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EFFECT OF DL-á-DIFLUOROMETHYLORNITHINE ON BIOCHEMICAL CHANGES IN BABOONS (*Papio anubis*) EXPERIMENTALLY INFECTED WITH *Trypanosoma brucei gambiense*

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SUMMARY

An experimental infection of sixteen baboons (Papio anubis) with Trypanosoma brucei gambiense strain NITR/ABRAKA was undertaken. A uniform pre-patent period of 3-4 days was observed in all infected groups followed by a peak parasite count of 500 ± 1.89/µL by week 10 postinfection in the infected untreated controls. Those, however, treated by week 8 post-infection at the onset of somnolence (hypersomnia) with á-difluoromethylornithine (DFMO) or diminazene aceturate (Berenil®) eliminated the parasites from peripheral blood, until relapse occurred between weeks 18-20 post-infection for both drugs. The infection in infected untreated baboons generally produced a significant ($P<0.05$) decline in total serum protein and glucose, but a significant ($P<0.05$) increase in alanine amino transferase (ALT), aspartate amino transferase (AST), bilirubin, alkaline phosphatase and creatinine remained unabated till all the baboons in this group died by week 10 post-infection at the peak of hypersomnia. All biochemical parameters, however, in the primates treated with either DFMO or Berenil® returned to pre-infection levels by week 20 post-infection, with the exception of serum glucose level. Similarly, pre-infection serum bilirubin concentration was attained for DFMO but not for Berenil®. The results showed that T. b. gambiense caused severe biochemical alterations in primates, which was modulated to pre-infection status more effectively with DFMO than Berenil®.

KEY WORDS: - Biochemical changes, DL-á-ifuoromethylornithine, T. brucei gambiense, Baboons

INTRODUCTION

The African human trypanosomosis or "sleeping sickness" due to *Trypanosoma brucei gambiense* is a debilitating and complex disease of man. The disease at the chronic (*menengio encephalitis*) stage, results in a major disruption of the circadian rythmicity of sleep and wakefulness in man (Radomski *et al.*, 1995). Many species of wild African ungulates such as the waterbuck (*Kobus deffasa*), eland (*Hypotragus derbisiensis*) and domestic dogs are important reservoir hosts to the Gambian form of the trypanosome (Kaguraka *et al.*, 1988).

The Gambian form of the disease caused by *T. brucei gambiense* has left an important imprint on the African continent with high prevalence in several parts of Central and West Africa (Solano *et al.*, 2003). The low human population of the middle belt areas of Nigeria is attributable to the endemicity of the disease. Although the disease is chronic in nature, a virulent course of human sleeping sickness following an outbreak in Abraka in the southern part of Nigeria has been reported (Enwezor and Ukah, 2000).

In this study, baboons (*Papio anubis*) were challenged for the first time with the virulent strain (NITR/ABRAKA) of *Trypanosoma brucei gambiense* in order to study the effect of DL- á

difluoromethylornithine (DFMO) on the biochemical changes.

MATERIALS AND METHODS

Experimental animals

Sixteen (16) apparently healthy adult baboons weighing between 25 to 35 kg of both sexes were used for the study. They were acquired from the Sanda Kyarimi Park, Maiduguri, Nigeria. The animals were routinely dewormed orally with 300 mg/kg of pyrantel parmoate (Combatrim®, Pfizer Ltd., USA) and treated with oxytetracycline hydrochloride at 1 ml/10 kg body weight against rickettsial organisms and diminazene aceturate (Berenil®, Hoechst, Farbwerke, Germany) at 3.5 mg/kg body weight against trypanosomes. They were placed in squeeze cages in a fly-proof room. The primates were fed fresh fruits and vegetables, while water was provided *ad libitum* and they were allowed 4 weeks acclimatization period before the commencement of the experiment.

