Bima Journal of Science and Technology, Vol. 8(1A) Mar, 2024 ISSN: 2536-6041

DOI: 10.56892/bima.v8i1.587

# Effects of *Trypanosoma brucei* Infection on Haematological Profile, Testosterone Level, and Oxidative Stress Status in Wistar Rat (*Rattus norvegicus*)

Nasiru Ademola Adeyemi[1]*, Yunusa Adamu Wada[1], Sonnie Joshua Oniye[2], Muhammad Hussaini[3] and Abubakar Dundere Muhammad[4]

[1]Department of Zoology, Ahmadu Bello University, Zaria, P.M.B 06, Kaduna state, Nigeria
[2]Department of Biological Sciences, National Open University, Abuja P.M.B 1514, FCT, Nigeria
[3]Department of Biology, Ahmadu Bello University, Zaria, P.M.B 06, Kaduna state, Nigeria
[4]Department of Biotechnology, Nigerian Defense Academy, Kaduna, P.M.B 2109, Kaduna, Nigeria

Corresponding Author: adeyeminasiru03@gmail.com

## ABSTRACT

*Trypanosoma brucei brucei* is associated with a number of disease conditions in cattle which has caused a huge loss to the agricultural sector. In this study, male Wistar rats were experimentally infected with *T. b. brucei* to observe changes in haematological profiles, serum testosterone level, and oxidative stress in the testicles. Group I was not infected, group II and III were infected intraperitoneally with 0.75 x$10^6$ and 1.0 x $10^6$ doses of *T. b. brucei* respectively. Mean PCV, RBC, Hb, and MCV of group II and III decreased progressively, culminating in anaemia when compared with the control group. However, there was a significant ($p$<0.05) increase in total leucocyte count of group II and III. The testosterone level evaluated in serum using Enzyme-Linked Immunosorbent Assay showed a significant decrease from group II to III. Still, the control group had the highest level of serum testosterone. Biomarkers of oxidative stress, such as Malondialdehyde (MDA), significantly increased in the infected groups. At the same time, reduced Glutathione (GSH), Catalase (CAT) and Superoxide dismutase (SOD) activities were statistically reduced in the testicles of experimental animals. The result suggests that oxidative stress in testicles may be related to the pathology in the testes of rats infected with the parasite.

**Keywords**: Oxidative stress, Trypanosomosis, Haematology, Pathology, Testosterone

## INTRODUCTION

Trypanosoma *brucei brucei* is a protozoan parasite and one of the causative agents of Animal African Trypanosomosis (Ponte-Sucre, 2016). Other parasites of the same genus responsible for similar disease conditions are *Trypanosoma congolense* and *Trypanosoma vivax* (Spickler, 2018). *Trypanosoma b. brucei* is the major cause of African trypanosomosis in several countries in sub-Saharan Africa, and these countries are marked as poorly developed countries in the world. The parasite infects large ruminants such as cattle and game animals, causing a form of trypanosomosis called 'Nagana', and it hinders agriculture and economic progress thereby contributing to the extreme poverty observed in affected areas (Odediran, 2018).

The clinical symptoms that occur in animal trypanosomiasis are abnormal haematological profile, emaciation, intermittent fever, edema, neurosis, abortion, decreased fertility, genital disorders, and death (Desquesnes *et al*., 2022). Infection by the parasite causes reproductive issues in male animals, and the associated infertility is linked to the local inflammatory response from immune cells such as macrophages and T-lymphocytes in the lumen of the epididymal ducts of the testis (Carvalho *et al*., 2018).

DOI: 10.56892/bima.v8i1.587

Oxidative stress refers to the imbalance between free radicals and antioxidants present within host tissues (Dabo *et al*., 2019). This condition has been implicated in several mechanisms of pathogenesis of reproductive dysfunctions in humans, other than in animal or livestock models (Ribas-Maynou and Yeste, 2020). It has long been known that reactive oxygen and nitrogen species are the major culprits behind oxidative stress in response to parasitic infections that also affect the host (Dabo *et al*., 2019). The aim of this study was to determine the effect of different dose of *T. b. brucei* parasite on body weight and rectal temperature, parasitaemia, haematological profile, serum testosterone level and hepatic biomarkers of oxidative stress in experimental Wistar rat.

