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Effect of cysteine protease inhibitor (E64) on haematological changes of New Zealand rabbits experimentally infected with *Trypanosoma brucei brucei*

SA Malgwi*, AW Mbaya, AA Biu & MK Zango

Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria

*Correspondence: Tel.: +2348039746636; E-mail: samsonmalgwi@unimaid.edu.ng

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Abstract

This study was conducted to determine the *in-vivo* effect of cysteine protease inhibitor (CPI) E64 on the clinico-haematology of New Zealand rabbits experimentally infected with *Trypanosoma brucei brucei.* A total of forty (40) New Zealand rabbits of both sexes were divided into 8 groups (A – H) of 5 rabbits each. Group A was infected but untreated (infected control), group B was uninfected and untreated (normal control). Group C was infected/treated pre-infection with CPI (E64) at 0.5ml/kg once daily for 5 days, while group D was infected and treated from 14 days post infection with CPI at 0.5ml/kg once daily for 5 days. Group E was uninfected and treated with CPI at 0.5ml/kg once daily for 5 days, while group F was infected and treated with a single standard dose of 3.5mg/kg of diminazene aceturate (Veriben®) by day 14. Group G was uninfected and treated with a single standard dose of Veriben® at 3.5mg/kg, while group H was infected and treated with of Veriben® at 3.5mg/kg and CPI at 0.5ml/kg once daily for 5 days at the peak of parasitaemia by day 14 post-infection (P.I). The animals were monitored for parasitaemia, haematological parameters such as red blood cell count, packed cell volume and haemoglobin concentration. The result showed that the animals became parasitaemic 7 days P.I with mean values of 7.75 ± 0.95, 8.50 ± 0.57, 8.00 ± 0.15, 8.00 ± 0.15 and 8.50 ± 0.57, in Groups A, C, D, F and H, respectively. Clinical signs such as anorexia, pyrexia, alopecia, and emaciation were seen. Haematological changes noticed as parasitaemia progressed include anaemia characterized by a significant decline in mean packed cell volume, haemoglobin concentration and red blood cell count was noticed in infected groups. Cysteine protease inhibitor (E64) alone was ineffective in ameliorating the deleterious effect of *Trypanosoma brucei brucei* especially against parasitaemia and haematological parameters.

Keywords: Anaemia, Cysteine protease inhibitor, Haematology , Parasitaemia, *Trypanosoma brucei brucei*

Introduction

African trypanosomosis is a vector-borne parasitic disease caused by a flagellated protozoon of the genus *Trypanosoma* (Soulsby, 1982). These infections are more prevalent in the rural areas (Hoet *et al*., 2007), and are transmitted through the bite of infected tsetse flies (*Glossina* spp.). Trypanosomosis is of great significance to human health and animal production in Africa (Bizimana *et*

al., 2006). Tsetse fly (*Glossina*) is responsible for biological (Cyclical) transmission while haematophagus arthropod vectors of the family; *Tabanidae, Stomoxynae* and *Hippoboscidae* are responsible for its mechanical transmission (Soulsby, 1982). *Trypanosoma congolense*, *T. vivax* and *T. brucei* have been reported to cause Nagana in cattle while *T. evansi* causes Surra in camels (*Camelus dromedarieus*) (Mbaya *et al*., 2010). In humans, *Trypanosoma brucei gambiense* and *Trypansoma brucei rhodesiense* are responsible for human sleeping sickness in West and East Africa respectively. In Nigeria, more than four-fifth (80%) of grazing land is rendered unsuitable for livestock rearing as a result of the disease (Solano *et al*., 2003). Trypanosomosis is a disease that results in annual losses in agricultural production of up to 4.5 billion U.S Dollars in Africa alone (Sorden & Adreasen, 2008). Clinical signs of tsetse transmitted trypanosomosis may include intermittent fever, oedema, abortion, decreased fertility and emaciation. Anaemia usually develops in affected animals and is followed by loss of condition, reduced productivity and often mortality (Soulsby, 1982).