Source of trypanosomes

Trypanosoma brucei gambiense strain NITR/ABRAKA used for the study was initially isolated from a human case of sleeping sickness at Abraka, Nigeria. The isolates were confirmed to be *T. b. gambiense* using the Serum Incubation and Infectivity Test (SIIT) (Owen and Gillette, 1992). They were maintained by serial passages in rats and produced acute and sub-acute infections with pre-patent period of 3 to 4 days in a donor baboon kept separately from the ones used for the experiment. The experimental baboons were each infected with 0.5 ml of blood from the donor diluted in phosphate-buffered saline glucose (pH 7.2) containing 1.5×10^3 *T. b. gambiense*. Parasite counts were estimated using the rapid matching technique of Herbert and Lumsden (1976).

Test drug

DL-á-difluoromethylornithine (DFMO) was obtained from Merrill Dow Research Institute, Ohio, USA in a white crystalline form.

Experimental protocol

The baboons were randomly separated into four cages (A, B, C and D) of four primates each. Primates in groups A, B and C were each infected with 0.5 ml of blood from the donor containing 1.5×10^3 *T. b. gambiense*. Those in group A were, thereafter, treated orally with a standard dose of 300 mg/kg of DL -á-difluoromethylornithine (DFMO) in drinking water for four consecutive days at the onset of somnolence by week 8 post- infection. Primates in-group B were treated intramuscularly with a single standard dose of diminazene aceturate (Berenil®) at 3.5 mg/kg body weight at the onset of somnolence by week 8 post-infection. Baboons in groups C and D served as infected untreated and uninfected controls, respectively.

Biochemical analysis

Blood samples (3 ml) for biochemical analysis were collected weekly from the brachial vein into vacutainer tubes (without anti-coagulant) and allow 1 to separate at room temperature (35^0C) to obtain serum samples, which were stored at 4^0 C until used. Total protein was determined by the biurette reaction method (Afonja, 1997); aspartate (AST) and alanine (ALT) amino transferase were estimated by colorimetric method using commercial kits (Randox Laboratories Limited, United Kingdom) (Reitman and Frankel, 1957). Total serum glucose was determined by the glucose oxidase procedure of Folin-Wu (Coles, 1980). Total bilirubin was estimated using commercially available reagents (Sigma-Aldrich Fine Chemicals) (Coles, 1980). The spectrophotometer (Boehringer, 4010 West Germany) adjusted at various optical densities (O.D.) (transmittance) was used to read the results, while calculation of values was by standard formulae (Coles, 1980).

Statistical analysis

Data collected were analyzed using twoway analysis of variance (ANOVA) to detect variations between groups at 95% confidence limit (Macd and Curnow, 1983).

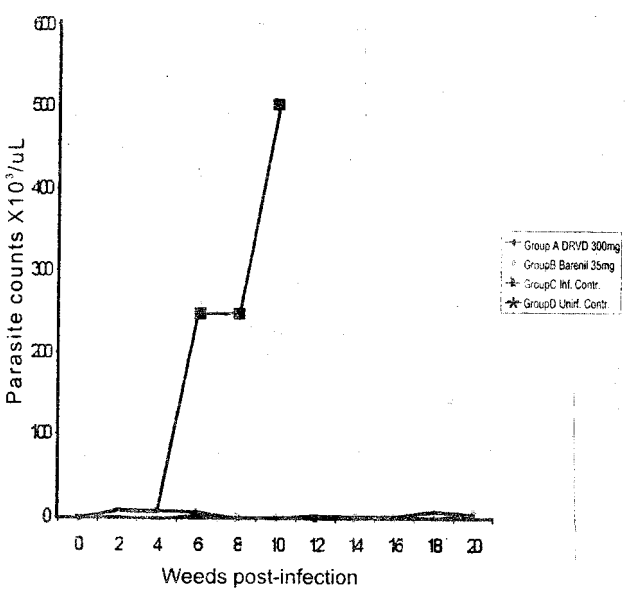
RESULTS

Infection of the baboons (*Papio anubis*) with *Trypanosoma brucei gambiense*, produced a uniform pre-patent period of 3-4 days, which rose to a peak count of 500 ± 1.89/μL by week 10 post-infection, which led to the death of all the untreated controls at that period. Parasite counts in the primates treated with either DFMO or Berenil® were effectively modulated to pre-infection levels, until relapse infection occurred with both trypanocides between weeks 18 to 20 post-infection (Fig. 1).

