Original Research Articles

THE EFFECT OF EXPERIMENTAL TRYPANASOMOSIS INFECTION AND PANTIZEN TREATMENT ON THE HAEMATOLOGICAL PROFILE OF ALBINO RATS.

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 cell counts (WBC), were monitored weekly.

Result: 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, 3, 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, 11, 12). *Trypanosome* parasites invade blood and produce numerous changes in the cellular and biochemical constituents of blood (13, 14, 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 291grams 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 x 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 0.1ml 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. RESULT

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. (figure 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 (figure 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 (figure 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 (figure 4).

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).

3.6 Neutrophil counts

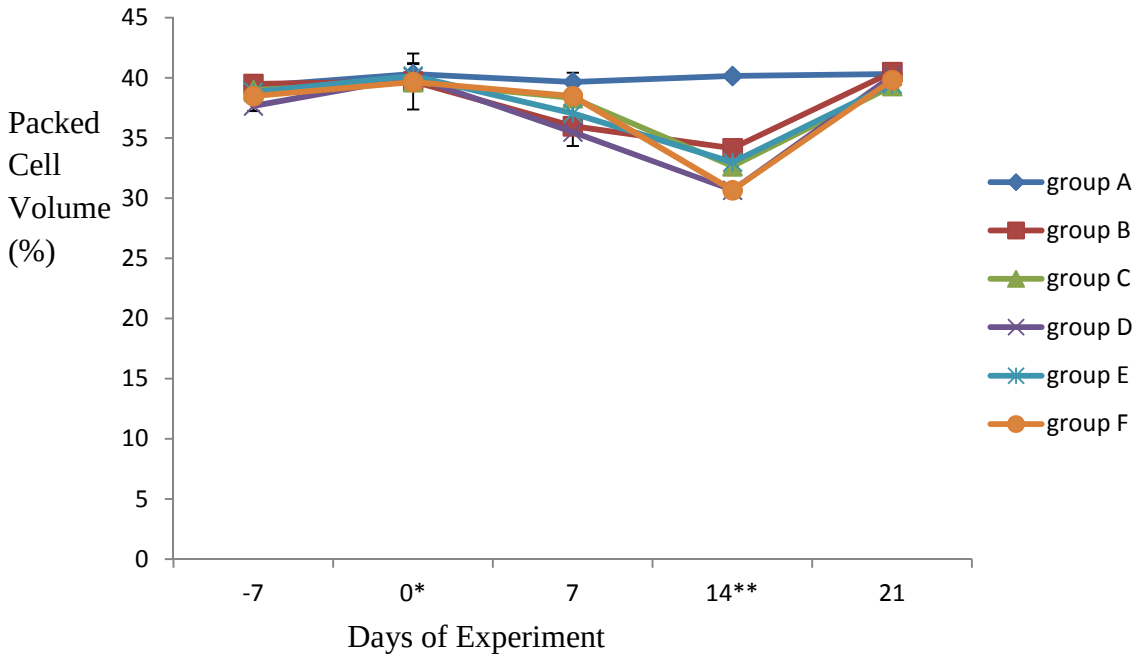
The trypanosomal infection caused significant ($P < 0.05$) decrease in the neutrophil counts in all the infected groups at days 7 PI and in groups B, D and E at day 14 PI especially in group E. However, there was significant increase in neutrophil counts in group F following treatment when compared to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

3.7 Monocyte counts

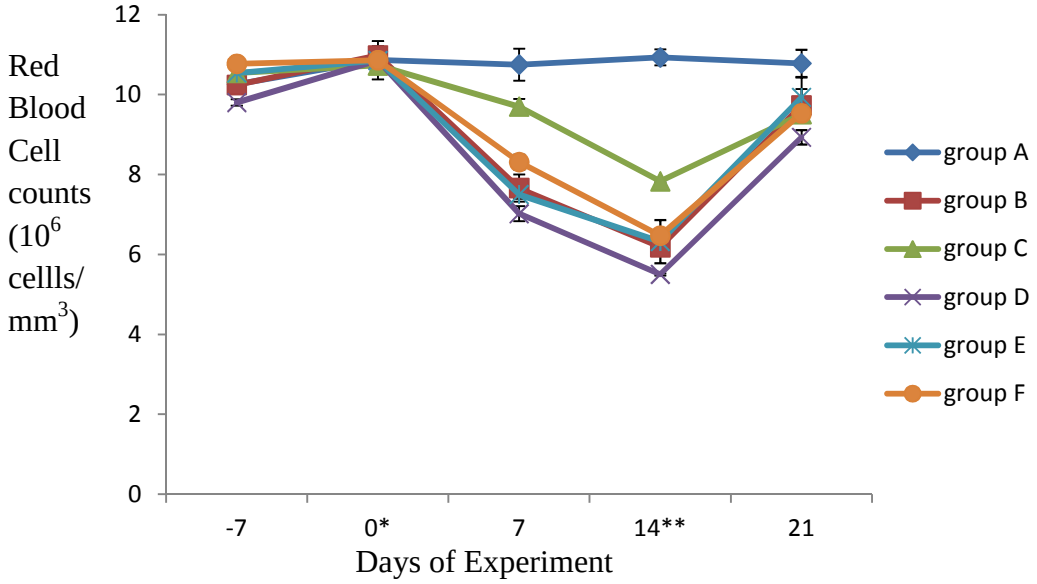
There was no significant ($P > 0.05$) variation in mean monocyte counts in all groups post infection. Following treatment, mean monocyte counts significantly ($P < 0.05$) increased in groups C and F at day 21(Table 1).

