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A Preliminary Trypanocidal Study of Natural and Synthetic Supplementation of Zinc and Magnesium in Combination with Diminazene Aceturate in Wistar Rats

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ABSTRACT

Eighty healthy Wistar albino rats were used to investigate the trypanocidal effect of natural and synthetic supplements of zinc and magnesium in combination with diminazene aceturate in *Trypanosoma congolense*-inoculated rats. The rats were randomly divided into eight groups in study '1' (involving the natural supplements of zinc and magnesium) and eight groups in study '2' (the synthetic supplements $ZnCl_2$ and $MgCl_2$), each group of both studies having five rats. Hence, studies 1 and 2 had groups A - H each. Parasitaemia of trypanosome parasite was assessed using 'wet mount method' after inoculation of all test groups except group 'G' of both studies (the normal, not infected, not treated control). Group 'E' of both studies in which combinations of natural source of zinc ion (maize bran) and magnesium ion (wheat bran) were combined with subtherapeutic dose of diminazene aceturate at 1.75 mg/kg cleared the trypanosome parasites with no relapse. The salts supplements of $ZnCl_2$ and $MgCl_2$ with the same subtherapeutic dose of diminazene aceturate also elicited an effect similar to the group 'E' in natural supplementation study. There was thus, no significant difference ($p>0.05$) between the PCV and RBC of normal group 'G' and the natural and synthetic supplemented groups with the trypanocide. The groups B, C, and D of both studies however, only prolonged the live of the animals (though with relapse occurring) there was still the death of the animals before the end of the experiment. The improvement of PCV and RBC values of treated groups 'E' in the two studies towards the normal gave credence to the fact that the supplements of Zn^{2+} and Mg^{2+} with the subtherapeutic dose of 1.75 mg/kg had a better trypanocidal and rejuvenating tendency and could be used for treatment.

Key words: Trypanosome, zinc, magnesium, diminazene aceturate.

INTRODUCTION

Trypanosomosis is a disease often accompanied by serum trace-element deficit. Amongst the ions depleted are zinc, magnesium, calcium and iron (Zia-Ur-Rahaman *et al.,* 1996; Egbe-Nwiyi *et al.,* 2003, 2004; Biobaku *et al.,* 2008a).

It is adduced that ionic imbalance coupled with the depletion of antioxidants could increase the free radicals of the antigenic factors of the trypanosome parasite in the host resulting in haemolysis (Igbokwe, 1994). The remedy to African animal trypanosomosis still remains chemotherapy and chemoprophylaxis thus, this accounts for an overall annual expenditure of not less than 44% of total veterinary drugs which is estimated to be over US$ 20 million (Ezeokonkwo *et al.,*2007). At present, the frequently used chemotherapeutic agents in the treatment of trypanosomosis are diminazene (an aromatic diamidine), homidium (a phenanthridine) and isometamidium (a phenanthridine aromatic amidine) (ILRAD, 1990). Of all these, diminazene aceturate is the most commonly used therapeutic agent (Egbe-Nwiyi *et al.,* 2006).

Natural mixed infections result to multiple species relapse of infections after treatment in the fields, these with the toxicity associated with the agents, justify the fact that several combinations are required to broaden the spectra of therapeutic effect and minimize toxicities associated with the trypanocidal agents (Ajagbonna and Olaniyi, 1999).

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For this reason, this study is embarked upon to study the trypanocidal effect of diminazene aceturate in combination with supplementation of natural and synthetic zinc and magnesium sources. Previous works had shown that magnesium chloride, a synthetic exogenous salt decreased the severity of pathogenicity of infection in inoculated wistar rats (Egbe-Nwiyi *et al.,* 2003; Biobaku and Ajagbonna, 2008a; Biobaku, *et al.,* 2008b). In the same vein, zinc chloride and its combination with diminazene aceturate (Olurode *et al.,* 2009) and zinc chloride alone orally decreased the severity and had effect on the pathogenicity of the disease (Egbe-Nwiyi *et al.,* 2004). Therefore, this study is a preliminary study to explore the natural and synthetic combinations of $Zn^{2+}$and Mg^{2+} from maize and wheat brans and their chlorides (i.e., $ZnCl_2$ and $MgCl_2$) in combination with diminazene aceturate on parasitaemia and its effect on erythrocytes as a method of assessing its therapeutic use.

