International Journal of TROPICAL DISEASE & Health
13(4): 1-9, 2016, Article no.IJTDH.22862
ISSN: 2278–1005, NLM ID: 101632866

SCIENCEDOMAIN *international*
www.sciencedomain.org

The Comparative Effect of Experimental Trypanasomosis Infection and Pantizen Treatment on the Haematological Profile of Albino Rats

O. S. Chukwu[1] and C. O. Ukwueze[2*]

[1]Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.
[2]Department of Veterinary Surgery and Theriogenology, Michael Okpara University of Agriculture, Umudike, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author OSC designed the study, wrote the protocol, managed the animals, collected all data and wrote the first draft of the manuscript. Author COU performed the statistical analysis, did the literature search and also wrote part of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2016/22862
Editor(s):
(1) Janvier Gasana, Department of Environmental & Occupational Health, Robert Stempel College of Public Health & Social Work, Florida International University, USA.
Reviewers:
(1) Jerry Abenga, Nigerian Institute for Trypanosomiasis Research, Nigeria.
(2) K. W. Nkpaa, University of Port Harcourt, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/13035

Original Research Article

Received 2nd November 2015
Accepted 24th December 2015
Published 20th January 2016

ABSTRACT

Objectives: Experimental infections of *Trypanosoma brucei* and *Trypanosoma congolense* in albino rats was done to compare their haematologic effects and then treated with Pantizen®, a brand of diminazene aceturate to ascertain its therapeutic effect on the heamatological value of the infected rats.
Methods: Six groups (groups A, B, C, D, E, and F) of six albino rats each were used for the study. Group A was uninfected control. Groups B and C were single infections of *T. brucei* and *T. congolense* respectively. Group D rats were infected with *T. brucei* and *T. congolense* the same day. Group E rats infected with *T. brucei* first and *T. congolense* 7 days after. While Group F rats were infected with *T. congolense* first and *T. brucei* 7 days after. The infected rats were treated on day 14 post infection and monitored for 7 days post treatment. Packed cell volume (PCV), haemoglobin concentration (Hb), Red Blood Cell (RBC) counts, total and differential White Blood

**Corresponding author: Email: ukwezecele@yahoo.com;*

Cell counts (WBC), were monitored weekly.
Results: There was significant ($P < 0.05$) decrease in the erythrocyte values (PCV, Hb and RBC counts) and leucocytes values, more in group D followed by groups B and E. This indicates aneamia and immunosupression respectively. However, there was no significant variation ($P > 0.05$) in the PCV, RBC counts and Hb conc**,** total leucocyte, lymphocyte, neutrophil and monocyte counts of the infected animals treated with Pantizen® especially in group E.
Conclusion: It was thus concluded that the therapeutic effect of Pantizen® treatment may reverse the depletion effect of both single and mixed infections on haemo-immune system of the animal taking into consideration the parameter measured.

Keywords: T. brucei and T. congolense; single and mixed infections; PCV; RBC; WBC; anaemia; Pantizen®.

1. INTRODUCTION

Trypanosomosis is a parasitic disease that affects domestic and wild animals, and humans. It is usually fatal if the host is not treated [1]. The trypanosome species affecting man and domestic animals have been classified into two groups: The haematic group (*Trypanosoma congolense* and *Trypanosoma vivax)* which are intravascular organisms and the humoral (tissue invading) group (*T. brucei, T. evansi, T. gambiense and T. rhodesiense*) which dwell both in extra and intravascular spaces [2-4].

Trypanosomosis is an important constraint to livestock and mixed farming in tropical Africa.

The economic impacts of trypanosomosis in Africa are diverse and complex, having direct effects on animal production and human health, as well as indirect effects on settlement patterns, land use, draught power use, animal husbandry and farming [5,6]. Direct losses in meat production and milk yield, loss in traction power and control programs are estimated to cost over 500 million US dollars each year [7,8]. The greatest socio-economic impacts occur in sub-Saharan Africa where a suitable environment exists for the survival of tsetse fly; the vector responsible for cyclical transmission of both human and animal trypanosomosis [9].