Proteases are enzymes that catalyze the hydrolysis of peptide bonds (Chapman *et al*., 1997). Proteolytic enzymes are present in all organisms and constitute approximately 2% - 4% of encoded gene products (Pandey, 2013). Since the hydrolysis of the peptide bond by proteases is essentially irreversible an extensive regulatory network of protease inhibitors has evolved to ensure targeted spatial and temporal control of their activity (Fear *et al.,* 2007). Proteases are grouped according to the key catalytic group in the active site; serine (Ser), threonine (Thr), cysteine (Cys), aspartate (Asp), gluthamate (Glu) or zinc in metalloprotease (Siklos *et al*., 2015). Since proteases activate an irreversible event, their activity must be tightly controlled for that purpose, hence nature has developed a number of strategies to control proteolysis, including zymogen activation, proteases degradation and the inhibition of active proteases by its macromolecular inhibitors. These macromolecular inhibitors of proteolytic enzymes regulate proteolysis and prevent effects of excess endogenous or exogenous proteases (Pandey, 2013). Proteolytic inhibition by protease inhibitors occur via two (2) mechanisms namely; irreversible trapping reactions and tight binding reactions (Rawlings, 2004). As modulation of protease activity with synthetic inhibitors has proven to be clinically useful for treating HIV and hypertension and shows potential for medical application in cancer, obesity, cardiovascular, inflammatory, neurodegenerative diseases and various infectious and parasitic diseases (Fear *et al*., 2007). Despite the promising potential of cysteine protease inhibitor (E64) as an antagonist of cellular damage coupled with the fact that trypanosomosis causes significant tissue damage, there is a dearth of information on the effect of cysteine protease inhibitor on the haematological parameters during *T. b. brucei* infection in rabbits.

Materials and Methods

Experimental animals

Forty (40) healthy New Zealand rabbits of both sexes weighing between 0.9 – 2.3kg were purchased commercially from rabbit farmers within Maiduguri. The rabbits on arrival were kept in the hutchery section of the Large Animal Clinic, University of Maiduguri, Nigeria. The rabbits were placed in a clean constructed cage which was separated into 8 different groups (A - H) by portioning, each partition comprised of 5 rabbits each. These rabbits were routinely screened for blood, intestinal and external arthropods parasites using standard criteria (Mbaya *et al*., 2009). The animals were fed pelleted commercial growers mash (Vital Feeds LTD, Jos, Nigeria). Fresh green vegetables and water were also provided *ad libitum*. The rabbits were allowed 14 days to acclimatize to their new environment before commencement of the experiment. All experiments were carried out in accordance with international guidelines for the use of animals for biomedical research and welfare (Murray & Jennings, 1983; Ochei & Kolhatkar, 2000).

Trypanosomes

Trypanosoma brucei brucei, Federe strain used for this study was obtained from the Nigeria Institute for Trypanosomosis and Onchocerciasis Research (NITOR) in Jos, Nigeria. The stabilates were passaged twice in donor rats. Tail blood from the donor rats was diluted with phosphate buffered saline glucose (PBSG) pH 7.2. The rabbits were infected intra-peritoneally with blood from the donor rats containing 1.5 $x10^6$ trypanosomes. The initial detection of parasitaemia was by the wet mount and haematocrit buffy-coat methods (Murray & Jennings, 1983) after collection of 2mls of blood sample via the ear vein. The degree of parasitaemia was estimated by the rapid matching method (Herbert & Lumsden, 1976).

Cysteine protease inhibitor (E64)/diminazene aceturate (Veriben®)

Cysteine protease inhibitor was commercially procured from Sigma Aldrich International® while diminazene aceturate was procured from veterinary pharmaceutical store in Maiduguri precisely in cattle market area, and were both reconstituted according to manufacturer's instruction.

In vivo experimental design

The 40 rabbits were weighed and randomly separated into eight (8) groups (A, B, C, D, E, F, G and H). The groups were infected and treated as follows:

Group A: Infected and untreated control (negative control).

Group B: Uninfected and untreated control (normal control).

Group C**:** Infected and treated pre-infection with cysteine protease inhibitor (E64) orally at 0.5ml/kg bodyweight once daily for 5 days.

Group D: Infected and treated post-infection with cysteine protease inhibitor (E64) orally at 0.5ml/kg bodyweight once daily for 5 days by day 14.

Group E: Uninfected and treated with cysteine protease inhibitor (E64) orally at 0.5ml/kg once daily for 5 days.

Group F: Infected and treated with a single standard dose of diminazene aceturate (Veriben®) at 3.5mg/kg bodyweight I.M by 14 days post infection.

Group G: Uninfected and treated with a single standard dose of diminazene aceturate (Veriben®) at 3.5mg/kg bodyweight I.M by 14 days post-infection.