MATERIALS AND METHODS

Experimental animals

Eighty healthy Wistar albino rats of both sexes, weighing between 190 g and 230 g, were purchased from the Biological Science Experimental Animal Unit of Usman Danfodiyo University, Sokoto, Nigeria. Animals were fed *ad libitum* and were left to acclimatize for two weeks. The rats were screened for haemoparasites using Giemsa staining method and were clinically examined to ensure that they were healthy and free from other diseases. The animals were handled according to the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 1985).

Experimental design

There were two phases of the study, namely study-1 and study-2 respectively in the design. The rats were divided into 8 groups of 5 rats each (A-H).

Study 1 – (natural supplementation study)

Group A: Infected not treated (positive control).
Group B: Infected supplemented with 50 percent of daily food required with maize bran per body weight.
Group C: Infected supplemented with 50 percent of daily food required with wheat bran per body weight.
Group D: Infected supplemented with 25 percent of daily food required with maize bran and 25 percent of wheat bran per body weight.
Group E: Infected supplemented with 25 percent maize bran, 25 percent wheat bran and treated with 1.75 mg/kg with of diminazene i.m. once.
Group F: Infected and treated with 1.75 mg/kg of diminazene i.m. once.
Group G: Not infected control.(normal, negative control)
Group H: Infected treated with 3.5 mg/kg diminazene i.m. once.

Study 2 – (synthetic supplementation study)

Group A: Infected not treated (Positive control).
Group B: Infected supplemented with zinc chloride
Group C: Infected supplemented with magnesium chloride 100 mg/kg
Group D: Infected supplemented with magnesium chloride and zinc chloride at 50 mg/kg.
Group E: Infected supplemented with $MgCl_2$ at 50mg/kg and 50 mg/kg $ZnCl_2$ treated with 1.75 mg/kg i.m. once
Group F: Infected and treated with 1.75 mg/kg i.m. once.
Group G: Not infected control (normal, negative control).
Group H: Infected with 3.5mg/kg diminazene i.m. once.

Trypanosome stock

T. congolense was obtained from the Nigerian Institute for Trypanosomosis and Onchocerciasis Research, Vom, Nigeria. The tail blood from a donor rat infected with *T. congolense* was collected and diluted in cold phosphate buffered saline. All rats in the infected group were infected by intraperitoneal injection of diluted blood containing (1×10^6) trypanosomes. Parasitaemia was estimated using rapid matching method technique. The tail blood from each infected rat was examined daily for thirty days to assess parasitaemia using standard wet mount method as previously adopted by (Ajagbonna *et al.,* 2005; Biobaku *et al.,* 2008b, 2009)

Parameter for assessing the therapeutic activity

Wet mount method was adopted in which glass slides; cover slips and tail blood were used to assess therapeutic activity of the administered treatment (Ajagbonna *et al.,* 2005; Ezeokonkwo *et al.,* 2007; Biobaku *et al.,* 2009; Olurode *et al.,* 2009). Blood was taken from the tip of the tail in order to assess for parasitaemia and the motility of the trypanosome in the microscopic field was noticed. If there is no motility in the wet mount slide field, it is

considered negative and if there is motility it is positive.

Natural sources of magnesium and zinc

The wheat and maize brans crown® brand were purchased from Sokoto Central market. The feed was supplemented with wheat bran and maize bran respectively, considering the basic average daily feed requirement in rats as adopted by Reuter and Suckow (2004).

The ratio used was 50% normal standard feed to 50% wheat or to maize bran. Similarly, 25% wheat to 25% maize to 50% feed was used as adopted previously by Biobaku and Ajagbonna (2008a).

Magnesium and zinc chloride

Analar® Brand of magnesium chloride and zinc chloride salts prepared by BDH and used as previously adopted (Egbe-Nwiyi *et al.,* 2003; Biobaku *et al.,* 2008b). In the administration of the combination of the two exogenous salts, 50mgkg^{-1} of $MgCl_2$ and $ZnCl_2$ of 50mgkg^{-}1were given orally, the method adopted was as previously described in the trials of Egbe-Nwiyi *et al.* (2003; 2004).

