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EFFICACY STUDY OF ZINC CHLORIDE AND DIMINAZENE ACETURATE ON *Trypanosoma brucei* INOCULATED RATS

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ABSTRACT

The effect of Zinc Chloride ($ZnCl_2$) and Diminazene aceturate in experimental *Trypanosoma brucei* infected rats was investigated. Six groups (A-F) consisting of five rats each were used. A and F were negative and positive controls, while B, C, D, and E were treated groups, respectively. The infection was achieved by inoculating ($1x10^6$) of the parasite and rapid matching method was used to estimate the parasitaemia in the host. Parasitaemia was monitored for 30 days using wet mount method. The inoculated treated groups progressively showed parasitaemia five days post inoculation that caused significant decrease ($p<0.05$) in packed cell volume (PCV) and white blood cell (WBC) count. After treatment with $ZnCl_2$ and Diminazene Aceturate (Group D), there was a remarkable improvement towards the normal (Group A). This study shows that supplemental $ZnCl_2$ could be used to alleviate the severity of the infection in Trypanosomosis.

Keywords: Diminazene aceturate, Parasitaemia, Rat, *Trypanosoma brucei,* $ZnCl_2$

INTRODUCTION

Trypanosomosis is a major factor that has devastated the livestock industry in 10million km^2 (Kinabo and Bogan, 1988). It is a disease caused by haemoprotozoan parasites normally transmitted by Glossina (Finelle,1983), Tabanid in semi-arid region and arid zones of sub-saharan Africa, especially in camels, coitus in horses; dourine disease and recently transplacental transmission in cattle was incriminated (Urquhart *et al*.,1989; Radostitis *et al*., 2003; Ate *et al*., 2007). Trypanosomosis is considered as one of the most neglected diseases (Truc, 2003). It poses as a re-emerging zoonosis which had recently received little attention from the international community and with over 500,000 people already carrying trypanosomes and would die if left untreated (WHO, 2005).

Currently, an annual loss of US $ 1.2billion directly from cattle death, reduced meat and milk production, decreased productive capacity is incurred (Kristjianson *et al*., 1999). The disease in man is accompanied by lymphadenopathy, non-specific signs of pyrexia, winterbottom's sign which later progresses to the late encephalitic mental dysfunction phase. In animals, the disease is character-

ized by various non-specific clinical signs such as fever, lacrimation, anaemia, jaundice, wasting of muscles, infertility, low milk production and sometimes geophagia (Radostitis *et al*., 2003; Kennedy, 2004).

Chemotherapy and chemoprophylaxis remains the main methods of control of the disease (Dolan *et al.*, 1990). Most drugs used were produced half a century ago and resistance and toxicities are associated with most (Ajagbonna and Onyeyili, 2003; Ezeokonkwo *et al*., 2007). Some workers advocated for the use of Magnesium supplementation and trypanocidal drug combination (Egbe-Nwiyi *et al*., 2003) and some trials with these regimens were investigated to have improved the severity of the pathogenicity of the trypanosome infection (Biobaku *et al*., 2008), while some other workers advocate combinations of various mineral supplements and vitamins with trypanocides (Ajagbonna *et al*., 2008), hence this study is aimed at investigating the trypanocidal efficacy of the combination of Zinc Chloride exogenous salt and Diminazene aceturate in wistar albino rats inoculated with *Trypanosoma brucei.*

MATERIALS AND METHODS

Experimental animals

Thirty healthy adult albino rats of both sexes weighing 128-200g were obtained from the animal unit of Usmanu Dan Fodiyo University, Sokoto and were fed on commercial feed (Sanders® feeds) and provided with clean water *ad libitum*.

Experimental design

The animals were divided into the following groups:

Group A: Not infected non- treated control
Group B: Not infected supplemented for 10 days
Group C: Inoculated supplemented with $ZnCl_2$
Group D: Inoculated supplemented and treated with subtherapeutic dose of Diminazene aceturate (1.75 mg/kg)
Group E: Inoculated treated with full dose of Diminazene aceturate (3.5 mg/kg)
Group F: Inoculated, non-supplemented, non-treated control

Trypanosome infection

Trypanosoma brucei was obtained from Nigerian Institute for Trypanosomiasis Research (NITR) Vom, Nigeria and rats were inoculated using a method adopted by Egbe-Nwiyi *et al.* (2004).

Oral Zinc Supplementation

Zinc Chloride ($ZnCl_2$) solution (10% aqueous) was administered daily at 100mg/kg as adopted by Biobaku *et al.* (2008).

Diminazene aceturate administration

Diminazene aceturate was administered intramuscularly in the quadriceps muscle using a method adopted by Ezeokonkwo *et al.* (2007).