3.8 Eosinophil counts

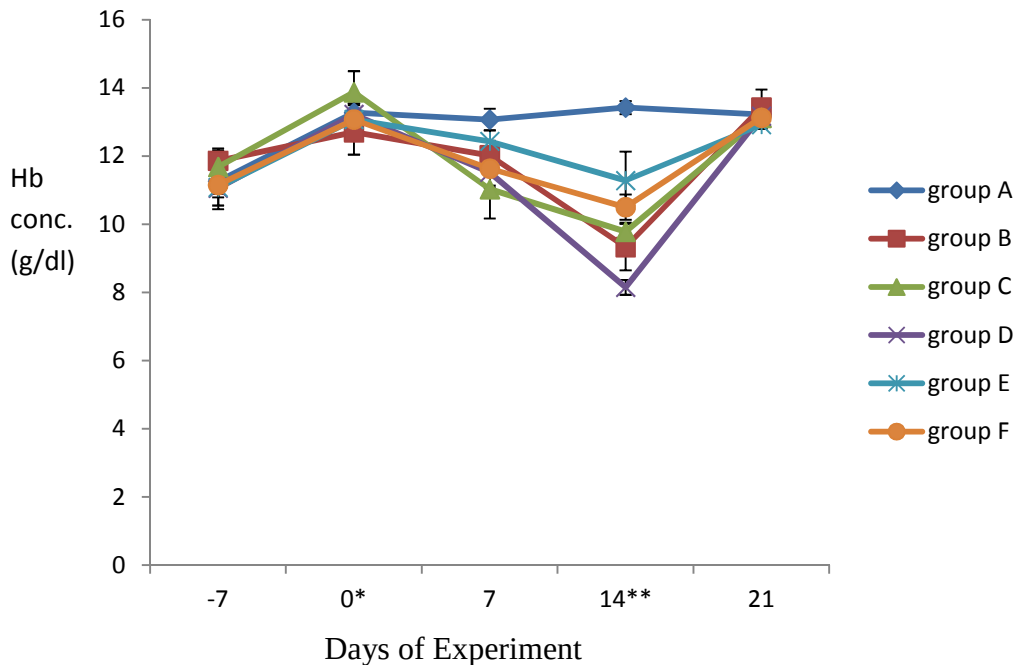
There was no significant ($P > 0.05$) variation in the mean eosinophil counts of all the groups post infection and post treatment.



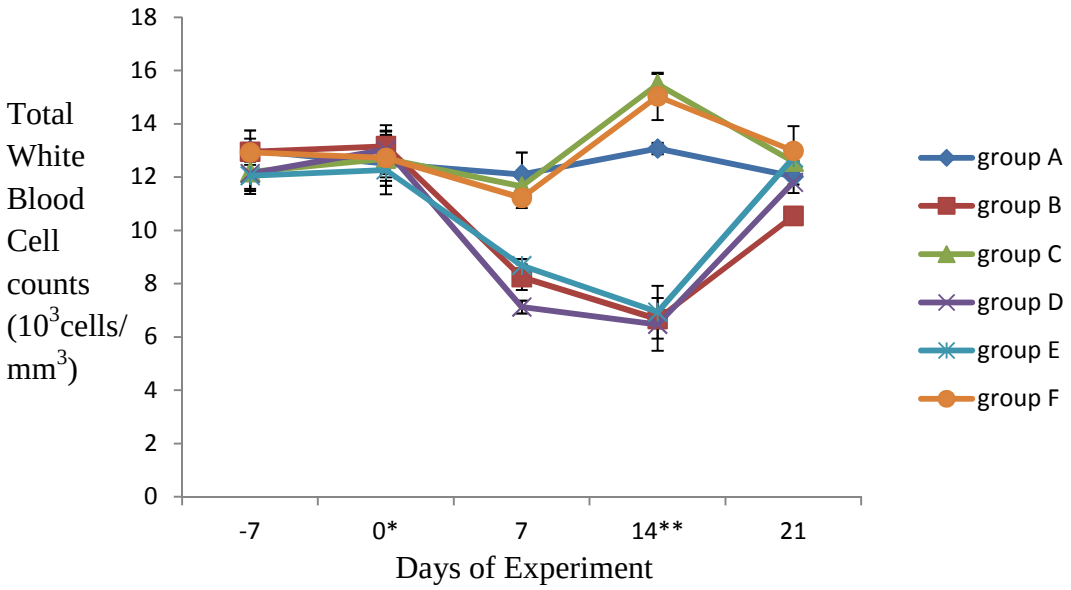
*Day of infection. **Day of treatment



*Day of infection. **Day of first treatment



*Day of infection. **Day of treatment.



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

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 ($P \leq 0.05$)
[*] Day of infection. **Day of first 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). The most severe effect observed in group D (infected with) 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 (34).

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.

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