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TRYPANOCIDAL EFFICACY STUDY OF NATURAL MAGNESIUM AND DIMINAZENE ACETURATE IN WISTAR ALBINO RATS INOCULATED WITH *TRYPANOSOMA BRUCEI*

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ABSTRACT

The effect of natural magnesium source wheat bran in combination with diminazene aceturate in wistar albino rats inoculated with *Trypanosoma brucei* was investigated. The infection with the parasite caused a progressive parasiteamia, a significant (P<0. 05) decrease in packed cell volume (PCV), Red blood cell count (RBC) and white blood cell (WBC) of infected not treated rats. This culminated in death of the rats by the 12th day post infection. These effects were significantly improved towards normal values by administration of sub therapeutic dose of 1.75 mg/kg diminazine aceturate and wheat bran supplement. Elemental analysis of wheat bran was carried out and was proved to have high proportion of magnesium. Although the combination and trial with wheat bran alone and did not clear the parasites completely yet it prolonged the survival time in the course of the experiment with remarkable improvement of hematological parameters towards the normal values.

Key words: Magnesium, wheat bran, and blood.

INTRODUCTION

Trypanosomiasis is a protozoan disease that affects man and animals (Radostitis *et al.,* 1994). This disease could be chronic and eventually fatal . In Africa it is termed the African sleeping sickness and in America is termed American sleeping sickness (Richard and Mycek, 2006).

Where trypanosomiasis is endemic, it has left the health of most afflicted societies precarious (Ghogomu, 1998) and their economies impoverished (Griffin and Allenby, 1979). A lot, as much as $1.3 billion is lost in Africa due to mortality and low production due to the trypanosome infected livestock (GFAR, 2000).

Chemotherapy and chemoprophylaxis are the methods of controlling Trypanosomiasis (Dolan *et al.,* 1990). Because of relatively limited markets in African and high cost of developing and licensing companies has shown little or no interest in developing new drugs especially trypanocides for use in either animals or human.

The current outstanding challenge to ma-

jority of African pastoralist therefore is to achieve optimal use of relatively cheap and existing measures and drugs in controlling the disease. Some suggested combination therapy (Ajagbonna and Olaniyi, 1999). The objective of this study is to determine the likely effects of wheat bran supplementation in daily feed intake of wistar albino rat in combination with diminazene aceturate a trypanocide, when the rats were challenged with *Trypanosoma brucei* and to explore the effect of the naturally existing magnesium in wheat bran on some basic hematological parameters in *T.brucei* inoculated rats. Thus, studying the trypanocidal efficacy of the combination.

MATERIALS AND METHODS

Experimental Animals

Thirty five healthy rats of both sexes weighing between 180 – 220 were purchased from Abeokuta, Ogun State, Nigeria.Each animal was fed *ad libitum* and was stabilized for two weeks. The rats were screened for haemoparasites using standard method.

Experimental Design

The rats were grouped into groups of 5 rats each:

Group A: Uninfected unsupplemented control.

Group B: Uninfected supplemented with 50 percent of daily food required with wheat 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 50 percent of daily food required with wheat bran per body weight in combination with 1.75 mg/kg diminazene acetrate.

Group E: Infected treated with 1.75mg/kg of diminazene aceturate.

Group F: Infected not treated unsupplemented control.

Group G: Infected treated with 3.5mg/kg of diminazene aceturate.

Trypanosome Stock

T.brucei used was obtained from Nigerian Institute for trypanosomasis and onchocerciasis research, Vom, Nigeria. The tail blood from a donor rat inoculated with T.brucei was collected and diluted in cold normal saline. All rats in the infected groups were infected by intraperitoneal injection of diluted blood containing $1x10^6$ tryanosomes. The tail blood from each infected rat was examined daily to access parasitaeamia using standard method as adopted by (Ajagbonna *et al.*, 2003, 2005).