## MATERIALS AND METHODS

### Ethical Statement and Laboratory Animal Maintenance

The experimental methodology and animal care were done per the guidelines of animal welfare committee, Ahmadu Bello University, Zaria, Kaduna State. Male Wistar rats were sourced from the Department of Human Anatomy, Faculty of Human Medicine, Ahmadu Bello University Zaria. The animals were housed in a neat iron cage and adequately fed at all time with molded chicken grower's marsh.

### *In vivo* Experimental design

The Nigerian Institute of Trypanosomiasis Research (NITR), Kaduna, Nigeria provided a Wistar rat with *T. b. brucei* that served as the parasite donor. Fifteen (15) male Wistar rats were laid out in a complete randomized design (CRD) of five rats each. Group 1 was the control group and was uninfected, while Group II and III (treatment groups) were inoculated with 2ml of blood from donor rat and normal saline containing 0.75 x $10^6$ and 1.0 x $10^6$ doses of *T. b. brucei,* respectively. Rapid matching technique of Herbert and Lumsden (1976) was used to estimate the inoculum.

### Assessment of Clinical Signs

The clinical symptoms evaluated in this study were body weight and rectal temperature and they were assessed every two days using a weighing scale and clinical digital thermometer respectively.

### Evaluation of Parasitaemia

Parasite count was estimated using the "Rapid Matching" as described by Herbert and Lumsden (1976). The haemoflagellates parasites was counted per microscopic field and compared with the logarithmic values of the respective counts on a reference table. The values were converted to antilog to provide absolute number of trypanosomes per ml of blood.

### Assessment of Haematological Parameters

The packed cell volume was determined every other day from the second-day post-infection to the end of the experiment using the method of Coles (1986). Haemoglobin (g/dl) was estimated by calculation and its value approximates to one-third (1/3) of the PCV value (Coles, 1986). The Huxtable (1990) approach was utilized to ascertain the overall Red Blood Cell (RBC) and White Blood Cell (WBC) count. Other absolute values of haematological parameters such as Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated using these formulars:

MCV ($um^3$) = PCV value (%) x 10/ red blood cell count (cells/$mm^3$),

MCH (Pg/cell) = Estimated haemoglobin concentration (g/100mL) x 10/ red blood cell count (cells/$mm^3$),

MCHC (g/100mL) = Estimated haemoglobin concentration (g/100mL) x 100/ PCV value (%).

DOI: 10.56892/bima.v8i1.587

### Evaluation of Testosterone hormone

The Wistar rats were anaesthetized using chloroform at the end of the experiment and blood was collected from the heart into plain bottles and centrifuged at 1000rpm for 15minutes. The testosterone level was determined in the serum using AccuBind ELISA microwell assay kit.

### Evaluation of Lipid Peroxidation and Bioactivity of Antioxidants

The testes of experimental Wistar rats preserved in formalin were rinsed properly with distilled water and 5g of tissue was weighed and later squashed with mortar and pestle after which the tissue was homogenized in cold phosphate buffer and centrifuged at 300rpm for 10 minutes. The resulting supernatant was dispensed into plain bottles and stored at -4$^{O}$C prior to analysis. The liver homogenate was used to determine the bioactivity catalase (Abei, 1974), superoxide dismutase (Fridovich, 1989), reduced glutathione (Ellman, 1959) and the extent of lipid peroxidation through the estimation of the level of malondialdehyde (Akanji *et al*., 2009).

### Data Analysis

The statistical difference in the serum testosterone, haematological parameter, rectal temperature, weight, and oxidative stress markers in the *in vivo* test was determine using One-way Analysis of Variance (ANOVA). Least Significant Difference (LSD) was used to separate the means where significant. Data was represented in tables, bar chart and line graph. Statistical Analysis Software package (version 9.3) for windows was used for the analysis while significance was set at $p<0.05$.