Trypanocide

The trypanocide used was diminazene aceturate made in India by Bio-Product, PVT India. The drug was administered at full therapeutic dose of 3.5mgkg-^{1} and 1.75mgkg^{-1} subtherapeutic dose respectively. Twenty-three (23)-gauge needles were used to administer the drug intramuscularly into the gluteus muscles as described by Ajagbonna *et al.* (2005), Ezeokonkwo *et al.* (2007) and Biobaku *et al.* (2009).

Blood sample collection

A 23-gauge syringe needle was used to carry out cardiac puncture in the rats surviving on the 30th day of the experiment. The blood was collected in ethylene diamine tetra acetic acid (EDTA) 20 mg/ml in purchased bottle. The packed cell volume (PCV) and the Red Blood Cell count were determined using standard method (Schalm *et al.,* 1975).

Statistical analysis

The data obtained were summarized as means and standard error of means. The means were compared by one way Analysis of Variance (ANOVA); p values of less than or equal to 0.05 (p=0.05) were considered significant. GRAPHPAD (1998) computer software was used to analyze the data.

RESULTS

Results in Tables 1 and 2 show that sequel to inoculation with *T. congolense,* parasitaemia was established on the 10th to 15th day post-infection. Although, the test group 'B' and 'C' recorded no absolute cure. The group B (the group supplemented with maize bran) and the group C (the group supplemented with wheat bran) showed that the animals survived for a relatively longer time of 15 and 20 days unlike the positive control, untreated infected group. The natural and synthetic supplementation with subtherapeutic doses each of diminazene aceturate of 1.75 mgkg^{-1} in group 'E' cured the rats with no relapse till the termination of the experiment.

Table 1. Effect of maize bran, wheat bran, diminazene aceturate and their combination on parasitaemia of rats infected with *T. congolense*

Days PI	Treatment groups							
	A	B	C	D	E	F	G	H
$0^{\pm}$	0/5	0/5	0/0	0/5	0/5	0/5	0/5	0/5
5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10	0/5	3/5	3/5	0/5	0/5	3/5	5/5	5/5
15	0/0	4/4	5/5	5/5	5/5	5/5	0/5	0/5
20	0/0	0/0	4/4	4/4	0/5	0/5	0/5	0/5
25	0/0	0/0	0/0	3/3	0/5	0/5	0/5	0/5
30	0/0	0/0	0/0	2/2	0/5	0/5	0/5	0/5

Days PI = No. of days post treatment; O^{+}_{-} = before infection; numerator = number of rats positive for *Trypanosome congolense;* denominator = number of rats surviving in a group

The groups 'F' of both studies showed relapse occurring in group 'F' of the natural supplementation study,

although all the rats survived till the last day of the experiment, 30-day post-infection. Group 'F' of the synthetic study (study 2) recorded only 2 rats surviving till the end of the experiment.

Table 2. Effect of $Mgcl_2$, $Zncl_2$, Diminazene aceturate and their combination on parasitaemia of rats infected with *T. congolense*

Days PI	Treatment groups							
	A	B	C	D	E	F	G	H
0	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
10	0/5	0/5	0/5	0/5	5/5	2/5	0/5	0/5
15	0/0	5/5	5/5	5/5	0/5	1/2	0/5	0/5
20	0/0	1/1	1/1	1/1	0/5	0/2	0/5	0/5
25	0/0	0/0	0/0	0/0	0/5	0/2	0/5	0/5
30	0/0	0/0	0/0	0/0	0/5	0/2	0/5	0/5

Days PI = No. of days post treatment; 0 = before infection; numerator = number of rats positive for *Trypanosome congolense;* denominator = number of rats surviving in a group

Tables 3 and 4 show that all animals in group A of both studies 1 and 2 had died before the end of the experiment. The groups 'D' of both studies recorded an absolute cure, hence none of the 5 rats survived till the end of the experiment.

