post-infection (pi) for biochemical analyses. Serum GPx activity had a significant ($p < 0.05$) positive correlations with liver ($r = 0.96$) and kidney GSH ($r = 0.93$) levels. All the measured serum parameters had significant ($p < 0.05$) negative correlations with serum GPx activity. In conclusion infection of rats with *T. b. brucei* caused a decrease in the serum GPx activities and organ GSH levels with increasing parasitaemia and duration of the infection.

Keywords: *Trypanosoma brucei brucei*; Oxidative stress; Glutathione peroxidase; Glutathione; Liver and kidney function

Introduction

African animal trypanosomosis is caused *Trypanosoma vivax (T. vivax), T. congolense* and *T. brucei* in cattle, sheep and goats, and *T. simiae* in pigs (Nantulya, 1990; Adamu *et al*., 2009), *T. cruzi* and *T. evansi* in dogs (Jones *et al.,* 2000) and *T. equiperdum* (Ahmed *et al*., 2018).

The clinical manifestations of the disease include anaemia, weight loss, weakness, anorexia, jaundice, abortion, low milk production, and decrease in reproductive capability (Radostis *et al*., 2003). These factors may emanate from the physical and metabolic activities of the Trypanosoma spp, trypanosome autolysates and oxidative lipoperoxidation (Igbokwe, 1994). The extent of tissue invasion varies with the Trypanosoma spp involved, with the T. brucei group being the most tissue invasive, followed by the T. vivax and T. congolense which are rather more restricted to the blood circulation (Igbokwe, 1994). Trypanosomes also produce protease, neuraminidase, phospholipase and some other toxic metabolites Igbokwe (1994).

The infection with trypanosomes results in the production of large amounts of reactive oxygen species (ROS) and free radicals which act as cytotoxic agents (Gutteridge, 1995) damaging vital components of the cell, including proteins and lipids (Murray *et al.,* 2003). Further reports have shown an important role of free radical induced oxidative stress in the pathogenesis of Trypanosomiasis (Ogunsanmi and Taiwo, 2007; Umar *et al*., 2007; Akanji *et al.,* 2009). Although several abnormalities in serum biochemical and oxidative stress markers have been identified in trypanosome-infected laboratory and domestic animals, attempts to investigate and correlate the changes in the concentration of these molecules in tissues sequel to infection is limited, hence the need for a research in this direction.

Glutathione (GSH) is a tripeptide (L-γ-glutamyl-L-cysteinyl-glycine) with multiple functions in living organisms (Vivancos *et al*., 2010). As a carrier of an active thiol group in the form of a cysteine residue, it acts as an antioxidant either directly by interacting with reactive oxygen/nitrogen species (ROS and RNS, resp.) and electrophiles or by operating as a cofactor for various enzymes (Duan and Chen, 2007; Cooper *et al*., 2011). The reduced and oxidized forms of glutathione (GSH and GSSG) act in concert with other redox-active compounds (e.g., NAD(P)H) to regulate and maintain cellular redox status (Jones *et al*., 2011). GSH is responsible for protection against ROS and RNS, and detoxification of endogenous and exogenous toxins of an electrophilic nature (). However, GPx are enzymes that catalyze the GSH-dependent reduction of many peroxides (reaction) (Cooper *et al*., 2011), they particularly involved in the removal of LOOH, thereby terminating lipid peroxidation chain reactions and protecting biological membranes (Toppo *et al*., 2009).

Materials and methods

Ethical Statement

The experimental protocol and procedures used in this study involving animals were obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with reference number ABUCAUC-2017-028.

Experimental Animals

Adult male rats (n = 40) were used for the experiment. The rats were kept in rat cages with wood shavings used as bedding. They were fed on pelleted grower mash (Vital Feed) and water was provided ad libitum. A period of 14 days was allowed for the rats to get acclimatized prior to the commencement of the experiment. During this period, the rats were exposed to the routine handling conditions they were subjected to during the experiment. On the day of commencement of the experiment, the rats were allocated, on basis of mean body weight, to two groups (infected and control) of 20 rats each.

Experimental Trypanosome Infection

Trypanosoma brucei brucei (Federi strain) was sourced from the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. The parasite was maintained by serial passages in donor rats until the day of inoculation in the experimental animals. Parasitaemia was monitored daily by preparing a wet mount of the blood collected from the tail vein of the infected rats according to the method of Woo (1969).

