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ISSN - 2277 - 0593  
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Journal of Natural  
Sciences, Engineer-  
ing and Technology

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

\*S.A. OLURODE<sup>1</sup>, O.P. AJAGBONNA<sup>2</sup>, K.T. BIOBAKU<sup>1</sup>, H. IBRAHIM<sup>3</sup>  
AND M.I. TAKEET<sup>1</sup>

<sup>1</sup>College of Veterinary Medicine, University of Agriculture, Abeokuta

<sup>2</sup>Department of Veterinary Pharmacology and Toxicology,  
Faculty of Veterinary Medicine, University of Abuja.

<sup>3</sup>Department of Veterinary Physiology and Pharmacology,  
Faculty of Veterinary Medicine, Usmanu Dan Fodio University, Sokoto

\*Corresponding author: olurodetona@yahoo.co.uk

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### ABSTRACT

The effect of Zinc Chloride (ZnCl<sub>2</sub>) 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<sup>6</sup>) 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<sub>2</sub> and Diminazene Aceturate (Group D), there was a remarkable improvement towards the normal (Group A). This study shows that supplemental ZnCl<sub>2</sub> could be used to alleviate the severity of the infection in Trypanosomosis.

**Keywords:** Diminazene aceturate, Parasitaemia, Rat, *Trypanosoma brucei*, ZnCl<sub>2</sub>

### INTRODUCTION

Trypanosomosis is a major factor that has devastated the livestock industry in 10 million km<sup>2</sup> (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 \$ 1.2 billion directly from cattle death, reduced meat and milk production, decreased productive capacity is incurred (Kristjansson *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<sub>2</sub>

**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<sub>2</sub>) 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  $\pm$  standard deviations. The means were compared by analysis of variance (ANOVA).

died prior to day-30 and precisely day-25.

## RESULTS

Table 1 shows trypanocidal efficacy of ZnCl<sub>2</sub> 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 30 days unlike group F the positive control where all rats

The result of the haematological values showed that group F manifested anaemia and leukopaenia with PCV and WBC significantly decreasing ( $p < 0.05$ ) from  $36 \pm 2.28$  and  $4.4 \pm 0.2$  to  $19 \pm 2.9$  and  $2.4 \pm 0.1$  respectively. The combination group of ZnCl<sub>2</sub> and Diminazene aceturate group (D) showed significant increase or leucocytosis.

**Table 1: Trypanocidal Efficiency of ZnCl<sub>2</sub> 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<sub>2</sub> 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 <sup>3</sup> /mm <sup>3</sup> )	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≤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 sig-

nificantly 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.

## ACKNOWLEDGEMENT

We wish to acknowledge the technical assistance rendered by Mallam Idris Ngaski of

the Department of Veterinary Physiology, Pharmacology and Biochemistry, Usmanu Dan Fodiyo University, Sokoto.