Trypanosoma brucei and *T. congolense* are the major species involve in animal trypanosomosis in Eastern Nigeria. Trypanosomosis in domestic animals such as dogs, cats, pigs, bovine, sheep, goat and horses has been reported [10-12]. *Trypanosome* parasites invade blood and produce numerous changes in the cellular and biochemical constituents of blood [13-15]. *Trypanosoma brucei and T. congolense* infection like other trypanosome infections precipitate increased red blood cell destruction which results in anaemia as well as tissue damage [16]. Anaemia is of a major clinical importance in trypanosomosis [17]. It is observed as pale mucous membranes resulting from the decline in the number of red blood cells and packed cell volume and is a primary criterion for assessing the severity of trypanosomosis [18].

Nevertheless, the use of trypanocidal drugs is the most widely adopted and utilized means of controlling trypanosomosis [19]. Trypanocidal drugs such as Isometamidium and Homidium are mainly used as prophylaxis. Treatment of animal trypanosomosis also depends on novidium® (homidium chloride) and berenil® (diminazene aceturate) which are relatively old compound use in veterinary practice pending the emergency of the new method of treatment [20,21].

The present study was carried out to compare the heamatopathology effects of single and mixed infection of *T. brucei* and *T. congolense*, and ascertain the therapeutic effect of Pantizen® (diminazene aceturate) on heamatological parameters.

2. MATERIALS AND METHODS

2.1 Animals

A total of 36 male albino rats weighing between 101 and 291 grams were used for the study. The rats were kept in clean cages and housed in well ventilated fly proof experimental animal house. They were fed with commercial growers ration (Grand Cereals Ltd) and water was supplied *ad libitum.* The rats were allowed to acclimatize for two weeks before the commencement of the study.

2.2 Parasite

Trypanosoma congolense obtained from National Institute for Trypanosomiasis and Onchocerchaciasis Research (NITOR) Vom, Plateau State (isolate from a cow) and *T. brucei*

isolate from a pig in Nsukka, and maintained by animal passage method in the Department of Veterinary Parasitology and Entomology laboratory, University of Nigeria, Nsukka) were used for the study. The trypanosomes were maintained in rats from which the experimental rats were infected.

2.3 Experimental Procedures

Rats were randomly assigned into six groups (A, B, C, D, E and F) of six rats each. Each experimental rat in groups (B, D, C, E and F) was inoculated with 0.2 ml of PBS diluted blood containing 1.0×10^6 trypanosomes intraperitoneally. Group A was uninfected control. Group B rats were infected with *T. brucei* only. Group C rats were infected with *T. congolense* only. Group D rats were infected with *T. brucei* and *T. congolense* the same day. Group E rats infected with *T. brucei* first and *T. congolense* 7 days after. While Group F rats were infected with *T. congolense* first and *T. brucei* 7 days after.

2.4 Drug and Drug Administration

Diminazene aceturate (Pantizen®, **Pantex B.V Holland**) was used in the experiment. The stock solution used was prepared immediately before administration by dissolving 2.36 g of the granule in 12.5 ml of sterile distilled water and stirring vigorously to achieve complete dissolution. The resultant clear solution was dark orange. A 100-fold dilution of the stock solution was prepared and administered at the dose rate of 0.1 ml drug solution per 10 g body weight (equivalent to a dosage of 7 mg/kg body weight). All the infected groups were treated on day 14 post infection.

2.5 Blood Collection

The orbital bleeding technique was used to collect blood for haematology. About 0.5 ml of blood was collected from the medial cantus of the eye of each rat into sample bottles containing EDTA. The blood was used to determine the Red Blood Cell (RBC) counts, Total White Blood Cell (TWBC) and Differential White Blood Cell (DWBC) counts described by Schalm et al. [22]. Packed cell volume (PCV) was done as described by Coles, [23], while heamoglobin concentration (Hb) was done according to Kachmar [24].

2.6 Data Analysis

The results of the study were analyzed using one way Analysis of Variance (ANOVA) and variant means separated using Duncan's multiple range Test. Probability values less than or equal to 0.05 ($P \leq 0.05$) were considered significant.