Inoculations of rats in the infected group were carried out as described by Ekanem and Yusuf (2005). In brief, when the donor rat was at the peak of parasitaemia, tail blood was collected and diluted with physiological saline to obtain a trypanosome concentration of 1 x 10^6 trypanosomes per ml. The diluted infected blood (1.0 mL) was injected intraperitoneally to each rat in the infected group. After infection of the rats the animals were monitored for the onset of parasitaemia.

Determination of Parasitaemia

Throughout the course of the experiment, blood was collected from the tail and examined by wet mount under the light microscope at x 40 magnification to monitor and score parasitaemia according to the rapid matching method described by Herbert and Lumsden (1976).

Serum and Tissue Sample Collection

On days 0, 3, 5 and 7 post-infections, 4 rats each from the infected and control groups were euthanized to obtain blood sample. The blood was collected in plain sample bottle without anticoagulant and allowed to clot, so that serum was obtained thereafter. Liver and kidney were collected and washed with phosphate buffered saline (PBS). The tissue sample (1.0 g) was macerated and homogenized with 5.0 ml of PBS. The homogenate was stored at 4ºC until it was used for biochemical analysis.

Biochemical Analyses

Serum aspartate aminotransferase (AST) activity was determined using the standard colorimetric method as described by Reitman and Frankel (1957). Serum alanine aminotransferase (ALT) activity was determined using the standard colorimetric method of Reitman and Frankel (1957). Determination of serum alkaline phosphatase (ALP) activity was done using the phenolphthalein monophosphate method (Klein *et al*., 1960). Serum urea was determined using Urease Berthelot method (Fawcett and Scott, 1960) while determination of serum creatinine was based on the modified Jaffe method (Fossati *et al*., 1983). Tissue homogenate Glutathione and serum glutathione peroxidase activity (GPx) was estimated with an assay kits (Northwest Life Science Specialist, Vancouver, Canada).

Statistical Analyses

GraphPad prism (version 4.2) was the computer software used for the statistical analysis. Data were summarized and presented as mean ± standard error of mean (± SEM). Means were compared by Student's t-test to assess the significant difference. Pearson's correlation analysis assessed the relationships between variable. $P < 0.05$ was considered significant.

Results

Parasitaemia was detected in four of the infected rats on day 3 post infections (pi) and by day 4 pi, all the rats in the infected group were parasitaemic, thus, giving a pre-patent period of 3.75 ± 0.11 days. The mean parasitaemia in the infected rats on days 3, 5 and 7 pi were 2.24 ± 0.63 x 10^6 (log10=6.30 ± 0.13), 107.50 ± 10.62 x 10^6 (log10=8.03 ± 0.04) and 403.60 ± 48.79 x 10^6 (log10=8.68 ± 0.017) parasites/ml of blood, respectively (Figure 1).

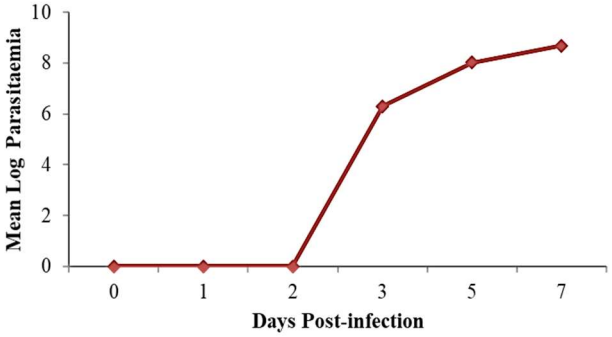

Figure 1: Mean Log_{10} Parasitaemia in Rats Experimentally Infected with *T. brucei brucei*

The mean serum GPx activities in the infected and control rats were presented in Figure 2. The day 0 pre-infection mean serum GPx activities in the infected and control group of rats were 43.00 ± 2.14 and 39.66 ± 4.58 U/ml, respectively. After the infection, the mean serum GPx activity in the infected rats progressively decreased to a lowest value of 32.59 ± 3.36 U/ml on day 7 pi which was not significant ($p > 0.05$) from the corresponding value in the control group.

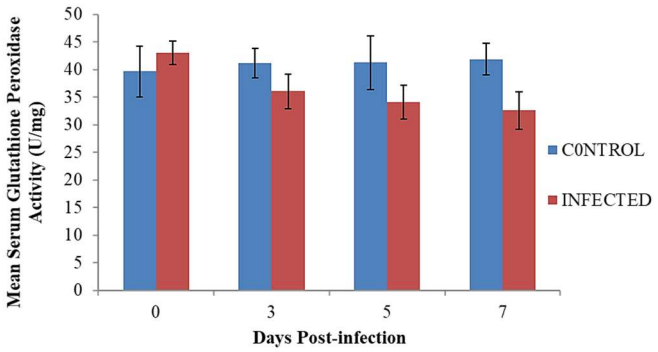

Figure 2: Serum Glutathione Peroxidase Activity of *T. brucei brucei*-infected and Control Rats.