Table 3. Summary of total number dead, cleared of parasites and average survival period in test groups using natural supplement of zinc and magnesium in *T. congolense* infected rats

Indices	Treatment groups							
	A	B	C	D	E	F	G	H
Total number of animals dead	5	5	5	3	0	0	0	0
Number of animals cleared of parasitaemia	0	0	0	2	5	5	NP*	5
Average days of survival	10	15	20	30	30	30	30	30

NP* = No parasitaemia not infected non treated control

Table 4. Summary of total number dead, cleared of parasites and average survival period in test groups using synthetic zinc and magnesium supplement in *T. congolense* infected rats

Indices	Treatment groups							
	A	B	C	D	E	F	G	H
Total number of animals dead	5	5	5	5	0	3	0	0
Number of animals cleared of parasitaemia	0	0	0	0	5	2	NP*	5
Average days of survival	10	20	20	20	30	30	30	30

NP* = No parasitaemia in the negative control

Table 5 shows that in group 'A' there was a significant ($p<0.05$) decrease of PCV and RBC in both studies before the end of the experiment, while group 'E' of the two studies showed no significant ($p>0.05$) difference when means were compared with the normal control.

DISCUSSION

The study gives credence to the fact that the trypanosome parasite is lethal if not treated. This is due to the severity of parasitaemia thus, causing parasitic haemolysis as a result of parasitic lysis that might predispose other erythrocytes to osmotic lysis thereby resulting in anaemia (Olayemi and Oyewole, 2002; John *et al.,* 2006).

Table 5. Effect of treatment with natural and synthetic supplements of magnesium, zinc, diminazene aceturate and their combination on erythrocytes values in *T. congolense* infected rats

Indices	Treatment groups							
	A	B	C	D	E	F	G	H
Study 1								
PCV %	26.40* ± 0.20	-	-	38.32 ± 0.30	42.34 ± 0.20	43.20 ± 0.20	44.33 ± 0.32	39.32 ± 0.20
RBC ($\times 10^6/\mu$l)	2.56* ± 0.13	-	-	3.42 ± 0.32	3.77 ± 0.23	4.32 ± 0.20	4.03 ± 0.10	4.23 ± 0.32
Study 2								
PCV %	22.80* ± 3.2	-	-	37.33 ± 0.00	38.22 ± 0.23	38.24 ± 0.32	39.23 ± 0.22	39.05 ± 0.22
RBC ($\times 10^6/\mu$l)	2.32* ± 0.22	-	-	3.32 ± 0.00	3.24 ± 0.32	3.22 ± 0.24	3.24 ± 0.20	3.22 ± 0.10

* = $p<0.05$ values are summarized as mean ± SEM; - = rats died before end of experiment (before 30 days); Study 1 = study with natural supplements of zinc and magnesium supplements; Study 2 = study with synthetic, exogenous salts supplement of zinc and magnesium supplement; asterisks on values indicate significance ($p<0.05$) decrease in values

The natural magnesium supplement in wheat bran and zinc in maize bran as previously adduced by Hecker *et al.* (1991), Egbe-Nwiyi *et al.* (2004) and Biobaku *et al.* (2008a) did not cure the parasitaemia but, most likely improved the level of the already depleted Mg^{2+} and Zn^{2+} in the sera during the course of the disease as stated by Zia-Ur-Rahaman *et al.* (1996). The magnesium must have acted through its antioxidative property by mopping and scavenging the free radicals from the antigenic factors of the parasite, these with its action on the ATPase would have made the erythrocytes to withstand the stress of the parasites, magnesium also decreases the severity of the disease conditions by its rejuvenating effect on the tissues (Delbet, 1992; Egbe-Nwiyi *et al.,* 2004; Biobaku *et al.*, 2009; Maduike, 2009). The zinc from the natural and synthetic exogenous sources concomitantly given with magnesium orally would act most probably by optimization of enzymatic action and improvement of the immune status of the animals, thus this action would have resulted in the prolongation of the lives of animals post infection. These allows the erythropoetic centres to become active again and produce red blood cells and other cells required for normal physiological take off, these assumptions are in concordance with the works of Awolaja *et al.* (1997), and Olurode *et al.* (2009). In the same vein, Anosa (1988) also reported that erythrocyte Zn^{2+} levels are higher in Keteku, a trypanotolerant breed of cattle.