3. RESULTS

The mean pre-patent periods (PPP) of the infections were 4.33±0.21, 9.50±0.22, 2.00±0.00, 4.33±0.21 and 8.50±0.22 days for groups A, B, C, D and E, respectively.

3.1 Packed Cell Volume (PCV)

There was significant ($P < 0.05$) decrease in PCV in groups B, and D at day 7 post infection when compared to the control (group A). Also, the PCV of all the infected groups were significant ($P < 0.05$) decreased by day 14 PI when compared to the control. Group D was mostly affected followed by groups B and F. However, no significant ($P > 0.05$) variation was recorded in all groups post treatment (day 21) when compared to the control. Post treatment response was high in Groups B and D when compared to other groups (Fig. 1).

3.2 Red Blood Cell (RBC) Counts

The result of the mean RBC counts of rats showed significant ($P < 0.05$) decrease in groups B, D and E by day 7 post infection when compared to the control (group A). Also, the mean RBC counts of all the infected groups were significant ($P < 0.05$) decreased by day 14 PI with group D mostly affected followed by groups B, E and F. However, no significant ($P > 0.05$) variation was recorded in all groups at day 7 following treatment when compared to the control (Fig. 2).

3.3 Haemoglobin Concentration

There was significant ($P < 0.05$) reduction was in the mean haemoglobin concentration of all the infected groups by day 14 PI. The decrease was significantly ($P > 0.05$) high in group D when compared to other groups. However, no significant ($P > 0.05$) variation in the mean Hb was recorded in all the groups by 7 day after treatment (day 21) when compared to the control (Fig. 3).

3.4 Total White Blood Cell (TWBC) Counts

There was significant ($P < 0.05$) reduction by day 7 PI in the TWBC counts of rats in groups B, D and E. Trypanosome infections led to a further

significant decline in the TWBC in groups B, D and most especially group E by day 14 PI.

However, groups C and F rats showed remarkable increase in TWBC by day 14 PI when compared to the control. The total white blood cell counts obtained 7 days post trypanocidal treatment in all the infected groups showed no significant ($P > 0.05$) variation when compared to the control (Fig. 4).

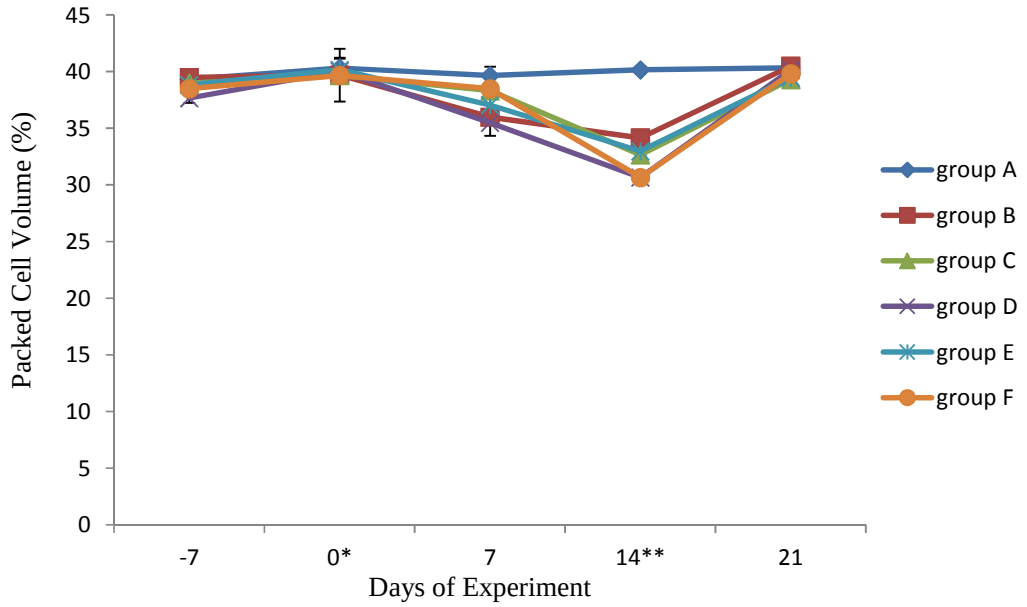

Fig. 1. Mean (±SEM) packed cell volume (%) of single and mixed infections of *T. brucei* and *T. congolense* infected rats treated with Pantizen®