Parameter for Assessing the Therapeutic Activity

Wet mount method was used in which glass slides; cover slips and tail blood were used for 30days to assess the therapeutic activity of the administered treatment (Mikhail et al., 2002; Ajagbonna *et al.*, 2003). Blood was therefore, taken from tip of tail, hence blood was sampled for 30 days of experiment to test for motility of the trypanosome. If there is no motility in the wet mounted slide then it is negative. This was used to assess the therapeutic effect of the diminazene and wheat supplementation.

Wheat Bran

The wheat bran was purchased from Uni-

versity of Agriculture Abeokuta, Ogun State feed mills. The feed was supplemented with wheat bran using the average daily need requirement in rats as adopted by Reuter and Suckow (2004). The ratio used was 50% normal feed to 50% wheat supplement.

Elemental Analysis

Wheat bran was taken for elemental analysis in the laboratories of the Faculty of Agriculture, Usmanu Dan Fodio University, Sokoto, Nigeria. 10 gram of the sample was digested and diluted with 30mls of distilled water. The number of Milligrams of the elements per gram of the Wheat bran were analyzed namely; potassium, magnesium, calcium, sodium were determined using atomic absorption spectrophotometer and titration method as described earlier in (Mikhail *et al.,* 2002; Ajagbonna and Oyeyili, 2003).

Trypanocide Administration

The trypanocide used was diminazene aceturate made in India Bio-product PVT limited. The drug was administered intramuscularly and the drug was diluted with distilled water at the dose of 3.5 mg/kg for full therapeutic dose and 1.75 mg/kg for subtherapeutic dose. The drug was administrated in the gluteal group of muscles using 23 gauge syringe needle.

Blood Collection

Blood was collected after anaesthetizing the rats using chloroform in a jar; cardiac blood was collected by extraction of blood via aspiration by cardiac puncture using 21 gauge syringe needle. The group F; the positive control were sacrificed at peak parasitaemia, 12 days after infection. While blood was taken at peak parasitaemia and 30 days after treatment in all groups. A total of 20 rats were sacrificed in all, these represent the rats that survived after the 30 days of therapy.

Analysis of Hematological Parameters

Blood was analyzed for packed cell volume, white blood cell count and red blood cell count respectively using standard procedure and as adapted by (Egbe-Nwiyi *et al.,* 2003).

Statistical Analysis

The data obtained were summarized as means, standard deviations and means were compared by Analysis of variance (ANOVA); $P<0.05$ was considered the level of significance.

RESULTS

In this study supplementation with wheat bran did not affect the prepatent period. Parasitological examination of the blood samples from the entire infected but supplemented group showed peak parasitaemia between 6 – 7 days. Wheat bran and a sub therapeutic dose of diminazene aceturate prolonged the life of the animals through out the 30 days as shown in (Table 1 and Table 2) the rats that received the supplementation alone were not cured and the parasite was not cleared from the blood. In the infected but untreated group the rats exhibited progressive parasitaemia that resulted in early death of the rats 12 days post infection.

Anaemia occurred in all the infected unsupplemented rats as shown in Table 3 and the animals reflected this by a significant ($P<0.05$) decrease in packed cell volume and Red blood cell. The white blood cell

also decreases significantly. But these haematological parameters later improved towards the normal value, in the combination groups. The same thing occurred in the white blood cell count. The elemental analysis shows that wheat bran constitutes magnesium in high proportion, 2,520 mg/g as shown in Table 4.

DISCUSSION

The present study gives credence to the well established fact that trypanosomiasis is usually accompanied by haematological depression this is in agreement with the work of Anosa (1988), Okochi *et al.* (2003).

The plane of nutrition is an important factor in the management of trypanosomosis infection as previously discussed by (Otesile, 1992; Igbokwe, 1995). Wheat bran is rich in magnesium (Egbe-Nwiyi *et al.,* 2003). Anosa (1988) reported decreased serum magnesium level in *Trypanosoma congolense* infected cattle. Oladele and Antia (2000) also reported slightly higher serum magnesium level in trypanotolerant keteku than trypanosusceptible white Fulani breeds of cattle. Thus, the result from this study indicate that wheat bran, a natural magnesium source when supplemented in feed had some effect on the dynamics of the parasitaemia in the infected animal in the experimental groups.