The synthetic supplementation of $ZnCl_2$ and $MgCl_2$ also might have probably acted in similar mechanism, but the variation in the resurgences of parasites and course of the disease must have been probably due to the variation of bioavailability of the supplements and most likely interaction of the salts taken orally. Dietary supplementation however is an important factor to be considered in ameliorating the disease. This is due to the fact that the previous documentations showed that the nutritional status of the host affects the dynamics of parasitaemia. Thus, the host immune system would be able to withstand the challenge especially in tsetse endemic foci if properly fed or given supplements (Fagbemi 1990; Hecker, *et al.,* 1991; Umar *et al.,* 1999).

The groups treated with both Mg^{2+} and Zn^{2+} sources with diminazene aceturate at a subtherapeutic dose of $1.75mgkg^{-1}$ offered a potential therapeutic value to the therapeutic rationale by decreasing the trypanocide thus, minimizing toxic tendency of diminazene and acting as a supportive therapy source, this is evident by the improvement of PCV and RBC to normal range in these groups, thus using these parameters as indicators of the severity of the disease, it could be considered that these supplements' sources might be useful in the therapy of trypanosomosis.

CONCLUSION

The natural and synthetic supplements alone did not cure the disease condition, but rather prolonged the post infection period. Prolonging the post-infection period in a natural disease condition might help the host defence system to overwhelm the disease.

The use of mineral supplement and a trypanocide at a subtherapeutic dose could offer a cure with less tendency of side effect of the drug so that minimal cost could be achieved, as well as reducing drug toxicity and maximizing potentiation tendency due to effective and rational combination.

Further work is therefore required on the biochemical and hormonal parameters as regards magnesium and zinc supplementation in combination with diminazene.

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REFERENCES

Ajagbonna, O.P. and Olaniyi, M.O. (1999). Combination therapy of diminazene aceturate and steroidal anti-inflammatory drugs on trypanosomiasis infection in mice. *Proceedings of 26th Nigerian Society of Animal Production Conference*, Ilorin, Nigeria. pp. 366-367.

Ajagbonna, O.P., Biobaku, K., Adeneye, A. A., Igbokwe, V., Mojiminiyi, F. B. O. and Ameh, I.G. (2005). Effects of combination of diminazene aceturate and *Khaya senegalensis* bark extract in *Trypanosome brucei*. Infection of rats. *Bull. Sci. Assoc.* (Nigeria) 26, 606-613.

Anosa, V. O. (1988). Haematology and biochemical changes in human and animal trypanosomiasis. Part II. *Rev. elev. Med. Pay Trop.* 41(2), 151-164.

Awolaja, O. A., Antia, R. E. and Oyejide, A. (1997). Trace elements levels in plasma/serum and erythrocytes of Keteku and white Fulani cattle. *Trop. Anim. Hlth. Prod.* 29, 2-6.

Biobaku, K. T. and Ajagbonna, O.P. (2008a). A trypanocidal efficacy study of natural magnesium and diminazene aceturate in Wistar albino rats inoculated with *Trypaosoma brucei*. *J. Agric. Sci. Sci. Environ. Technol.* A 8(1), 41-49.

Biobaku, K.T., Ajagbonna, O.P., Ajayi, O.L. and Adebowale, O.O. (2008b). Efficacy of magnesium chloride supplement and diminazene aceturate in rats inoculated with *Trypanosima brucei*. *J. Agric. Sci. Sci. Environ. Technol.* B7(1), 64-70.

Biobaku, K. T., Abodunrin, C. O., Ajibola, E. S., Adenubi, O. T., Olukunle, O.J., and Thomas, F. C. (2009). Trypanocidal effect of aqueous extracts of *Khaya senegalensis* and *Allium sativum* with natural magnesium supplement in rats. *Nig. J. Parasitol.* 30(2), 90-95

CIOMS (1985). *International Guiding Principles for Biomedical Research Involving Animals*. Council for International Organizations of Medical Sciences, WHO, Geneva, Switzerland.