## RESULTS

### Clinical Observations

The mean body weight of experimental Wistar rats is shown in Fig 1. For Day 0 post-infection, the body weight of the randomly assigned Wistar rats in control group was significantly ($p<0.05$) lower than group 2 and 3. The mean body weight of animals in the infected groups kept reducing but was still significantly ($p<0.05$) higher than the control up till day 3 post-infection. The mean body weight of the infected groups was reduced significantly ($p<0.05$) than the control group on day 5 and 6 post-infection. The rectal temperature of experimental animal is presented in Fig 2. There was a marked significant ($p<0.05$) increase in mean rectal temperature of infected groups compared to the control group from day 5 to day 11 post-infection.

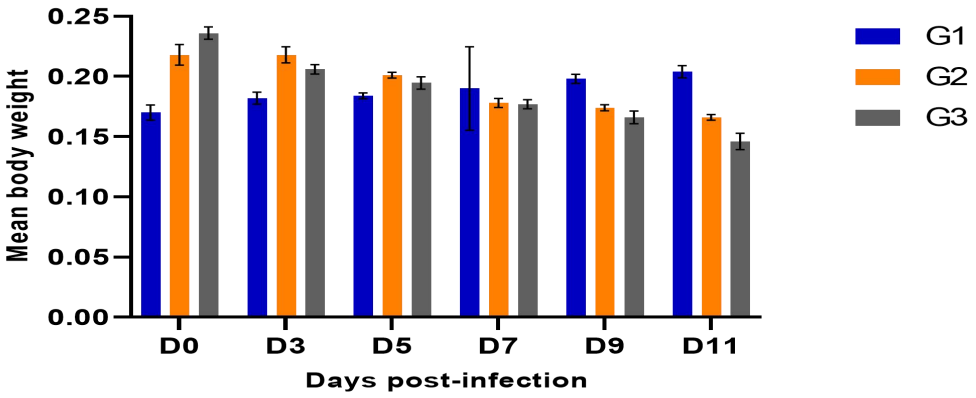


**Figure 1:** Mean body weight of experimental Wistar rats

**NOTE:** Data are expressed as mean ± SEM, n=5

Keys: G1(Control group), G2 and G3 are groups infected with 0.75 x $10^6$ and 1.0 x $10^6$ dose of the parasite respectively.

Bima Journal of Science and Technology, Vol. 8(1A) Mar, 2024 ISSN: 2536-6041

DOI: 10.56892/bima.v8i1.587

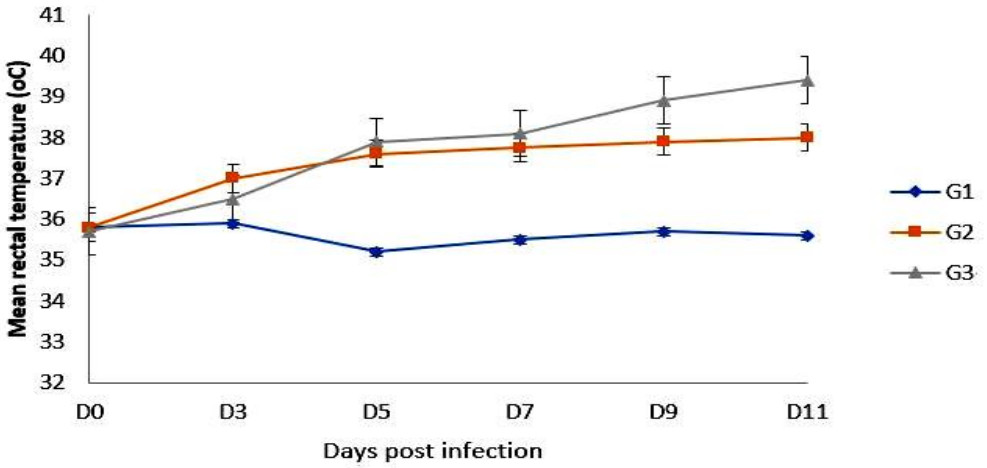


**Figure 2:** Mean rectal temperature of experimental Wistar rats

**NOTE:** Data are expressed as mean ± SEM, n=5

Keys: G1(Control group), G2 and G3 are groups infected with 0.75 x $10^6$ and 1.0 x $10^6$ dose of the parasite respectively.