**Day of infection. **Day of treatment*

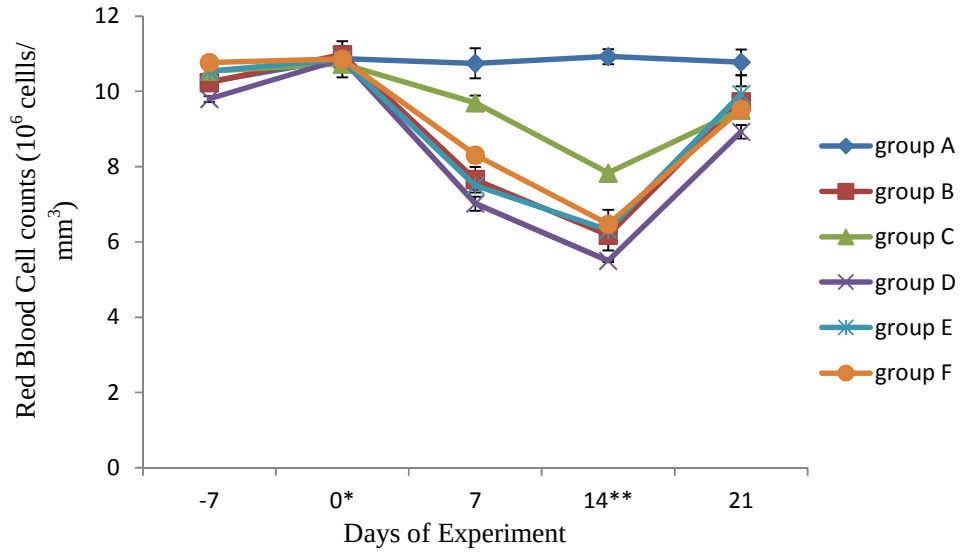

Fig. 2. Mean (±SEM) red blood cell counts of (10^6 cellls/ mm^3)) single and mixed infections of *T. brucei* and *T. congolense* infected rats treated with Pantizen®

**Day of infection. **Day of first treatment*

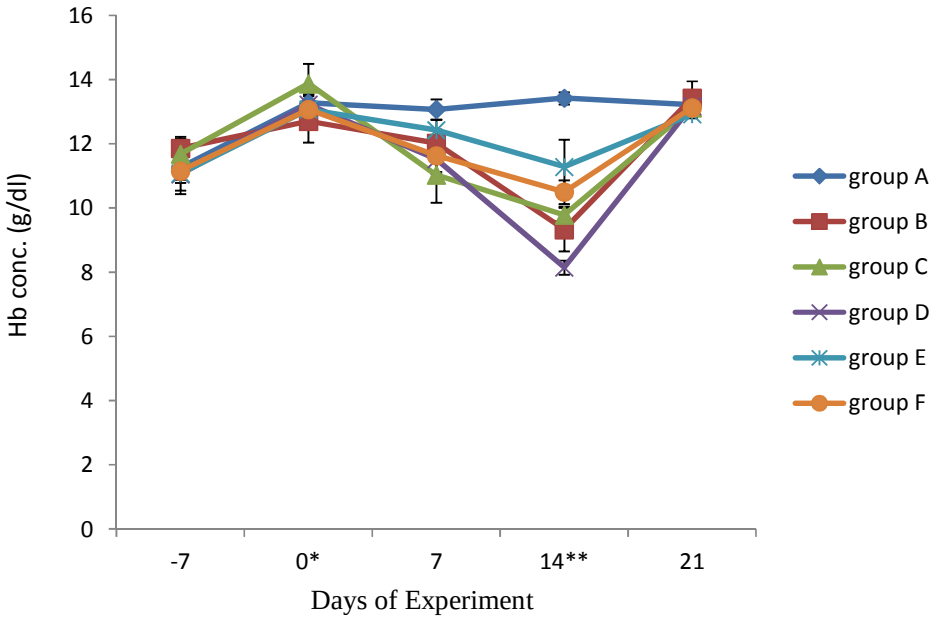

Fig. 3. Mean (±SEM) haemoglobin concentration (g/dl) of single and mixed infections of *T. brucei* and *T. congolense* infected rats treated with Pantizen®

