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BIOTECHNOLOGY AND SUSTAINABLE DEVELOPMENT IN NIGERIA.

Edited by:
Eyo A. A., P. O. Aluko, S. A. Garba, U.D. Ali, S. L. Lamai and S. O. Olufeagba.

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SOME SERUM BIOCHEMICAL AND HAEMATOLOGICAL CHANGES IN EXPERIMENTAL TRYPANOSOMA BRUCEI BRUCEI INFECTION OF RABBITS.

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ABSTRACT.

Serum biochemical and haematological changes were studied for 5 consecutive weeks in male adult rabbits experimentally infected sub-cutaneously with T.b. brucei. They were treated with Berenil 3 weeks post infection. The infection produced significant decreases in mean PCV, Hb and RBC count values (P<.05). There were significant elevation of mean total serum protein between 2 – 3 weeks and mean total alkaline phosphatase at week 3 post infection (P<0.05). Following treatment with Berenil all these parameters returned to control level after 2 weeks. No significance change was found in acid phosphatase activities (P>0.05). The implication of these results are briefly discussed.

INTRODUCTION

The more important trypanosome species affecting man, domestic and experimental animals have been sub-divided into two groups, the haematonic group *(Trypanosoma congolense*, T. vivax) which remains in the plasma and the tissue-invading group (*T. brucei, T. evansi, T. gambiense, T. rhodesiense* and *T. equiperdum*) which is found extravascularly and intravascularly (Anosa, 1988).

Trypanosoma brucei infections have been reported to cause disease in ruminants, horses, dogs, cats and rodents (Losos and Chouinard, (1978). The level of parasitaemia in infected domestic animals is not related to the course of the disease whereas massive parasitaemia could be related to an acute course in rodents (Losos and Ikede, 1972). The parasite is known to invade the connective tissues causing foci of degeneration and necrosis of interstitial and parenchyma cells as well as induce an extensive infiltration by lymphocyte macrophages and plasma cells (Losos and Ikede, 1972).

Pathological changes in the organs lead to alterations in their functions. It was the opinion of Seed and Hall (1985) that greater emphasis should be given to the physiological changes which occur in the infected host in order to reach a better understanding of patient management. Therefore, they summarized reports on the changes in the biochemical parameters of infected laboratory animals which indicated liver dysfunction.

Anaemia is a consistent finding in various trypanosome infections. It usually sets in during the first wave of parasitaemia (Anosa, 1988) subsequent development of the anemia is determined by the frequency and intensity of the parasitaemia.

The present study refers to some serum biochemical and haematological changes in T. brucei intravenous experimental infection of rabbit with a view to determine the effect of the parasite on phosphatases and blood parameters since these animals are known to have highly efficient immune system.

MATERIALS AND METHODS

Adult male rabbits weighing between 1.6 – 1.8 kg. were used. They were purchased from parasitology section of the Nigerian Institute for Trypanosomiasis Research (NITR) Vom and housed in cages. They were given water *ad libitum* and fed with diet composed of maize offals, soyabeans and fish meal. They were screened for infection by physical, blood and faecal examinations and allowed to acclamatize for 3 months before the experiment.

Experimental design.

Four rabbits were infected sub-cutaneously with 1.25×10^5 of T. brucei brucei strain, NITR/Federe /CT/28 from the liquid nitrogen of Veterinary and livestock studies division, NITR, Vom. It was sub-passaged thrice in rats before use for this studies. Another group of 4 rabbits served as uninfected controls. Infection period was 3 weeks after which the animals were treated with diminazene aceturate (Berenil) at 7mg/kg body weight.

Five millilitres of blood were collected once a week for 3 weeks post infection and 2 weeks post treatment from the jugular veins of the control and infected rabbits. 1.0ml was placed in bottles containing ethylene diamine tetraacetic acid (EDTA) as anticoagulant and the remaining 4.0ml was left to clot in clean sterile bijou bottles at room temperature for 1 hour. The clear serum was separated using a pasteur pipette after centrifugation at 2000g for 10 minutes and used immediately for the experiment. The packed cell volume (PCV) was determined by the microhaematrocrit method, while the red blood cell (RBC) count and haemoglobin concentration were determined by haemocytometer and cyanomethaemoglobin methods respectively (Schalm et al, 1975). Total serum protein (TSP) was measured by the method of Lowry *et al* (1957), while the activities of acid and alkaline phosphatases were determined by the methods of Wright *et al*, (1978; 1978). The weight were also determined using Beanch – Top balance. The data were summarized as mean ± standard deviation (SD) and significant difference was assessed after a paired student's t-test adapted for small size (Mead and Curnow, 1983).

RESULTS

The T. brucei strain, NITR/Federe/CT/28 was highly infective. The infected rabbits developed parasitaemia at about 4 days post infection. The changes in the mean haematological and biochemical parameters of the control and infected rabbit, for 1 to 3 weeks post infection and 1 to 2 weeks post treatment and their control are presented in Table 1. The mean PCV, Hb and RBC counts of infected rabbits significantly decreased from 2 to 3 weeks PI ($P<0.05$). There was a gradual increase in ALP activities from weeks 1 to 3. The increase was statistically

significant at week 3 PI ($P<0.05$). The mean TSP of infected rabbits also significantly increased from 2 to 3 weeks. ($P<0.05$). Following treatment with Berenil all these parameters returned to preinfection or control level after 2 weeks. There was no significant changes in the mean ACP activities of rabbit at PI and PT when compared to control ($P>0.05$).