**Parasitaemia in Infected Wistar Rats**

The parasites were first observed in the peripheral circulation using wet mount by day 3 post infection (p.i.) in the infected Wistar rat with a low parasitaemic score. The number of the circulating flagellates increased progressively over time up to day 11 post-infection, when the parasitemia was the highest within rats in group three (Fig 3).

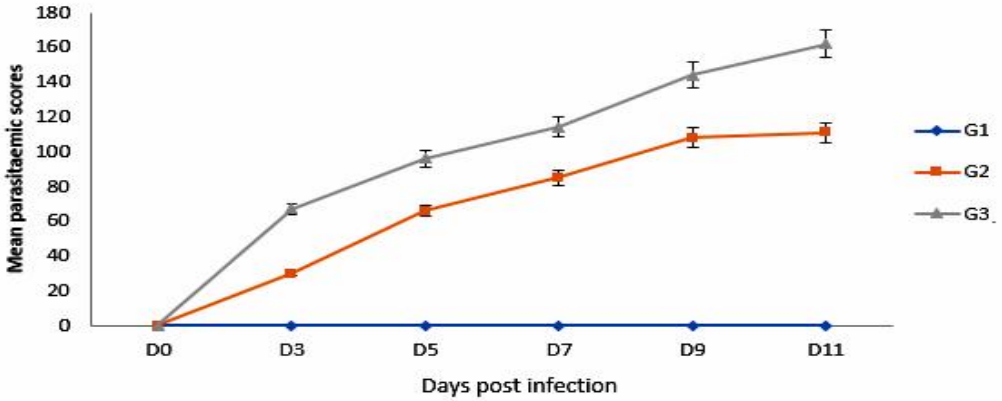


**Figure 3:** Mean parasitaemic scores of experimental Wistar rats

**NOTE:** Data are expressed as mean ± SEM, n=5

Keys: G1(Control group), G2 and G3 are groups infected with 0.75 x $10^6$ and 1.0 x $10^6$ dose of the parasite respectively

**Haematological Profile of Infected Wistar rats**

The result of the haematological parameters shown in Table 1 reveals that group II and III had a decrease in the level of PCV, RBC, HB, MCV at the end of the experiment when compared with the control group (group I). There was observed increase in the level of WBC in group II and III when compared with the control group (Table 1). However, statistics suggest no significant difference in the treatment groups' haematological parameters except WBC at $p<0.05$.

Bima Journal of Science and Technology, Vol. 8(1A) Mar, 2024 ISSN: 2536-6041

DOI: 10.56892/bima.v8i1.587

**Table 1:** The haematological parameters of infected and control Wistar rats

| Parameters | G1 | G2 | G3 | *p*-value |
|---|---|---|---|---|
| PCV (%) | 44±0.87[a] | 39.5±4.29[b] | 35.25±5.39[c] | 0.042 |
| HB (g/100mL) | 14.75±0.41[a] | 13.17±1.43[b] | 11.75±1.80[c] | 0.031 |
| WBC (cells/mm$^3$) | 9.59±0.13[b] | 10.83±0.22a | 10.92±0.39[a] | 0.012 |
| RBC (cells/mm$^3$) | 4.82±0.02[a] | 4.60±0.09[ab] | 4.32±0.20[b] | 0.045 |
| MCV (um$^3$) | 10.54±1.53[a] | 8.54±0.87[b] | 8.05±0.89[b] | 0.31 |
| MCH (Pg/cell) | 27.46±2.85[a] | 28.68±2.87[a] | 26.81±2.95[a] | 0.89 |
| MCHC (g/100mL) | 33.35±0.02[a] | 33.33±0.004[a] | 33.33±0.005[a] | 0.54 |