**Day of infection. **Day of treatment*

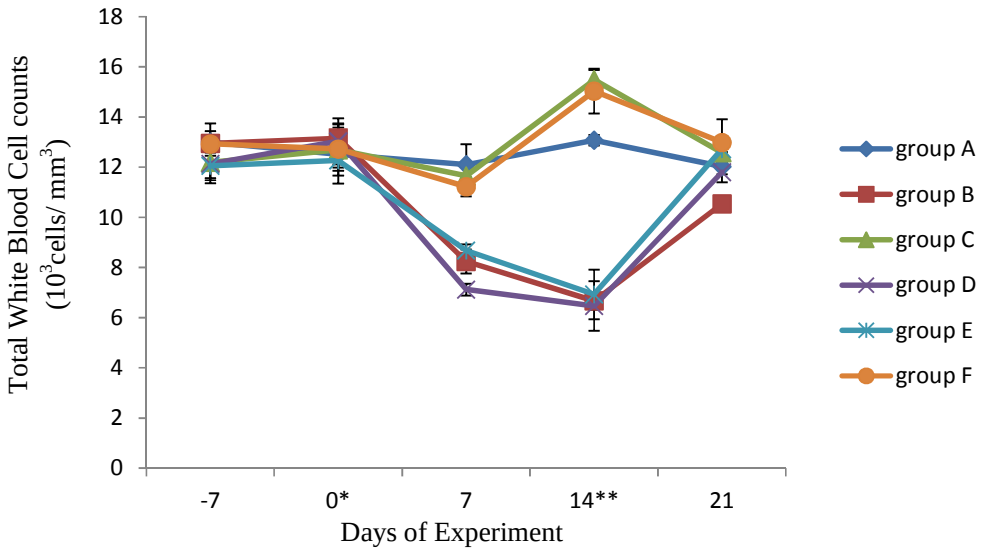

Fig. 4. Mean (±SEM) Total White Blood Cell counts (10^3cellls/ mm^3) of rats infected with single and mixed infections of *T. brucei* and *T .congolense* and treated with Pantizen®

** Day of infection. **Day of first treatment*

3.5 Lymphocyte Counts

The mean lymphocyte counts of the rats in groups B and E were significantly ($P < 0.05$) low but significantly ($P < 0.05$) high in groups C and F by day 14 PI when compared to the control. However, there was no significant variation in the mean lymphocyte counts in groups B, D and E post treatment (by day 21) while the values remain significantly ($P < 0.05$) high in groups C and F when compared to the control (Table 1).

Table 1. Mean (±SEM) differential white blood cell counts (10^3cells/ mm^3) of rats infected with single and mixed infections of *T. brucei* and *T. congolense* and treated with Pantizen®