Sample Collection

Blood was collected and parasitaemia assessment was carried out using the method previously adopted by Mikailu *et al*. (2002) and Ajagbonna *et al*. (2005). Analysis for Packed Cell Volume and white blood count was done using a method adopted by Jain and Carrol (1975). The blood was analysed at peak parasitaemia and post treatment.

Statistical analysis

The data were summarized as means ± standard deviations. The means were compared by analysis of variance (ANOVA).

RESULTS

Table 1 shows trypanocidal efficacy of $ZnCl_2$ and Diminazene aceturate in *T. brucei* inoculated rats with test groups C, D, E having 1, 3 and 2 rats alive.

Tables 2 shows that test groups C, D and E had average survival days of 30days unlike group F the positive control where all rats died prior to day-30 and precisely day-25.

The result of the haematological values showed that group F manifested anaemia and leukopaenia with PCV and WBC significantly decreasing ($p< 0.05$) from 36 ± 2.28 and 4.4 ± 0.2 to 19 ± 2.9 and 2.4 ± 0.1 respectively. The combination group of $ZnCl_2$ and Diminazene aceturate group (D) showed significant increase or leucocytosis.

Table 1: Trypanocidal Efficiency of $ZnCl_2$ and Diminazene in *T. brucei* inoculated rats

Days	Groups	A	B	C	D	E	F
0		0/5	0/5	0/5	0/5	0/5	0/5
5		0/5	0/5	4/5	5/5	5/5	3/5
10		0/5	0/5	4/5	5/5	5/5	5/5
15		0/5	0/5	4/5	3/5	4/5	5/5
20		0/5	0/5	4/5	2/5	3/5	4/4
25		0/5	0/5	2/2	2/5	3/5	3/3
30		0/5	0/5	0/1	0/3	0/2	0
Number Alive		5	5	1	3	2	0

Table 2: Summary of Total Number of Deaths, Cleared of parasites and Average Survival Period in test groups

Indices	A	B	C	D	E	F
Total Number of Deaths	0	0	4	2	3	5
No. of animal cleared of Parasitaemia	5	5	1	3	2	0
Average survival in days	30	30	30	30	30	25

Table 3: Effect of $ZnCl_2$ and Diminazene aceturate on Haematological values 18-20days Post- infection and after treatment (Mean ± SEM)

Haematological indices	A	B	C	D	E	F
PCV(%)	36±2.28	33±2.0	29.8±3.7	32±1.82	33±1.32	19±2.9*
WBC(10^3/mm^3)	4.4±0.2	4.2±2.3	4.3±2.1	5.3±0.2*	2.3±3.2	2.4±0.1*

*$p \leq 0.05$

DISCUSSION

The result obtained from this study is in agreement with the findings of Egbe-Nwiyi *et al.* (2004) that investigated the effect of Zinc Chloride on the severity of *T. brucei and T. congolense*. The study shows that zinc chloride improved the parameters of group D towards the normal group A. In a previous study carried out on camels in Pakistan, the animals showed a marked decrease in zinc ion, calcium ion and other ions in the sera of the animals (Zia-ur-Rahman *et al*., 1996). In a similar vein, Anosa (1988) adduced that trypanotolerant cattle and small ruminants showed marked increase in zinc and magnesium ions, thus this study exploits the supplementation of a microelement zinc in zinc chloride exogenous salt to control the decline in the serum deficit of zinc. Previous study on zinc in maize bran by Hecker *et al*. (1991) showed that the dynamics of parasitaemia was affected via prolonging the prepatent period of the disease in tse-tse infested zone. The zinc chloride studied by Egbe-Nwiyi *et al*. (2004) must have enhanced the enzymatic, hormonal and immune systems of the animals (McDonald *et al*., 1995), thus the metabolic process and cell-mediated immunity were improved, thereby increasing the WBC significantly as shown in this study, these with its enhancement of intermediate metabolic process must have improved the PCV by probably enhancing the erythropoietic centres to produce more reticulocytes and red cells (Kaneko, 1989). This is also in agreement with the study carried out by supplementation of magnesium chloride in which its supplementation reduced the severity of the pathogenicity of *T. brucei* infection in rats (Biobaku *et al*., 2008). Zinc chloride exogenous salt with the trypanocidal effect of Diminazene aceturate must have probably improved the haematological values of the test groups towards normal, thus prolonging the live of the rats. This study, therefore would further explain that supplementation of microelements in combination with trypanocide would maximize efficacy by the supplement acting as being supportive thus minimizing toxicity and side effects of the drug and thereby aiding the plane of mineral imbalance associated with the disease which could be a potential measure in the management and control of African animal trypanosomosis.

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