The mean liver GSH levels in the infected and control rats were as presented in Figure 3. The pre-infection mean liver GSH content in infected and control groups of rats were 19.53 ± 0.23 and 19.27 ± 0.55 µg/ml, respectively. After the infection, the mean liver GSH content in the infected rats progressively decreased to a lowest value (18.53 ± 0.86 µg/ml) on day 5 pi, and remained until termination of the experiment. The hepatic GSH concentration in the infected group was only significantly ($P < 0.05$) lower than in the control group on day 5 pi.

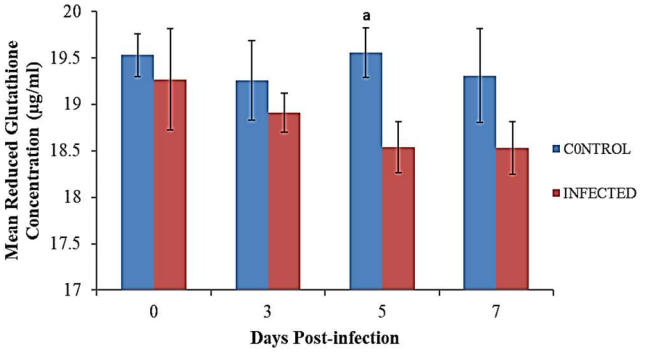

Figure 3: Reduced Glutathione Levels in the Liver of *T. brucei brucei*-infected and Control Rats.

The mean kidney GSH levels in the infected and control rats were as presented in Figure 4. The pre-infection mean kidney GSH level in the infected and control group of rats were 24.34 ± 2.17 and 25.82 ± 1.031µg/ml, respectively. After the infection, the mean kidney GSH level in the infected group decreased to the lowest value of 19.09 ± 0.70 µg/ml on day 7. The mean kidney GSH level remained relatively unchanged in the control group with only insignificant fluctuations.

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

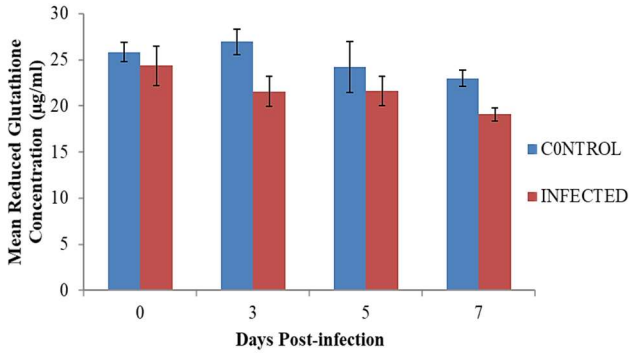

Figure 4: Reduced Glutathione Levels in the Kidney of *T. brucei brucei*-infected and Control Rats.

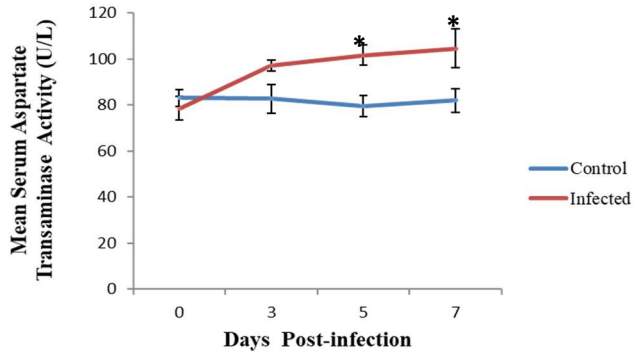

Figure 5: Serum Aspartate Aminotransferase Activity of *T. brucei brucei*-infected and Control Rats. * Significantly ($P < 0.05$) higher when compared to the pre-infection and controls.