DISCUSSION

It has now been definitively established that the measurement of anaemia gives a reliable indication of the disease status (Murray, 1979) and productive performance (Morrison et al; 1981) of trypanosome – infected animal. The anaemia of trypanosomosis usually starts during the first wave of parasitaemia (Anosa, 1980). According to Anosa, (1983) several factors, especially haemolysis, contribute to anaemia. The exact incidence of each of these factors is not known, but the fact that the PCV, RBC and Hb values decreased sharply in periods of high parasitaemia but maintained a gradual decrease during the period of low parasitaemia shows a direct relationship between anaemia and parasitaemia. Similar fluctuations in erythrocyte values have been demonstrated in *T. brucei* infection of dogs (Kaggwa *et al;* 1984) and *T. brucei* and other trypanosome infections of various other animals (Anosa, 1974).

It was observed in this study that there was an increase in total serum protein. The elevated serum protein could probably be due to depression of albumin levels and elevation of globulin levels (Anosa, 1988). There have been reports of decreased plasma albumin concentrations in several trypanosome infections (Anosa, 1988) and it is thought that the decrease could be due to plasma expansion (Anosa, 1988), proteinuria (Bruijn 1987) or hepatocellular damage (Saror, 1980; Anosa and Isoun, 1983).

In the present study, serum ALP activities increased significantly at week 3 post infection (PI). Arowolo *et al* (1988) have shown that there may be depressed liver function in *T. brucei* infection of rabbit after observing an increase in serum alkaline phosphatase and a decrease in serum cholinesterase. Furthermore hypercholesterolaemia has been reported in rabbits infected with *T. gambiense* (Diahl and Risby, 1974) and T. brucei (Goodwin and Guy, 1973). Hypercholesterolaemia was thought to indicate an energy deficit due to liver dysfunction in trypanosome infected animals (Seed and Hall, 1985). The increase in ALP activities in our study may suggest, hepatic dysfunction.

We observed that the infected rabbits did not show weight gain, as against the controls. This may lead to lowered productivity, which is another feature of African animal trypanosomiasis (Dolan, 1987). They regained weight after the treatment.

Table 1: Changes in the mean biochemical and haematological paramters in the serum of rabbits during courses of infection with *T. b. brucei* and after treatment with Berenil.

Parementers	Preinfection	Weeks Post Infection (PI)			Weeks Post treatment.	
Paremeters*	Preinfection	1	2	3	1	2
ALP	+ 12.41±2.5 ++12.71±4.4	12.39±2.2 14.32±0.4	12.17±4.9 15.52±4.4	13.04±4.9a 19.11±5.8b	9.56±2.5 16.5±3.0	11.4±1.2 10.4±2.7
ACP	15.9±4.5 14.2±1.9	16.5±1.2 16.7±4.0	12.1±7.3 13.4±6.9	13.8±0.8 12.7±2.1	12.7±5.4 14.2±2.2	15.5±2.9 12.5±0.4
TSP (mg/ml)	13.1±1.8 12.4±0.9	11.3±1.7 14.5±0.4	10.5±1.0a 15.8±1.3b	10.9±0.3a 16.9±0.7b	11.6±0.6 11.7±0.2	11.4±01 11.5±0.5
PCV (%)	33.0±4.2 33.0±3.6	32.5±3.5 27.0±6.5	31.0±0.1a 23.5±6.2b	31.5±2.1a 19.5±1.3b	30.4±1.2 31.0±4.8	33.0±2.8 35±3.4
Hb (g/dl)	10.0±0.3 11.4±1.2	11.2±0.0 9.6±1.7	10.5±0.0a 7.5±2.3b	10.5±0.6a 5.9±1.1b	10.5±0.6 10.2±1.1	11.1±0.8 12.3±0.6
RBC)x106/ml)	4.14±0.07 4.48±0.4	5.23±0.4 4.17±0.9	5.14±0.1a 3.77±1.0b	4.31±0.5a 1198±0.3b	4.81±0.4 3.68±0.2	4.33±0.5 4.04±0.2

* Alkaline Phosphatase (ALP); Acid Phosphatase (ACP); Total Serum Protein (TSP); Packed cell volume (PCV); Haemaglobin (Hb); Red blood cell (RBC).
All enzyme activities wsere expressed as nmol/min/ml
A,b, mean +SD with different superscripts are significantly different ($P<0.05$
+ Control; ++ infected.

CONCLUSION

We showed that a high *T. brucei* experimental infection of rabbits caused decreased values of PCV, RBC count and Hb concentration. There is increase in ALP activities and total serum protein concentration. However no alteration was found in ACP activities. These findings seem to suggest anaemia and hepatic impairments. The result of this study has also shown that the drug diminazene aceturate clears the parasitaemia, abolishes the clinical symptoms and also improves the depressed function of the liver.

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