Group H: Infected and treated with cysteine protease inhibitor at 0.5ml/kg orally and diminazene aceturate (Veriben®) at 3.5mg/kg bodyweight I.M by day 14 post infection.

Monitoring infected and control animals

The experimental rabbits were monitored daily for the development of clinical signs of trypanosomosis including mortality. Initial detection of parasitaemia was by wet mount and buffy coat examination (BCE) using blood samples already collected via the ear vein. (Murray *et al*., 1990), while the degree of parasitaemia was estimated by rapid matching technique (Herbert & Lumsden, 1976). About 3mls of blood was collected for the determination of haematological parameters such as red blood cell count, packed cell volume and haemoglobin concentration were determined every 7 days (Coles, 1986).

Statistical analysis

Data generated from the study were expressed as mean ± standard deviations (S.D) using 2 way analysis of variance (ANOVA) and $p < 0.05$ was considered significant (GraphPad Instat, 2009).

Results

The clinical signs seen include pyrexia, weakness, anorexia, emaciation, increased respiration, alopecia and corneal opacity. The mean parasitic counts of the New Zealand rabbits infected with *Trypanosoma brucei brucei* with their controls is presented in Table 1. In group A; infected with *Trypanosoma brucei brucei* but untreated, a mean parasite count of 7.75±0.95, was obtained after a prepatent period of 7 days post infection (P.I). The parasitic count continued to appreciate significantly ($p<0.05$) without abating to 59.00 ± 2.94 by day 35 (P.I) when all the rabbits (n=5) died of the infection. In group B (uninfected/untreated control), E (uninfected/treated with CPI (E64) at 0.5ml/kg) and G (uninfected and treated with 3.5mg/kg of Veriben®) no parasite was detected throughout the study period. Group C (infected with *Trypanosoma*

Table 1: Effect of cysteine protease inhibitor (E64) on the mean parasite counts ($x10^3$/uL) of New Zealand rabbits experimentally infected with *Trypanosoma brucei brucei* with their controls

Groups	Days	Day of treatment					
(n=5)	0	7	14	21	28	35	42
A	0	7.75 ± 0.09*	19.75 ± 0.95*	39.00 ± 1.63*	50.50 ± 1.29*	59.00 ± 0.05*	-
B	0	0	0	0	0	0	0
C	0	8.50 ± 0.57*	21.00 ± 1.55*	33.50 ± 0.57*	44.50 ± 0.57*	51.50 ± 0.57*	61.00 ± 2.30*
D	0	8.00 ± 1.15*	21.00 ± 1.15*	35.50 ± 0.57*	46.50 ± 0.57*	52.00 ± 1.15*	57.50 ± 0.57*
E	0	0	0	0	0	0	0
F	0	8.00 ± 1.15*	20.50 ± 1.29*	5.00 ± 0.81*	0	0	0
G	0	0	0	0	0	0	0
H	0	8.50 ± 0.57*	20.50 ± 0.57*	3.75 ± 0.50*	0	0	0

*Statistically significant within and across the tables ($P < 0.05$)

brucei brucei and treated pre-infection with CPI (E64)), a mean parasitic count of 8.50 ± 0.57 was obtained after a prepatent period of 7 days (P.I). Parasitaemia began to appreciate significantly ($p<0.05$) thereafter, to a count of 61.00 ± 2.30 by day 42 P.I when all the rabbits (n=5) died of the infection.In group D; infected with *Trypanosoma brucei brucei* and treated post infection with CPI (E64) at 0.5ml/kg, a mean parasite count of 8.00 ±1.15 was observed 7 days P.I, this continued to appreciate significantly ($p<0.05$) to 57.50 ± 0.57 by day 42 P.I when the rabbits (n=5) died of the infection. In group F; infected with *T. b brucei* and treated with 3.5mg/kg of Veriben® parasitaemia was not detected in peripheral circulation, until a count of 8.00 ± 1.15 was obtained by day 7 (P.I). This count rose significantly ($p<0.05$) to 20.50 ± 1.29, following treatment with 3.5mg/kg Veriben® by day 14 (P.I). The parasite count began to decline significantly ($p<0.05$) until they could no longer be detected in peripheral circulation by day 28 (P.I) or by day 14 post treatment (P.T). In group H; infected and treated with both 3.5mg/kg of Veriben® and CPI (E64) at 0.02ml/kg, a mean parasite count of 8.50 ± 0.57 was recorded by day 7 (P.I). The parasitaemia continued to appreciate significantly ($p<0.05$) till day 14 P.I when treatment was instituted, and this resulted to a significant decline ($p<0.05$) until parasitaemia was not detected by day 28 P.I or day 14 (P.T).