**NOTE:** Data are expressed as mean ± SEM, n=5

Keys: G1(Control group), G2 and G3 are groups infected with 0.75 x $10^6$ and 1.0 x $10^6$ dose of the parasite respectively, PCV=Packed Cell Volume, MCH=Mean Corpuscular Haemoglobin, HB=Haemoglobin Concentration, MCHC= Mean Corpuscular Haemoglobin Concentration, WBC=White Blood Cell Count, RBC=Red blood Cell count, MCV=Mean Cell Volume, means with different superscript within a row are statistically significant at $p<0.05$.

### Serum Testosterone in Infected Wistar rats

The serum testosterone concentrations of groups II and III decreased significantly ($p<0.05$) at the end of the experiment when compared with the control group I. The animals in group III had the lowest level of serum testosterone (Fig 4).

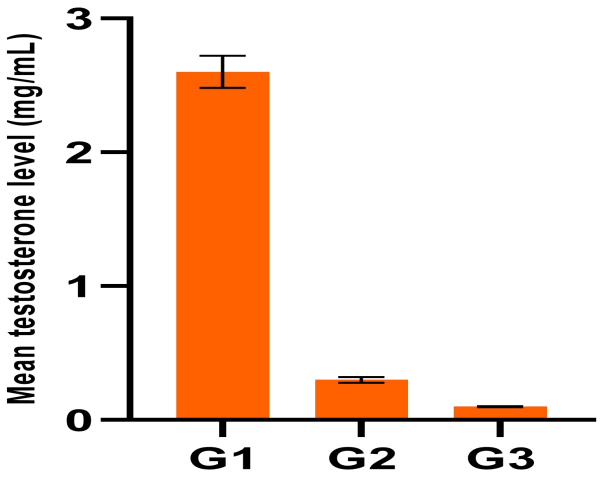


**Figure 4:** Serum testosterone concentration of experimental animals

**NOTE:** Data are expressed as mean ± SEM, n=5

Keys: G1(Control group), G2 and G3 are groups infected with 0.75 x $10^6$ and 1.0 x $10^6$ dose of the parasite respectively.

### Biomarkers of Oxidative Stress in Infected Wistar rats

Lipid peroxidation was greater in reproductive tissue of group II (85.5±1.574nmols/mg protein) and III (104.5±2.89nmoles/mg protein) as evidenced by their elevated MDA levels in comparison to the control group (74.7±2.899nmols/mg protein) that showed a low level of MDA value (Fig. 4.5). Significantly lower level ($p<0.05$) was observed in the archive of SOD, Catalase, reduced Glutathione in the testicles of group II and III when compared with the control group (Fig 5).