## REFERENCES

- Ajagbonna, O.P., Onyeyili, P.A.** 2003. Effects of aqueous extract of *Allium Sativum* (Garlic) on haematological and biochemical parameters in rabbits infected with *Trypanosoma brucei*. *Proc. 8<sup>th</sup> Ann. Conf. Anim. Sci. Assoc. of Nig.* (ASAN) Sept. 16<sup>th</sup>-18<sup>th</sup>, Minna.
- Ajagbonna, O.P., Biobaku, K.T., Adeneye, A.A., Igbokwe, V., Mojiminiyi, F.B.O., Ameh, I.G.** 2005. Effects of the combination of Diminazene aceturate and *Khaya senegalensis* Stem bark extract in infection of rats. *Bulletin of Science Association of Nigeria*, 26: 606-613.
- Ajagbonna, O.P., Edeifo, W.U., Onakpa, M., Biobaku, K.T.** 2008. Evaluation of orally administered vitamin C on the efficacy of Diminazene aceturate (Berenil®) in experimental Trypanosomosis in rats. *Book of Abstracts of International Conference on Science and Technology*, Abuja, Nigeria.
- Anosa, V.O.** 1988. Haematology and Biochemical changes in human and animal trypanosomosis. II. *Revue Eleve. Med. Vet pays trop.*, 41(2): 151-164.
- Ate, I.U., Rekwot, P.I., Nok, A.J., Tekdek, L.B., Luga, I.I.** 2007. On farm Transplacental transmission of *Trypanosoma vivax* in a Bunaji calf in Zaria, Northern Nigeria. *Nigerian Veterinary Journal*, 27(3): 95-98.
- Biobaku, K.T., Ajayi, O.L., Ajagbonna, O.P., Omotainse, S.O.** 2008. Effect of Magnesium Chloride and Diminazene aceturate on the Pathogenicity of Trypanosome infection in rats. *Vom Journal of Veterinary Sciences*, 5(1): 47-53.
- Dolan, R.B., Okeh, G., Alushula, H., Mutugi, M., Stevenson, P., Sayer, P.D., Njoku, A.R.** 1990. Homidium bromide as a chemoprophylactic agent for cattle trypanosomiasis in Kenya. *Acta Trop*, 43: 137-144.
- Egbe-Nwiyi, T.N.C., Nwaosu, S.C., Tsuya, R.D.** 2003. Effects of high Oral Magnesium Chloride Supplementation in Pathogenicity of *Trypanosoma brucei* and *T. congolense* infection in rats. *Tropical Veterinarian*, 2(3): 152-159.
- Egbe-Nwiyi, T.N.C., Aliyu, M.M., Igbokwe, I.O.** 2004. Effects of Oral Zinc Chloride Supplementation on severity of *Trypanosoma brucei* and *T. congolense* infections in rats. *Sahel Journal of Veterinary Science*, 3: 39-43.
- Ezeokonkwo, R.C., Okoro, F.C., Ezeh, I.O.** 2007. The efficacy of increasing doses of Samorenil® in the treatment of *Trypanosoma brucei* infected Albino rats. *Nigerian Veterinary Journal*, 28(2): 24-32.
- Finelle, P.** 1983. African Trypanosomiasis. In: *FAO Animal Production paper*, 37: 19-22.
- Hecker, P.A., Coulibaly, L., Rowlands, G.J., Nagda, S.M., d'Ieteren, G.D.M.** 1991. Effect of plane of nutrition on trypanosome prevalence and mortality of Djallonke Sheep exposed to high tsetse challenge. In: *21<sup>st</sup> Meeting of the International Scientific Council of Trypanosomiasis Research Council and Control*, Yamoussoukro, Cote d'Ivoire, 21-25 October.
- Jain, N.C., Carrol, E.J.** 1975. *Veterinary*

*Haematology*, 3<sup>rd</sup> edn. Lea and Febiger, Philadelphia. P. 15-81.

**Kaneko, J.J.** 1989. *Clinical Biochemistry of Domestic animal*. 4<sup>th</sup> Ed. Academic Press Inc. America. P. 196-206.

**Kennedy, P.G.E.** 2004. Human African trypanosomiasis of the CNS. Current issues and challenges. *The Journal of Clinical Investigation*. 113(4): 496-504.

**Kinabo, I.D.B. Bogan, J.A.** 1988. The Pharmacology of Isomethamidium. *Journal of Vet. Pharmacol. Therap.*, 11: 233-245.

**Kristjanson, P.M., Swellow, G.J., Rowland, R.L., Kruska Deleeuw, P.N.** 1999. Measuring the Cost of African animal Trypanosomosis, the potential benefits of control and returns to research. *Agric. Syst.*, 59: 79-98.

**McDonald, P., Edward, R.A., Greenhalgh, J.F.D., Morgan, C.A.** 1995. *Animal Nutrition*, 5<sup>th</sup> edn. Pearson Education Ltd., UK. P. 176-180.

**Mikailu, H.G., Ajagbonna, O.P., Muhammed, B.Y., Onyeyili, P.A.** 2002. Garlic - a natural agent for treatment of Trypanosomiasis. *Proceedings of 39<sup>th</sup> Nigerian Veterinary Medical Association Conference*, Sokoto.

**Radostitis, O.M., Gay, C.C., Blood, D.C., Hinchclift, K.W.** 2003. *Diseases of Domestic Animals*. 9<sup>th</sup> ed. Britain. P. 1330-1334.

**Truc, P.** 2003. About *Trypanosoma brucei gambiense*, the causative agent of the chronic form of Human African Trypanosomiasis: Some findings and proposals. *African Journal Biotech.*, 2(12): 657-661.

**Urquhart, G., Armour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W.** 1989. *Veterinary Parasitology*. 3<sup>rd</sup> ed. Bath Press, Avon Great Britain.

**World Health Organisation** 2005. Control and Surveillance of African Trypanosomiasis. *W.H.O. Technical Report Series* 881. W.H.O., Geneva, P. 1-113.

**Zia-ur-Rahman, A.A., Asif, I.U., Haq, I.U., Ahmad, A., Asgab, M., Shaukat, S.A.** 1996. Concentration of serum micro- and macro-elements in sera of control and Trypanosome-infected camel. *Trop. Vet.*, 14: 133-136.

(Manuscript received: 1st April, 2009; accepted: 8th July, 2009).