The mean serum ALT activities in the infected and control rats were as presented in Figure 6. The pre-infection mean serum ALT activities of the infected and control groups were 39.35 ± 3.02 and 41.33 ± 2.60 U/L, respectively. After the infection, there was a progressive increase in the mean serum ALT activity in the infected rats, with the highest activity, 57.20 ± 3.26 U/L, recorded on day 7 and was significantly ($p< 0.05$) higher than the pre-infection or corresponding control group value. The mean ALT activity in the control group did not vary significantly up to day 7 of the experiment

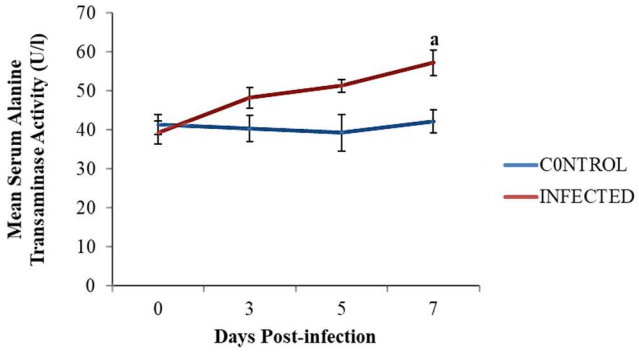

Figure 6: Mean ± SEM Alanine Aminotransferase Activity of *T. brucei brucei*-infected Rats and Control. [a] Significantly ($P < 0.05$) higher when compared to the pre-infection and controls.

The mean serum ALP activities in the infected and control rats were as presented in Figure 7. The mean pre-infection serum ALT activities of the infected and control groups were 104.80 ± 5.76 and 117.50 ± 2.10 U/L, respectively. Following infection there was a progressive increase in the mean serum ALP activity up to the end of the experiment. Although all the mean serum ALP activities obtained post-infection were not statistically different ($p > 0.05$) from the corresponding control values, they had a consistent and sequentially higher value than those of the control group, which remained relatively unchanged throughout the study.

The mean serum urea concentrations in the infected and control rats were as presented in Figure 8. The mean pre-infection serum urea concentrations of the infected and control groups were 6.45 ± 0.47 and 6.77 ± 0.64 mmol/L, respectively. Following the infection there was a progressive increase in the mean serum urea concentration of the infected rats, with the highest concentration of 9.32 ± 0.98mmol/L, attained on day 7 pi which was significantly ($p< 0.05$) higher than the corresponding control group value. The mean serum urea concentration of the control group remained relatively unchanged up to termination of the experiment.

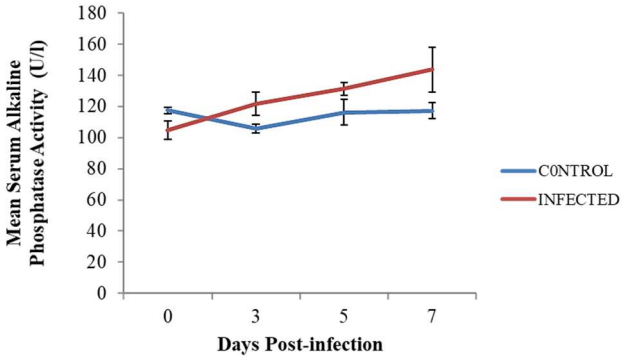

Figure 7: Mean ± SEM Serum Alkaline Phosphatase Activity of *T. brucei brucei*-infected and Control Rats.

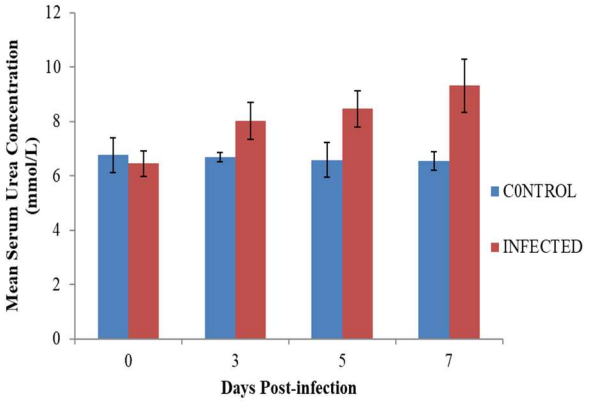

Figure 8: Mean ± SEM Serum Urea Concentration of *T. brucei brucei*-infected and Control Rats.

The mean serum creatinine concentrations in the infected and control rats were as presented in Figure 9. The mean pre-infection serum creatinine concentrations of the infected and control groups were 76.24 ± 3.28 and 74.92 ± 2.78 µmol/L, respectively. After the infection, a progressive increase in the mean serum creatinine concentration was observed in the infected rats, with the highest level of 111.10± 6.09 µmol/L, recorded on day 7 pi, which was significantly ($p< 0.05$) higher than the corresponding control group value. The mean serum creatinine concentration of the control group remained relatively unchanged up to termination of the experiment.