Bima Journal of Science and Technology, Vol. 8(1A) Mar, 2024 ISSN: 2536-6041

DOI: 10.56892/bima.v8i1.587

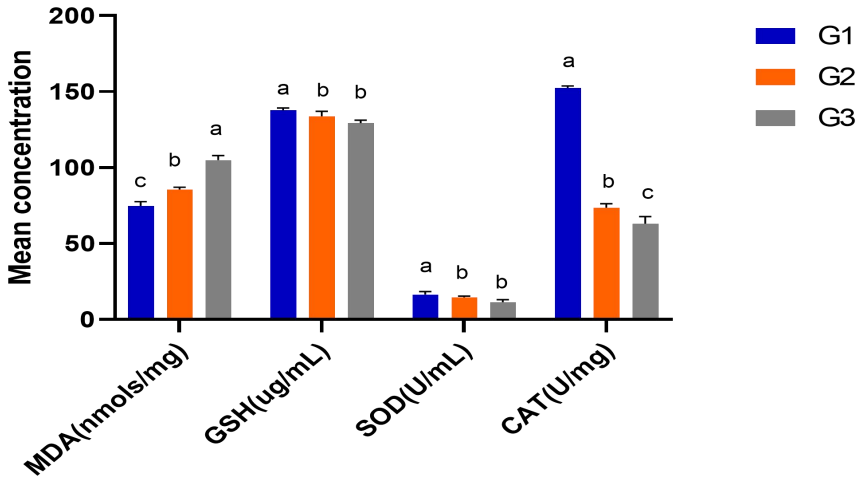


**Figure 5:** Mean concentrations of malondialdehyde, reduced glutathione, superoxide dismutase and catalase in testes of experimental rats

**NOTE:** Data are expressed as mean ± SEM, n=5

Keys: statistical analysis performed amongst G1(Control group), G2 and G3 are groups infected with 0.75 x $10^6$ and 1.0 x $10^6$ dose of the parasite respectively, groups with different letters on bars are statistically significant at $p<0.05$.

## DISCUSSION

This experimental study observed a decrease in body weight in Wistar rats infected with *T. b. brucei* and the reduced body weight may be due to anorexia caused by the interference in energy either by the parasite or Tumor Necrosis Factor-alpha (TNF-α) released by the immune system (Fidelis Junior *et al.*, 2016). A similar symptom occurred in guinea fowl and rabbit experimentally infected with the same parasite (Oyeyemi and Adeyemo, 2022). The observed decrease in rectal temperature of the infected rats upon inoculation is consistent with *T. brucei* parasite in sheep (Wada *et al.*, 2016). The release of pro-inflammatory cytokines caused by the activities of the hemoflagellate in the blood circulation and body tissues is suggested to be related to the increased temperature observed (Fidelis Junior *et al.*, 2016).

The pre-patent period in this present study was three days in all infected rats, which coincides with that observed by Nwoha and Anene (2016). The parasite showed parasitic waves, which may be associated with the uncontrolled proliferation of the trypanosomes. The characteristic nature of the parasitemia may be influenced by the ability of the haemo-parasites to circumvent the immune system of the host through variable surface antigens (Rufa'i *et al.*, 2021).

This study has demonstrated a decrease in Red Blood Cell, Packed Cell Volume, Haemoglobin, and Mean Corpuscular Volume of the infected animals and may be linked with an increase in lipid peroxidation during the infection without sufficient antioxidant enzymes to prevent the damage. This is in accordance with the findings of Mahmood *et al.* (2014), who studied haematological and biochemical profile of camels naturally infected by *Trypanosoma evansi*. The increase in number of leukocytes may be due to an increase in a subtype of leukocyte called oesinophilia, a common feature of most parasitic diseases often associated with hypersensitivity or inflammatory reactions (Sivajothi *et al.*, 2014).

DOI: 10.56892/bima.v8i1.587

The reduced serum testosterone of the infected groups complies with the findings of Amin *et al*. (2020) on the pathogenesis of *Trypanosoma evansi* in male dromedary bulls. Testosterone is a natural steroidal hormone that is responsible for the development of male sexual attributes (Verhoeven *et al.,* 2010), and trypanosomosis infection impairs the axis of the endocrine system that controls the production of this hormone (Noakes *et al.,* 2009). The mechanism includes testicular oxidation, which leads to a reduction in testosterone production, either due to injury to the Leydig cells or endocrine structures such as the anterior pituitary (Zirkin and Chen, 2000; Turner *et al*., 2005).

Infection with *Trypanosoma b. brucei* in rats generates oxidative stress; evidenced by the increase in the level of MDA and the decrease in the level of antioxidant enzymes, which are biomarkers of oxidative stress in the testicles of infected animals, further indicates that *T. b. brucei* induces oxidative stress in the testes. A similar result was observed in the liver of albino rats infected with *T. brucei* but not treated (Ogunleye *et al*., 2020). The parasite accumulates in large numbers in organs, including the testes (Carvalho *et al*., 2018), which is indicative of the accumulation of MDA in tissue, resulting in oxidative stress and damage (Pawłowska *et al*., 2024).