Delbet, P.R. (1992). The art of getting well magnesium chloride hexahydrate therapy. Arthritic Thrust of America for Medical Information. 992pp.

Egbe-Nwiyi, T. N. C., Igbokwe, I. O. and Onyeyili, P. A. (2006). Relapse of infection in single and mixed trypanosome infections in rats after diminazene aceturate treatment. *Vet. Arhiv* 76(3), 255-262.

Egbe-Nwiyi, T. N. C., Aliyu, M.M. and Igbokwe, I.O. (2004). Effects of oral zinc chloride supplementation on severity of *Trypanosoma brucei* and *T. congolense* infection in rats *Sahel J. Vet. Sci.* 3, 39-43.

Egbe-Nwiyi, T. N.C., Nwaosu, S. C. and Tsuya, T. D. (2003). Effects of high oral magnesium chloride supplementation in the pathogenicity of *Trypanosoma brucei* and *T. congolense* infection in rats. *Trop. Vet.* 21(3), 152-159.

Ezeokonkwo, R. C., Okoro, F. C. and Ezeh, I.O. (2007). The efficacy of increasing doses of Samorenil® in the treatment of *Trypanosoma brucei* albino rats. *Nig. Vet. J.* 28(2), 24-32.

Fagbemi, B.O., Otesile, E. B., Makinde, M.O. and Akiboade, O. A.(1990). The relationship between dietary energy levels and the severity of *Trypanosoma brucei* infected growing pigs. *Vet. Parasitol*. 35, 29-42.

GRAPHPAD (1998). Graph pad software, Instat guide to choosing and Interpreting statistical tests, Graph Pad software, Inc., San Diego California USA, *www.graphpad.com*

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: *21st Meeting of the International Scientific Council for Trypanosomiasis and Control*, Yamoussoukro, Cot d'Ivoire, 21-25 Oct. (Abstract).

Igbokwe, I.O. (1994). Mechanisms of cellular injury in African trypanosomiasis. *Vet. Bull.* 64, 611-620.

ILRAD Reports (1990). Chemotherapy of trypanosomiasis. The International Laboratory for Research on Animal Trypanosomiasis in the Eastern Hemisphere, Nairobi, Kenya. *Pharmacol. Therap.* 13, 91-147.

John, M.K., John, K. T., Maina, N., Raymond, M., David, M.M. and Joseph, M.N. (2006). Haematology of experimental *Trypanosoma brucei* rhodesiense infection in vet monkeys. *Afr. J. Hlth. Sci.* 13(3/4), 59-65.

Maduike, E. (2009). *The Synthetic Aluminum Magnesium Silicate*. Great AP Express Publishers, Nsukka, Nigeria. pp. 14-15.

Olayemi, F. O. and Oyewole, J.O. (2002). Haematology of the Nigerian white Fulani cattle under two different management systems. *Trop. Vet.* 20(1), 17-21.

Olurode, S. A. Ajagbonna, O.P., Biobaku, K. T., Ibrahim, H. and Takeet, M.I. (2009). Efficacy study of zinc chloride and diminazene aceturate on *Trypanosome brucei* inoculated rats. *Assets J*. series A. 9(1). (In Press)

Reuter, J.D. and Suckow, M.A. (2004). International Veterinary Information Service, Itheca NV (www.ivis.org.) Biology and Medicine of rats.

Schalm, O.W., Jain, N.C. and Carroll, E.J. (1975). *Veterinary Haematology*, 3rd ed. Lea and Febiger, Philadelphia. pp. 15-81.

Umar, I.A., Wuro-Chekke, A.U., Gidado, A. and Igbokwe, I.O. (1999). Effects of combined parenteral vitamins C and E administration on the severity of *Trypanosoma brucei*-infected rabbits. *Vet. Parasitol.* 85, 43-47.

Zia-Ur-Rahman, A. A., Butt, M.M., Asif, I.U., Haq,, A., Ahmed, M,. Asghab, M. and Shaukat., S. A. (1996). Concentration of serum micro and macro elements in sera of control and trypanosome infected camels. *Trop. Vet*. 14, 133-136.