The effect of the infection on the total serum protein or glucose levels in the baboons (*Papio anubis*) treated with either DFMO or Berenil® and their controls are presented in Figs. 2 and 3, respectively. There was a gradual but significant ($P<0.05$) decline in total serum protein levels from week 4 post-infection and glucose from week 2 post-infection in relation to their uninfected controls. Following treatment with either DFMO or Berenil® at the onset of somnolence by week 8 post-infection, the total serum protein attained pre-infection levels by week 20 post-infection. Meanwhile, a pre-infection glucose level in all treatment groups was not attained. Similarly, the infected untreated baboons had an unabated decline in the values of total serum protein and glucose levels until the death of the baboons occurred by week 10 post-infection.

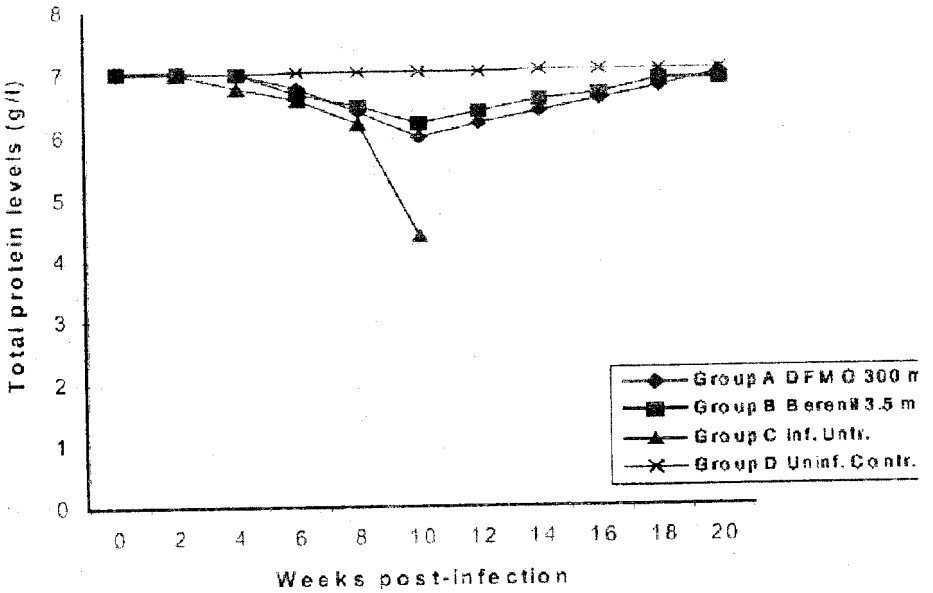
The infection induced a gradual but significant ($P<0.05$) increase in the levels of serum ALT, AST and total bilirubin by week 4 post-infection (Figs. 4, 5 and 6). Pre-infection values of ALT and AST were both attained by week 20 post-infection for baboons treated with either DFMO or Berenil®. Although the pre-infection value could not be attained for bilirubin after treatment with Berenil® by the end of the experiment, the value was attained by week 20 post-infection in baboons treated with DFMO. The infected and untreated controls, however, showed a significant ($P<0.05$) rise in the values of serum bilirubin. Relapse parasitaemia following the treatment with either DFMO or Berenil® did not significantly ($P>0.05$) affect the bilirubin values.