## CONCLUSION

*Trypanosoma b. brucei* infection causes a decrease in body weight and rectal temperature with an increased level of parasitaemia if left untreated. The infection is also detrimental to the haematological profile in Wistar rat models. *Trypanosoma brucei brucei* infection results in low serum testosterone levels in Wistar rat models, hence an important cause of animal infertility. *Trypanosoma b. brucei* infection induced oxidative stress in the testicles of Wistar rat, as evident in the increased level of malondialdehyde and reduced antioxidant enzyme level.

## REFERENCES

Abei, H.C. (1974). In: Bergmeyer HU, editor. *Methods of enzymatic analysis*. New York: Public Academic Press. pp 673-684.

Akanji, M.A., O.S. Adeyemi, S.O. Oguntoye and F. Sulyman (2009). *Psidium guajava* extract reduces trypanosomiasis associated lipid peroxidation and raises glutathione concentration in infected animals. *Experimental and Clinical Sciences International Journal for Advances in Sciences*, 8: 148-154.

Amin, Y. A., Noseer, E. A., Fouad, S. S., Ali, R. A., & Mahmoud, H. Y. A. H. (2020). Changes of reproductive indices of the testis due to *Trypanosoma evansi* infection in dromedary bulls (Camelus dromedarius): Semen picture, hormonal profile, histopathology, oxidative parameters, and hematobiochemical profile. *Journal of Advanced Veterinary and Animal Research*, 7(3), 537–545.

Carvalho, T., Trindade, S., Pimenta, S., Santos, A. B., Rijo-Ferreira, F., & Figueiredo, L. M. (2018). *Trypanosoma brucei* triggers a marked immune response in male reproductive organs. *PLoS neglected tropical diseases*, 12(8), e0006690. https://doi.org/10.1371/journal.pntd.0006690

Coles, E.H. (1986). *Veterinary Clinical Pathology* (4thEdition) Published by W.B. Sounders Company Philadelphia, London, Toronto, Mexico City Rio de Janeiro, Sydney, Tokyo Hong Kong. 486 pp.

Dabo, N. T., Maigari, A. K., & Zakari, A. (2019). Oxidative stress in trypanosomiasis. In Proceedings of the 3rd *International Conference on Biosciences and Biotechnology* (pp. 1-2). ResearchGate.

DOI: 10.56892/bima.v8i1.587

Desquesnes, M., Gonzatti, M., Sazmand, A., Thévenon, S., Bossard, G., Boulangé, A., Gimonneau, G., Truc, P., Herder, S., Ravel, S., Sereno, D., Jamonneau, Vincent., Jittapalapong, S., Jacquiet, P., Solano, P. and Berthier D. (2020). A review on the diagnosis of animal trypanosomoses. *Parasites and Vectors*, 15:64 https://doi.org/10.1186/s13071-022-05190-1.

Ellman, G.I. (1959). Tissue sulphydryl groups. *Archive of Biochemistry and Biophysics*. 82:70-77

Fidelis Junior, O. L., Sampaio, P. H., Machado, R. Z., André, M. R., Marques, L. C., & Cadioli, F. A. (2016). Evaluation of clinical signs, parasitemia, hematologic and biochemical changes in cattle experimentally infected with *Trypanosoma vivax*. *Brazilian Journal of Veterinary Parasitology*, 25(1), 69-81. http://dx.doi.org/10.1590/S1984-29612016013

Fridovich I. (1972). Superoxide radicals and superoxide dismutase. *Archive of Chemistry Resource.* 5:321

Herbert, W.J. and Lumsden, W.H.R. (1976). *Trypanosoma brucei*: a rapid "matching" method for estimation of the host parasitaemia. *Experimental Parasitology*, 40(3):427-431.