The liver and kidney GSH levels positively and significantly ($p < 0.05$) correlated with serum GPx activity in the infected rats (Table 1). Serum biochemical parameters (except creatinine) and enzymes activities were negatively and significantly ($p < 0.05$) correlated with the serum GPx activity in the infected rats (Table 1).

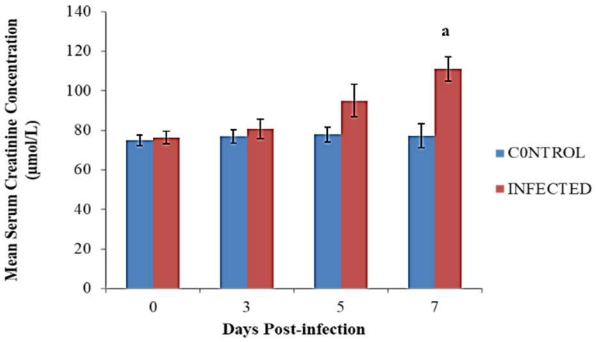

Figure 9: Mean ± SEM Serum Creatinine Concentration of *T. brucei b*-infected and Control Rats. [a] Significantly ($P < 0.05$) higher when compared to the pre-infection and controls.

Discussion

The mean prepatent period for the infection with *T. b. brucei* similar to the ones in previous reports (Umar *et al.,* 1999; Umar *et al.,* 2007; Kobo *et al*., 2014). The finding in this study of lower values, though not significant GPx activities following infection of the rats with *T. b. brucei* supports the fact that reactive oxygen species were released during the infection and the reduction may have been a consequence of GPx utilization in the mopping up of the oxidant substances (Igbokwe *et al.,* 1996; Omer *et al.,* 2007; Yusuf *et al.,* 2012).

The GPx catalyzes the conversion of H_20_2 to H_20 through the oxidation of GSH (Szkudeslski, 2001; Duarte *et al.,* 2001) and oxidative stress leads to the depletion of the endogenous antioxidant defense components (Igbokwe *et al*., 1996; Baydas *et al.,* 2002; Yusuf *et al.,* 2012), and this could be the possible reason for the observed reduction in serum GPx activities in this study.

The negative correlation of the level of parasitaemia with serum GPx activities and organ (liver and kidney) GSH levels, in the *T. b. brucei* infected rats, further buttresses the possible depletion of the host's antioxidant defense, corroborated by earlier findings (Igbokwe *et al*. 1996, Baydas *et al.* 2002, Yusuf *et al.* 2012). There was a decrease in the level of GSH in the liver and kidney of the *T. b. brucei* infected rats. The decrease was marginal and insignificant, but followed similar patterns observed by earlier research where significant decrease in GSH levels of the tissues and

serum of infected rats were reported (Igbokwe *et al.,* 1996; Saleh *et al*., 2009; Yusuf *et al*., 2012).

The positive correlations of the liver and kidney GSH levels with serum GPx activities in the *T. b. brucei*-infected rats, suggests that these variable are influence by similar factor of oxidative stress. Serum GPx being an enzymatic antioxidant and the body's first line of antioxidant defense, works in tandem with GSH, a non-enzymatic endogenous antioxidant. Serum GPx activity had a significant correlation with the liver and kidney GSH, indicating that organ GSH levels were closely related to serum GPx.

Elevated serum AST, ALT and ALP activities in the infected rats indicated liver damage from oxidative radicals which caused nonspecific oxidative injury (Lee *et al.,* 2004; Bouayed and Bohn, 2010; Ayla and Metin, 2015). Oxidative stress has been reported in trypanosomosis and has been implicated to play a contributory role in tissue damage and elevation of serum biochemicals parameters (Omer *et al.,* 2007; Umar *et al.,* 2007; Saleh *et al.,* 2009). The elevated serum levels of urea and creatinine in the *T. b. brucei*-infected rats indicated the involvement of renal damages in this study.

Table 1: Correlation of Serum GPx Activity with Organ GSH, Serum Biochemicals and Enzymes in *T. brucei brucei*-Infected Rats

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

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

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

Conclusion

This study has shown that there were decreases in serum GPx activities and organ GSH levels of *T. b. brucei*-infected rats which correlated to the biochemical derangements with corresponding increase parasitaemia.

Author Contribution

K. A. N. Esievo and S. Adamu designed and supervised the research. Fanaiye, O. G. sourced and bred the rats used. Erin, J.P. financed and carried out the experimental aspect of the research. Idris, S. Y., Erin, J.P. and Kolawole, B. J. contributed in the laboratory analysis of samples and data analysis.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

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