The result of this study also showed a significant ($P<0.05$) rise in the levels of alkaline phosphatase by week 6 post-infection in the infected untreated baboons, while the values fluctuated within normal range ($P>0.05$) in all treatment groups (Fig. 7). The serum creatinine levels significantly ($P<0.05$) increased from week 6 post-infection in all infected groups, but was effectively modulated to pre-infection values in groups treated with either DFMO or Berenil® by week 20 post-infection. This was in contrast to the infected untreated baboons, which experienced a significant ($P<0.05$) increase in creatinine values. Peak values of creatinine for the infected untreated control was attained by week 10 post-infection with the death of all baboons in this group occurring at this time. Similarly, relapse parasitaemia with either DFMO or Berenil® treatments did not significantly ($P>0.05$) affect the creatinine levels beyond this point (Fig. 8).



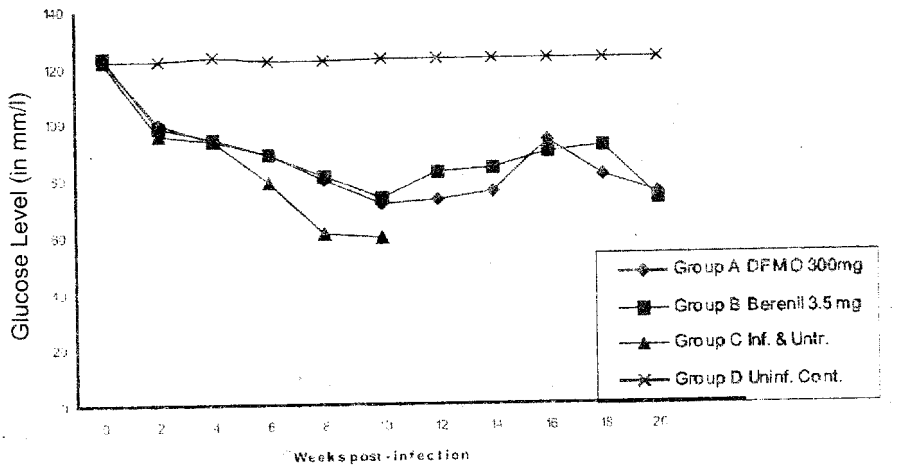
Key: Inf. Untr. Contr. = Infected Untreated Control
Uninf. Contr. = Uninfected Control
Point of treatment (arrowed)
Group A: n = 4, Group B: n = 4, Group C: n = 4, Group D: n = 4

Fig. 1: Parasite counts (x10³/μL) of baboons experimentally infected with *T. brucei gambiense* and treated with either DFMO or Berenil® and their controls



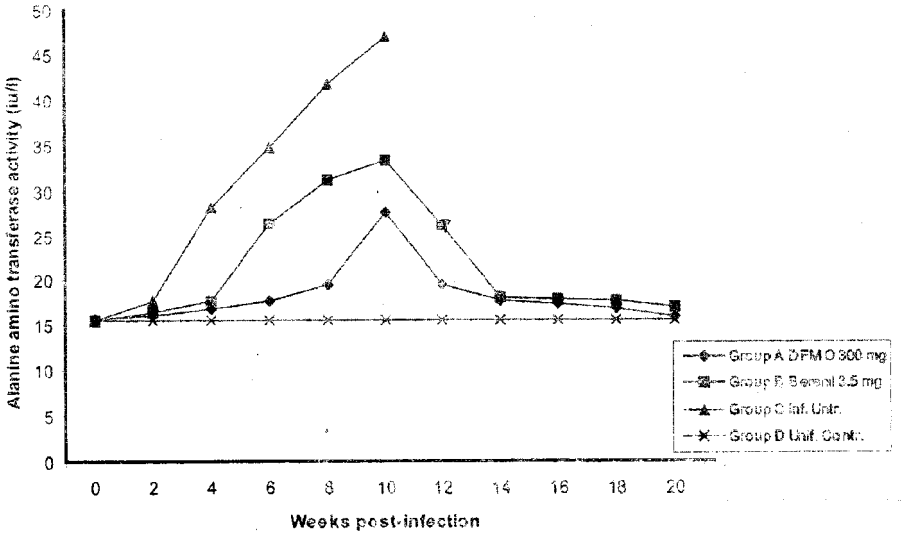
Key: Inf. Untr. Contr. = Infected Untreated Control
Uninf. Contr. = Uninfected Control
Point of treatment (arrowed)
Group A: n = 4, Group B: n = 4, Group C: n = 4, Group D: n = 4