Since the magnesium is from a natural source and toxicity due to magnesium is rare (Baron, 1982) the dosage of magnesium in the feed was safe and as such no observation of toxicity in the supplemented animals could be attributed to the magnesium source in the wheat bran. Magnesium is trace element which is a major cation of the cellular fluid and is involved in cellular respiration and many other cellular reactions (Wilson, 1960). The replacement of the magnesium lost in infection in combination with sub therapeutic dose of a trypanocide diminazene aceturate, must have probably improved the haematological parameters in the inoculated teste groups. The wheat bran supplementation in combination with diminazene aceturate is efficacious to some extent and shows potential in management of trypanosomiasis, thus more researches should be conducted on other feeds that could have potentials in combination with conventional drugs, so achieving minimal cost, minimizing drug toxicity and maximizing potentiation due to effective and rational combination.

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Table 1: Trypanocidal Efficacy of Wheat Bran Supplement and *Diminazine aceturate* in *T.brucei* Infected Rats

	GROUPS	PARASITAEMIA																DAYS OF INFECTION														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
A	Not infected, Not supplemented Not treated	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
B	50% Wheat Bran Supplement not infected	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
C	50% Wheat Bran and Infected	0/5	0/5	0/5	0/5	4/5	5/5	5/5	5/5	5/5	3/3	3/3	2/2	2/2	1/1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	50% Wheat Bran ½ dose Diminazine and Infected	0/5	0/5	0/5	0/5	5/5	5/5	5/5	5/5	5/5	5/5	4/4	4/4	4/4	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/2	2/2	1/1
E	Infected and ½ dose Diminazine	0/5	0/5	0/5	4/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	4/4	4/4	4/4	3/3	3/3	3/3	3/3	3/3	2/2	2/2	1/1	0	0	0	0	0	0	0
F	Infected Not treated control	0/5	0/5	0/5	3/5	4/5	4/5	5/5	5/5	5/5	4/4	4/4	3/3	1/1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G	Full dose of Diminazine	0/5	0/5	0/5	0/5	0/5	5/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

The table shows in each group the number of positive over total number in a group tested in parasitaemia of infection

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Table 2: Summary of Total Number of Deaths, Cured Animals and Average Survival Period in Treated and Untreated Groups in Wheat Supplemented Study

Indices Determination	A UNIN-FECTED untreated	B UNIN-FECTED 50% wheat supplemented	C INFECTED 50% wheat bran supple-ment	D INFECTED 50% wheat bran and ½ dose diminazine	E INFECTED treated with ½ dose of diminazine	F INFECTED Not treated control	G INFECTED treated with full dose of diminazine
Total Number of deaths	0	0	5	4	5	5	0
Number cleared of parasite	5	5	0	1	0	0	5
Average sur-vival days	30	30	13	30	24	12	30

Table 3: Effect of Wheat bran Supplementation, Diminazine aceturate and Its Combination on Haematological Values 11 days post infection and after treatment

Haematological indices	A	B	C	D	E	F	G
Pcv (^{0}/0)	44.8±3.5	44.3±3.0	37±5.5	43.6±0.5	40±1.0	19±2.9*	44.4±2.6
Rbc (10^6/mm^3)	6.2±0.4	6.3±0.3	5.6±0.2	5.9±0.1	6.3±0.8	2.7±0.2*	6.4±1.0
Wbc (10^3/mm^3)	4.4±0.2	4.2±0.3	4.6±0.1	4.7±0.2	5.4±0.2*	2.4±0.1*	4.6±0.8

*P<0.05 compared to control untreated group

Haematological values mean±SD

Table 4: Elemental Analysis of Wheat Bran

Elements	mg/g
K^+	94,500.0
Na^+	99.0
Ca^{++}	540
Mg^{++}	2,520