The mean PCV of the New Zealand rabbits infected with *Trypanosoma brucei brucei* with their controls is presented in Table 2. In group A, the pre-infection PCV values experienced a continuous but significant decline ($p<0.05$) from day 7 to day 35 (P.I) when all rabbits died of the infection. In groups (B, E and G) their pre-infection values remained fairly constant ($p>0.05$) throughout the study. In group C and group D the pre-infection value sex perienced a sharp and significant decline ($p<0.05$) from day 7 (P.I) to day 42 (P.I) when all the rabbits in both groups died of the infection. Meanwhile in group F and group H, the pre-infection values continued to decline significantly ($p<0.05$) following treatment, by day 14 (P.I) the values began to appreciate significantly ($p<0.05$) to its pre-infection PCV values by day 42 (P.I).

The mean haemoglobin concentration of the New Zealand rabbits experimentally infected with *Trypanosoma brucei brucei* with their controls is presented in Table 3. In group A the pre-infection value experienced a significant decline ($p<0.05$) from

Table 2: Effect of Cysteine Protease Inhibitor (E64) on the Mean Packed Cell Volume (%) Changes of New Zealand Rabbits Experimentally Infected with *Trypanosoma brucei brucei* with their Controls

Groups	Days	Day of treatment					
(n=5)	0	7	14	21	28	35	42
A	44.73 ± 0.095	34.00 ± 0.81*	28.75 ± 0.95*	25.00 ± 0.81*	21.50 ± 1.29*	16.50 ± 0.05*	-
B	45.25 ± 1.89	45.75 ± 1.70	45.75 ± 1.70	45.75 ± 1.70	45.75 ± 0.95	44.75 ± 0.95	45.50 ± 1.29
C	44.50 ± 0.57	33.75 ± 0.50*	28.00 ± 1.15*	26.50 ± 0.57*	26.50 ± 0.57*	26.00 ±1.15*	15.50 ± 1.29*
D	44.50 ± 1.73	34.50 ± 0.57*	28.50 ± 1.57*	29.00 ± 2.30*	25.50 ± 0.57*	26.50 ± 0.57*	15.50 ± 1.73*
E	44.50 ± 0.57	45.00 ± 1.15	44.50 ± 0.57	43.50 ± 0.57	43.50 ± 0.57	43.50 ± 0.57	44.50 ± 0.57
F	44.25 ± 0.95	34.00 ± 2.16*	28.75 ± 0.95*	32.00 ± 2.16*	33.75 ± 0.50*	38.50 ± 0.57*	44.25 ± 0.95
G	44.00 ± 0.81	44.00 ± 0.81	43.75 ± 0.95	43.75 ± 0.95	43.75 ± 0.95	44.00 ± 0.81	43.75 ± 1.25
H	44.50 ± 1.73	34.50 ± 0.57*	28.50 ± 0.57*	33.50 ± 0.57*	35.50 ± 0.57*	42.25 ± 0.50*	45.00 ± 1.15[a]

*Statistically significant within and across the tables ($P < 0.05$)

Table 3: Effects of Cysteine Protease Inhibitor (E64) on the Mean Haemoglobin Concentrations (g/dl) of New Zealand Rabbits Experimentally Infected with *Trypanosoma brucei brucei* with their Controls

Groups	Days	Day of treatment					
(n=5)	0	7	14	21	28	35	42
A	13.63 ± 0.66	8.30 ± 0.25*	7.12 ± 0.22*	6.30 ± 0.19*	5.68 ± 0.23*	4.08 ± 0.09*	-
B	14.05 ± 0.34	14.05 ± 0.35	14.00 ± 0.28	14.00 ± 0.28	13.90 ± 0.10	13.90 ± 0.10	13.80 ± 0.14
C	13.80 ± 0.23	10.10 ± 0.11*	7.65 ± 1.32*	6.30 ± 0.23*	5.90 ± 0.11*	4.10 ± 0.11*	3.90 ± 0.11*
D	13.90 ± 0.11	8.40 ± 0.23*	7.10 ± 0.11*	8.50 ± 1.73*	6.30 ± 0.28*	6.65 ± 0.17*	3.75 ± 0.95*
E	14.00 ± 0.23	13.90 ± 11	14.00 ± 0.00	14.00 ± 0.23	13.90 ± 0.34	14.10 ± 0.11	14.05 ± 0.17
F	13.95 ± 0.34	8.40 ± 0.28*	7.15 ± 0.19*	8.10 ± 0.11*	9.10 ± 0.21*	10.6 ± 012*	13.95 ± 0.37*
G	14.00 ± 0.16	13.90 ± 0.61	14.10 ± 0.25	14.05 ± 0.19	13.95 ± 0.10	13.90 ± 0.11	14.00 ± 0.16
H	14.00 ± 0.23	8.50 ± 0.34*	7.20 ± 0.23*	8.20 ± 0.00*	9.30 ± 0.11*	11.30 ± 0.28*	13.95 ± 0.28