Fig. 2: Mean total serum protein (g/l) of baboons experimentally infected with *T. brucei gambiense* and treated with either DFMO or Berenil® and their controls



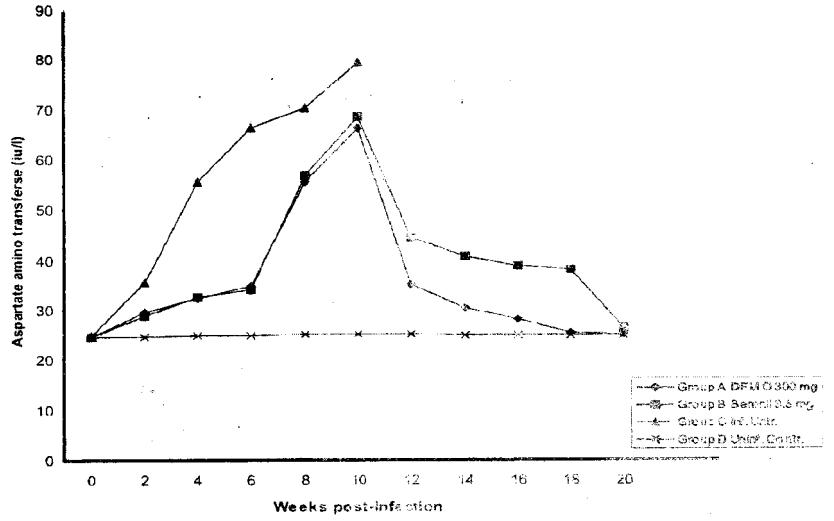
Key: Inf. Untr. Contr. = Infected Untreated Control
Uninf. Contr. = Uninfected Control
Point of treatment (arrowed)
Group A: n = 4, Group B: n = 4, Group C: n = 4, Group D: n = 4

Fig. 3: Mean serum glucose (mmol/l) of baboons experimentally infected with *T. brucei gambiense* and treated with either DFMO or Berenil® and their controls



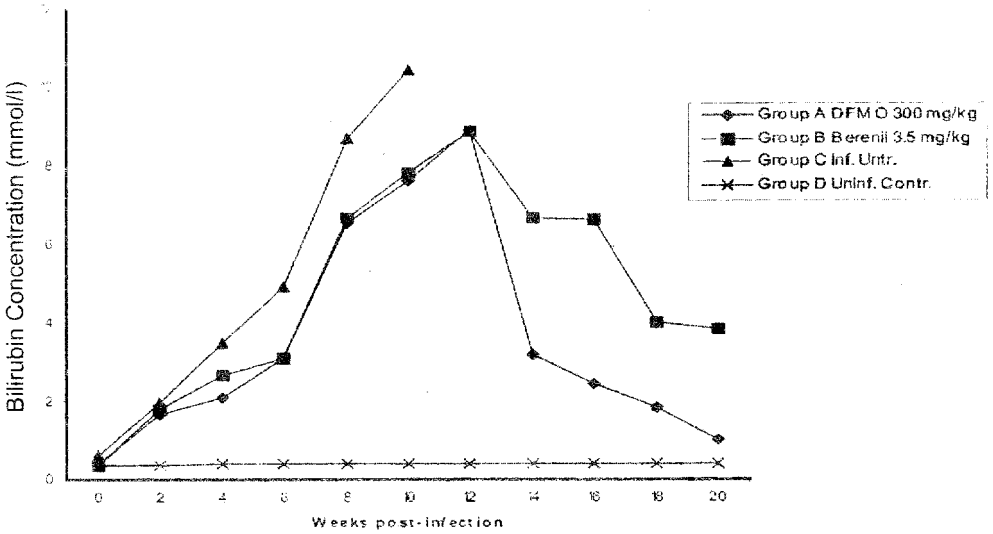
Key: Inf. Untr. Contr. = Infected Untreated Control
Uninf. Contr. = Uninfected Control
Point of treatment (arrowed)
Group A: n = 4, Group B: n = 4, Group C: n = 4, Group D: n = 4