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
Lymphocyte counts						
-7	6.75±0.55	6.21±0.35	6.74±0.61	6.33±0.44	6.93±0.69	6.47±0.28
*0	7.19±1.48	7.29±0.47	6.65±0.78	7.08±1.50	6.65±1.63	6.67±1.16
7	7.50±1.37	5.75±0.36	6.49±0.36	6.96±0.19	5.41±0.19	7.97±0.30
**14	6.84±0.14	4.22±0.30[a]	11.56±0.73[b]	5.28±0.73	4.37±0.33[a]	9.79±0.50[b]
21	7.74±0.25	7.31±0.17	9.19±0.83[a]	6.94±0.30	7.38±0.33	9.21±0.62[a]
Neutrophil counts						
- 7	2.21±0.17	2.20±2.81	2.34±0.19	2.00±0.13	2.27±0.22	1.99±0.07
*0	3.23±0.03	2.59±0.13	2.50±0.27	3.19±0.18	3.32±0.25	2.79±0.23
7	3.21±0.19	2.25±0.13[a]	2.67±0.12[b]	2.79±0.06[b]	2.09±0.08[a]	2.75±0.17[b]
**14	2.78±0.11	1.82±0.08[a]	3.12±0.18	2.03±0.22[a]	1.67±0.14[a]	2.74±0.16
21	2.85±0.09	2.90±0.07	3.06±0.22	2.59±0.10[a]	2.89±0.15	3.51±0.28[b]
Monocyte counts						
-7	0.15±0.02	0.14±0.04	0.14±0.03	0.12±0.02	0.16±0.07	0.14±0.03
*0	0.25±0.08	0.22±0.06	0.26±0.02	0.20±0.05	0.14±0.00	0.26±0.08
7	0.24±0.03	0.11±0.02	0.29±0.11	0.38±0.14	0.25±0.11	0.23±0.05
**14	0.11±0.00	0.10±0.02	0.41±0.10	0.12±0.03	0.11±0.03	0.36±0.10
21	0.16±0.04	0.16±0.02	0.24±0.02[a]	0.15±0.02	0.17±0.05	0.26±0.07[a]
Eosinophil counts						
-7	0.09±0.02	0.07±0.00	0.07±0.00	0.08±0.00	0.09±0.03	0.00±0.00
*0	0.12±0.01	0.15±0.04	0.11±0.003	0.13±0.01	0.14±0.00	0.12±0.01
7	0.06±0.06	0.08±0.009	0.16±0.07	0.20±0.07	0.06±0.00	0.11±0.00
**14	0.11±0.00	0.00±0.00	0.00±0.00	0.10±0.00	0.06±0.00	0.22±0.09
21	0.10±0.00	0.20±0.006	0.20±0.05	0.10±0.00	0.17±0.00	0.12±0.02

[a b]Figures with different superscripts within rows are significantly different (P ≤ 0.05)
*[*] Day of infection. **Day of first treatment*

3.6 Neutrophil Counts

The trypanosomal infection caused significant (P < 0.05) decrease in the neutrophil counts in all the infected groups at days 7 PI and in groups B, D and E at day 14 PI especially in group E. However, there was significant increase in neutrophil counts in group F following treatment when compared to the control (Table 1).

3.7 Monocyte Counts

There was no significant (P > 0.05) variation in mean monocyte counts in all groups post infection. Following treatment, mean monocyte counts significantly (P < 0.05) increased in groups C and F at day 21 (Table 1).

3.8 Eosinophil Counts

There was no significant (P > 0.05) variation in the mean eosinophil counts of all the groups post infection and post treatment.

4. DISCUSSION

The results of the haematological parameters (PCV, Hb and RBC) showed that all the infected groups had anaemia which was revealed by significant (P < 0.05) decrease in the values of each of the parameters especially prior to treatment. The cause of anaemia in trypanosomosis has been described as multifactorial including erythrocyte destruction by phagocytosis [25,26], disordered erythropoiesis [27] and increased plasma volume and haemodilution [28,29]. The most severe effect observed in group D (infected with with *T. brucei* and *T. congolense* the same day) shows that infection with *T. brucei* and *T. congolense* the same day caused more haematologic insult than that of other groups. However, treatment with Pantizen® reversed them to almost the pre-infection values. This shows that Pantizen® may be effective for the treatment of single and mixed infections of trypanosomosis.

The initial increase in total leucocyte (TWBC) counts in the group C (rats infected with *T. congolense* only) and group F (rats infected with *T. congolense* first and *T. brucei* after 7 days) prior to treatment may be due to the stimulation of the immune system by the trypanosomes leading to increased production of these cells in circulation [30,31]. The leucopaenia

seen in groups B (infected with *T. brucei* only), D (infected with *T. brucei* and *T. congolense* same day) and E (infected with *T. brucei* first and *T. congolense* after 7 days) prior to treatment may be attributed to ineffective or depressed granulopoiesis in the bone marrow [32]. However, Eze et al. [33] reported leukocytosis in *T. brucei* infected rats.

This study revealed that lymphocyte counts increased significantly following infection with *T. congolense* only (group C) and group F (*T. congolense* first day and *T. brucei* after 7 days). However, lymphocyte counts decreased significantly following infection with *T. brucei* only. The pattern of infection contributes a lot in the immuno-haematological effect of the parasite.