*Statistically significant within and across the tables ($P < 0.05$)

day 7 (P.I) to day 35 (P.I) when all the rabbits in the group died of the infection. For group B; group E and group G the pre-infection values remained fairly constant ($p<0.05$) throughout the study period. In group C and group D the pre-infection values decline sharply and significantly ($p<0.05$) from day 7 (P.I) to day 42 (P.I) respectively even after being treated with CPI (E64). In group F and group H, the pre-infection value decreased sharply and significantly ($p<0.05$) from day 7 (P.I) to day 14 (P.I) following treatment value began to appreciate significantly ($p<0.05$) to its pre-infection value by day 42 (P.I) or by day 28 (P.T).

The mean red blood cell count of the New Zealand rabbits experimentally infected with *Trypanosoma brucei brucei* with their controls is presented in Table 4. In group A; the pre-infection value experienced a significant decline ($p<0.05$) from day 7 (P.I) to day 35 (P.I) when all rabbits died of the infection. For group B, group E and group G.

The pre-infection values remained fairly constant ($p>0.05$) throughout the study period. In group C and group D the pre-infection values declined sharply and significantly ($p>0.05$) from day 7 (P.I) to by day 42 P.I, when all the rabbits in the groups died of the infection, after instituting treatment with CPI (E64) at 0.5ml/kg. In group F and group H, the mean RBC counts pre-infection decreased sharply and significantly ($p<0.05$) from day 7 to day 14 (P.I) but following treatments with 3.5mg/kg of Veriben® and 0.5ml/kg of CPI (E64) by day 14 (P.I) the values began to appreciate significantly ($p<0.05$) to the pre-infection values by day 42 (P.I) or day 28 (P.I).

Discussion

This study showed that the infected groups (A, C, D, F and H) presented physical signs characterized by pyrexia, weakness, anorexia, emaciation, increased respiration, alopecia and corneal opacity. However, following treatment in groups F (infected/treated with Veriben® at 3.5mg/kg) and group H (infected/treated with both Veriben® at 3.5mg/kg and CPI (E64) at 0.5ml/kg for 5 days), these signs were reduced or even reversed suggesting an attempt by the administered drugs and CPI (E64) to restore cellular functions to its pre-infection status. These reversals can be attributed to the efficacy of Veriben® in the treatment of trypanosomosis (Anosa, 1988; Mbaya *et al.,* 2009), and the ability of CPI (E64) to prevent tissue damage (Chapman *et al.,* 1997; Atkinson *et al., 2*009)

All the infected rabbits became parasitaemic 7 days post infection confirming the report of prepatent period of 5 – 10 days for the parasite (Sorden & Andreasen, 2008). Similarly, the prepatent period of 7 days observed among rabbits is in consonance with the report of Anosa (1988). However haemolytic anaemia and prepatent periods in most cases often depends on the species of trypanosomes involved and the route of inoculation of the parasite (Sorden & Adreasen, 2008). A significant decline in red blood cell counts (RBC), packed cell volume (PCV) and haemoglobin concentration (Hb) parameters were observed in the infected groups (A, C, D, F and H). This was indicative of anaemia which started at the onset of parasitaemia, from day 7 (P.I). This is in agreement with several reports that the anaemia in trypanosomosis often started during the 1st wave of parasitaemia which is haemolytic in nature (Anosa, 1988; Igbokwe, 1994; Mbaya *et al.,* 2011, 2012). However, the haemolytic nature of the anaemia in most cases would depend on the species of trypanosomes involved (Mbaya *et al.,* 2012). The expanded and active mononuclear phagocytic system (MPS) has been a major player in haemolytic anaemia in trypanosomosis through erythrophagocytosis which develops soon after infection and continued thereafter, in the various