Fig. 4: Mean alanine amino transferase (iu/l) of baboons experimentally infected with *T. brucei gambiense* and treated with either DFMO or Berenil® and their controls



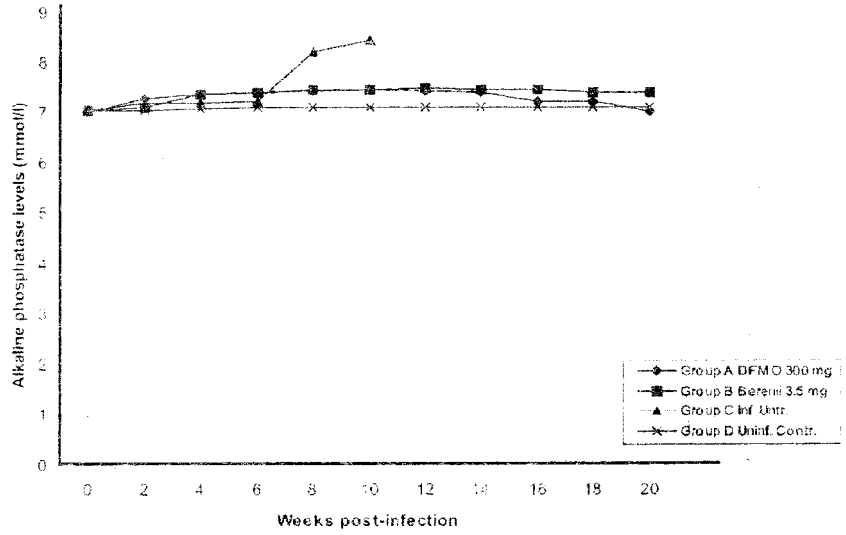
Key: Inf. Untr. Contr. = Infected Untreated Control
Uninf. Contr. = Uninfected Control
Point of treatment (arrowed)
Group A: n = 4, Group B: n = 4, Group C: n = 4, Group D: n = 4

Fig. 5: Mean aspartate amino transferase activity (iu/l) of baboons experimentally infected with *T. brucei gambiense* and treated with either DFMO or Berenil® and their controls