The neutropaenia present in groups B, C, D and E may have resulted from depression of bone marrow granulocyte precussors by *Trypanosoma* toxins or massive elimination of neutrophils when they engulf trypanosomes.

Treatment with Pantizen® may have some corrective effect on the immune system of the infected rats as evidenced in the result of this study.

5. CONCLUSION

The infections were characterized by significant ($P < 0.05$) decrease in erythrocytic values and leucopaenia. However, Pantizen® significantly returned the values to near normal. The pre treatment haematological changes were highest in group D followed by groups B and E but the post treatment response was highest in group E. It was thus concluded that both single and mixed infections may cause anemia and leucopaenia in animal and Pantizen® treatment may reverse the anemic condition and correct the immune system taking into consideration the parameter measured.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ohaeri CC. Histological changes in organs of rats with *Trypansoma congolense.* Res J. Anim Sci. 2010;4(1):50-52.
2. Losos GJ, Ikede BO. Review of pathology of the diseases in domestic and laboratory caused by *T. congolense T. brucei, T. rhodesiense and T. Gambiense.* Vet. Pathol. Supplemention ad. 1972;9:1-71.
3. Abubakar A, Iliyasu B, Yusuf AB, Igweh AC, Onyekwelu NA, Shamaki BiA, Afolayan DO, Ogbadoyi EO. Antitrypanosomal and haematological effects of selected Nigerian medicinal plants in wistar rats. Biokemistri. 2005;17: 95-99.
4. Ngure RM, Ndungu JM, Ngotho JM, Nancy MK, Maathai RG, Gateri LM. Biochemical changes in the plasma of ververt monkeys (*Chlorocebus aethiops*) experimentally infected with *Trypanosoma brucei rhodesiense.* J. Cell Animal Biol. 2008;2: 150-157.
5. Swallow BM. Impacts of trypanosomosis on African agriculture. Programme against African Trypanosomiasis (PAAT), Technical series, No. 2, FAO, Rome, Italy. 2000;52.
6. Bourn D, Grant I, Shaw A, Torr S. Cheap and safe tsetse control for livestock production and mixed farming in Africa. Aspects App. Biol. 2005;75:1-2.
7. ILRI. Livestock, people and the environment. International Livestock Research Institute (ILRI), Nairobi Kenya; 1997.
8. Kamunaga M. Socio – economic and cultural factors in the research and control of trypanosomiasis, pp. 1 – 10, Information division FAO, Rome; 2003.
9. Kristjanson P, Rowlands J, Swallow B, Kruska R, Deleeuw P, Nogda S. Using the Economic surplus model to measure potential returns to international livestock research the case of trypanosomosis vaccine Research. International livestock Research Institute (ILRI), Nairobi, Kenya. 1999;36.