Table 4: Effects of Cysteine Protease Inhibitor (E64) on the Mean Red Blood Cell Counts ($x10^3/mm^3$) of New Zealand Rabbits Experimentally Infected with *Trypanosoma brucei brucei* with their Controls

Groups (n=5)	Days	Day of treatment					
	0	7	14	21	28	35	42
A	7.83 ± 0.23	6.64 ± 0.11*	5.15 ± 0.12*	4.43 ± 0.09*	3.53 ± 0.35*	3.03 ± 0.12*	-
B	7.85 ± 0.34	7.85 ± 0.34	8.10 ± 0.25	8.10 ± 0.25	8.30 ± 0.25	8.15 ± 0.19	8.20 ± 0.16
C	7.80 ± 0.28	6.70 ± 0.05*	5.05 ± 0.05*	4.40 ± 0.05*	3.90 ± 0.173*	5.00 ± 0.23*	3.90 ± 0.11*
D	8.00 ± 0.23	6.70 ± 0.11*	5.15 ± 0.05*	6.60 ± 0.11*	4.83 ± 0.89*	5.00 ± 0.00*	3.42 ± 0.43*
E	8.00 ± 0.00	8.00 ± 0.23	7.90 ± 0.11	8.10 ± 0.11	8.10 ± 0.11	8.10 ± 0.11	7.95 ± 0.05
F	7.88 ± 0.29	6.63 ± 0.126*	5.08 ± 0.09*	6.40 ± 0.07*	6.68 ± 0.13*	7.27 ± 020*	7.90 ± 0.37
G	7.88 ± 0.29	7.85 ± 0.34	8.00 ± 0.16	7.68 ± 0.28	7.68 ± 0.28	7.68 ± 0.28	7.88 ± 0.37
H	7.80 ± 0.28	6.60 ± 0.11*	5.05 ± 0.05*	6.50 ± 0.00*	6.90 ± 0.05*	7.50 ± 0.00	7.80 ± 0.23

*Statistically significant within and across the tables ($P < 0.05$)

phases of the disease (Mbaya *et al.,* 2012). The presence of MPS might have been associated with increased demand on the system to remove dead red blood cells, tissue cells, trypanosome antigen-antibody complexes and to participate in immune response (Nwosu & Ikeme, 1992). The fact that the red blood cell parameters (PCV, RBC, and Hb) decreased sharply during bouts of parasitaemia but maintained a gradual increase during the periods of low parasitaemia showed an inverse relationship between parasitaemia and anaemia. This clearly shows that when level of parasitaemia is high there is a decrease decrease in PCV, RBC and Haemoglobin concentration. (Nwosu & Ikeme, 1992; Mbaya *et al*., 2009). In all cases, the rabbits treated with CPI (E64) had no significant effect on haematology with 100% mortality which showed that CPI (E64) is not a good candidate for the treatment of *Trypanosoma brucei brucei* infection alone in rabbits. The difference in groups treated with Veriben® alone and with combination with CPI was not significantly different. However, the study showed synergistic action of CPI (E64) and Veriben® with all haemotological parameters modulated to their pre-infection status with 0% mortality, it is important to note that the CPI did not act as an antagonist but rather a synergist. In practice it has been reported that, the best therapeutic activity of certain drugs could be achieved not alone but in combination with other chemical agents (Onyeyili & Anike, 1991; Mbaya *et al*., 2008). This might be the reason for the effectiveness of CPI (E64) in combination with Veriben® than CPI (E64) used as a single therapy. It is however important to note that all parameters evaluated during the course of this study remained fairly constant for the uninfected Groups B (uninfected/untreated group), Group E (uninfected/treated with CPI (E64)) and Group G (uninfected/treated with Veriben®).

In conclusion, the result of this study clearly shows that New Zealand rabbits are susceptible to *Trypanosoma brucei brucei* infection. CPI (E64) at a dose of 0.5ml/kg alone was ineffective in ameliorating the effect of *Trypanosoma brucei brucei* infection on haematology. A single standard dose of diaminazene aceturate at 3.5mg/kg or in combination with CPI (E64) at 0.5ml/kg worked synergistically by potentiating to ameliorate the deleterious effects of *T.b. brucei* on haematology of *Trypanosoma brucei brucei* infected rabbits.

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Conflicts of Interest

The authors declare they have no conflict of interest.

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