Huxtable R.J. (1990). Activation and pulmonary toxicity of pyrrolizidine alkaloids. *Pharmacological Therapy*, 47(3): 371-89

Mahmood, A, Khosro, G, Mohammed, M.D. (2014). Haematological and serum biochemical aspects associated with a camel (Camelus dromedaries) naturally infected by *Trypanosoma evansi* with severe parasitaemia in Semnan, Iran. *Asian Pacific Journal of Tropical Biomedicine*, 4(9): 743-745.

Noakes, D.E., Parkinson, T.J. and England G.C. (2009) Veterinary reproduction and obstetrics. 9th edn 868 pp. China: Elsevier.

Nwoha, R.I, and Anene, B.M. (2016). Clinical effect of mixed infection of Trypanosomes and Ancyclostoma caninum in Dogs and treatment with Dimiazene and Meendazole. *British Journal of Medicine and medicinal Research*, 11(9): 1-10

Odediran, P.O., Ademola, I.O., Macleod, E.T., Welburn, S.C. (2018). Bovine and small ruminant African animal trypanosomiasis in Nigeria- A review. *Veterinary Parasitology*: Regional Studies and Reports, 13:5-13

Ogunleye, O. O., Jatau, I. D., Natala, A. J., & Ola-Fadunsin, S. D. (2020). Effects of aqueous extract of fruit pulp of *Adansonia digitata* L. on the oxidative stress profile against *Trypanosoma brucei brucei* infection in albino rats. *Clinical Phytoscience*, 6(1), 57. https://doi.org/10.1186/s40816-020-00203-x

Oyeyemi, M. O., and Adeyemo, O. K. (2022). Comparative study of *Trypanosoma brucei brucei* infection in rabbits and guinea fowls. *European Journal of Microbiology and Immunology*, 12(1), 1-6. https://doi.org/10.1556/1886.2021.00033

Pawłowska, M., Mila-Kierzenkowska, C., Szczegielniak, J., & Woźniak, A. (2024). Oxidative Stress in Parasitic Diseases—Reactive Oxygen Species as Mediators of Interactions between the Host and the Parasites. *Antioxidants*, 13(1), 38. https://doi.org/10.3390/antiox13010038

Ponte-Sucre A. (2016). An overview of *Trypanosoma brucei* infections: an intense host-parasite interaction. *Frontiers of Microbiology*, 7:2126. doi: 10.3389/fmicb.2016.02126

Ribas-Maynou, J., and Yeste, M. (2020). Oxidative stress in male infertility: Causes, effects in assisted reproductive techniques, and protective support of

DOI: 10.56892/bima.v8i1.587

antioxidants. *Biology*, 9(4), 77. https://doi.org/10.3390/biology9040077

Rufa'i, F. A., Zakari, A. I., Umar, A., Shuaibu, M., & Sani, A. A. (2021). Clinical Signs and Pathogenesis of Trypanosomal Infection in Human and Animals. *Asian Journal of Pharmaceutical Research and Development*, 9(3), 1-7

Sivajothi, S., Sudhakara, R.B., Nalini-Kumari, K., and Rayulu, V.C. (2014). Haematological changes in *Trypanosoma evansi* infected cattle. *International Journal of Scientific World*, 2(1): 27-30.

Spickler, A. R. (2018). African Animals Trypanosomiasis. Retrieved from http://www.cfsph.iastate.edu/DiseaseInfo/factsheets.php

Turner, T.T., Bang, H.J., Lysiak, J.J. (2005) Experimental testicular torsion: reperfusion blood flow and subsequent testicular venous plasma testosterone concentrations. *Urology*, 65(2):390-394.

Verhoeven, G., Williams, A., Demalet, E., Swinnen, J.V and De Gendt, K. (2010). Androgens and spermatogenesis: lessons from transgenic mouse models. *Biological Science,* 365(1546): 1537-1556.

Zirkin, B.R. and Chen, H. (2000). Regulation of Leydig cell steroidogenic function during aging. *Biology of Reproduction*, 63(4):977-81.