10. Omamegbe JO, Orajaka LJE, Emehelu CO. The incidence and clinical forms of naturally occurring canine trypanosomiasis in two veterinary clinics in Anambra State of Nigeria. Bull. Anim. Health Prod. Afri. 1984;32:23-29.
11. Urquhart GM, Armour J, Dunn JL, Jennings FW. Veterinary parasitology. Blackwell science limited; 1998.
12. Hoare CA. Trypanosomes of mammals. A zoological monograph. Blackwell Scientific Publications Oxford; 1972.
13. Igbokwe IO, Mohammed A. Some plasma biochemical changes in experimental *Trypanosoma brucei* infection in Sokoto red goats. Rev Elev Med Pays Trop. 1992;45(3-40):287-90.
14. Taiwo VO, Olaniyi MO, Ogunsanmi AO. Comparative plasma biochemical changes and susceptibility of erythrocytes to in vitro peroxidation during experimental *Trypanosome congolense* and *T. brucei* infections in sheep. Israel J Vet Med. 2003;58(4).
15. Sulaiman FA, Adeyemi OS. Changes in haematological indices and protein concentrations in *Trypanosoma brucei* infected rats treated with Homidium chloride and Diminazene aceturate. EXCLI Journal. 2010;9:39–45.
16. Ekanem JT, Yusuf OK. Some biochemical and haematological effects of black seed (*Nigella sativa*) oil on *T. brucei*-infected rats. Afr J Biomed Res. 2008;11:79–85.
17. Jenkins GC, Facer CA. Haematology of African Trypanosomiasis. In: Immunology and pathogenesis of Trypanosomiasis (Ed. I. Tizard). Boca Raton, GRC Press. 1985; 13-44.
18. Murray M, Dexter TM. Anaemia in bovine African tryponosomiasis: A review. Acta Tropica. 1988;45:389-432.
19. Feldmann U, Heridrichs J. Integrating the sterile insect technique as a key component of area wide tsetse and trypanosomiasis intervention. PAAT Technical and scientific series, 3, Food and Agricultural organization, Rome. 2001; 1-66.
20. Geerts S, Holmes PH. Drug management and parasite resistance in Bovine trypanosomiasis in African PAAT technical and scientific series, no 1 FAO, Rome. 1998;22.
21. Adamu S, Barde N, Abenga JN, Useh NM, Ibrahim NDG, Esievo KAN. Experimental *Trypanosoma brucei* infection induced changes in the serum profiles of lipids and cholesterol and the clinical implications in pigs. J. Cell Animal Biol. 2009; 3(2):15-20.
22. Schalm OW, Jain NC, Carroll EJ. Veterinary haematology 3rd Ed. Lea and Febiger, Philadephia. 1975;19-25.
23. Coles EH. Determination of packed cell volume in Coles EH Ed, Veterinary Clinical Pathology, W.B Saunder & Co; Philadelphia. 1986;17-19.
24. Kachmar JE. Determination of blood haemoglobin by cyanomethaemoglobin procedure in Tictz NW, Ed, fundamentals of clinical chemistry, W.B sanders company, Philadelphia. 1970;268-269.
25. Walia PS, Kalra IS, Juyal PD, Sood N, Ahuja SP. Role of silidase activity of *Trypanosoma evansi* in inducing anaemia and immunomodulation in buffalo calves (*Bubalis bubalis*). J. Vet. Parasitol. 1996; 10:1–9.
26. Taylor K, Authie EML. Pathogenesis of animal trypanosomosis. In: Maudlin I, Holmes PH, Miles MA, (eds). The Trypanosomiases. CAB International, UK. 2004;331-353.
27. Andrianarivo AG, Muiya P, Opollo M, Logan-Henfrey LL. *Trypanosoma congolense*: Comparative effects of a primary infection on bone marrow progenitor cells from N'dama and Boran cattle. Exp. Parasitol. 1995;80:407–418.
28. Jennings FW. The anaemias of parasitic infections. In: Pathophysiology of parasitic infections, (ed.) Soulsby EJL. New York Academic Press. 1976;41–67.
29. Dwinger RH, Murray M, Moloo SK. Parasite kinetics and cellular responses in goats infected and superinfected with *Trypanosoma congolense* transmitted by *Glossina morstans centralis*. Acta Tropica. 1990;47:23-33.
30. Ndoutamia G, Mbakesse RN, Brahim A, Khadidja A. Influence of *T. congolense* infection on some haematological and serum biochemical parameters in sahelian goats. Revue de Medicine Veterinaire. 2002;153(6):395-400.
31. Anosa VO, Logan-Henfrey LL, Wells CW. The haematology of *T. congolense* infected cattle 11: Macrophages structure and function in adult Boran catlle. Comp. Haematology Internatl. 1997;7:23–29.
32. Eze JI, Anene BM, Chukwu CC. Effect of selenium supplementation on the efficacy of Diminazene aceturate or Isometamidium

chloride in chemotherapy of *Trypanosoma brucei* infected rats. Bulletin of Animal Health and Production African. 2009;57: 97–107.

33. Ezeokonkwo RC, Agu WE. Experimental infection of *Trypanosoma brucei* alone and in mixed infection of *T. brucei* and *T. congolense*: A comparative study proceedings of the 28th annual conference of the Nigerian Society of Annual Production, held at the Institute of Agricultural Research and Training (IAR&T) Moor plantation, Ibadan, March 16th 20th. 2003;17-21.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13035