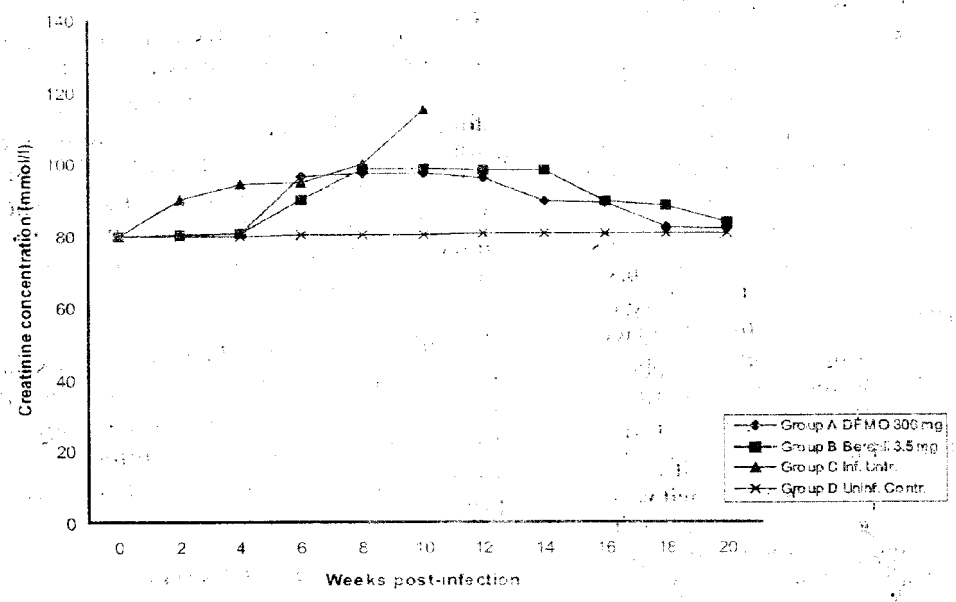
Key: Inf. Untr. Contr. = Infected Untreated Control
Uninf. Contr. = Uninfected Control
Point of treatment (arrowed)
Group A: n = 4, Group B: n = 4, Group C: n = 4, Group D: n = 4

Fig. 6: Mean serum bilirubin (mmol/l) of baboons experimentally infected with *T. brucei gambiense* and treated with either DFMO or Berenil® and their controls



Key: Inf. Untr. Contr. = Infected Untreated Control
Uninf. Contr. = Uninfected Control
Point of treatment (arrowed)
Group A: n = 4, Group B: n = 4, Group C: n = 4, Group D: n = 4

Fig. 7: Mean alkaline phosphatase levels (mmol/l) of baboons experimentally infected with *T. brucei gambiense* and treated with either DFMO or Berenil® and their controls



Keys: Inf. Untr. Contr. = Infected Untreated Control
Uninf. Contr. = Uninfected Control
Point of treatment (arrowed)
Group A: n = 4, Group B: n = 4, Group C: n = 4, Group D: n = 4

Fig. 8: Mean serum creatinine (mmol/l) of baboons experimentally infected with *T. brucei gambiense* and treated with either DFMO or Berenil® and their controls

DISCUSSION

Contrary to the typical chronic nature of *T. b. gambiense* infection in man (Scott, 1970), the course of infection in the baboons (*Papio anubis*) was acute and appear to be similar to experimental *T. brucei* infection in animals (Losos and Ikede, 1972). A similar course of an experimental *T. b. gambiense* infection was reported in vervet monkeys (*Cercopethicus aithiopes*) (Abenga and Anosa, 2006). This confirmed the existence of atypical type II of *T. b. gambiense* with resultant *T. b. rhodesiense*-like syndrome (WHO, 1998). This virulent form of sleeping sickness was first reported in an outbreak of sleeping sickness among natives in Abraka, Nigeria (Enwezor and Ukah, 2000).

The experimental infection in the baboons (*Papio anubis*) using the same isolate produced a gradual but significant decrease in total serum protein until death of the infected untreated controls. The biochemical change occurred at the stage of somnolence (hypersomnia), which corresponds with the typical "sleeping sickness" stage in man. Those infected but treated with either DFMO or Berenil® survived with the attainment of pre-infection values by week 20 post-infection. The reversal of the biochemical changes following diminazene aceturate therapy is consistent with *T. brucei* infection of Red Sokoto goats (Igbokwe and Mohammed, 1992) and in *T. brucei* infected red fronted gazelles (Mbaya *et al.*, 2007).

The changes in total serum plasma proteins may be associated with altered hepatic and renal function commonly encountered in trypanosomosis (Teitz, 1994).

The hypoglycaemia experienced in the study is in consonance with the results of previous investigations on trypanosomosis in puppies

(Kaushik *et al.*, 1989) and in red fronted gazelles (*Gazella rufifrons*) (Mbaya, 2007). Trypanosomes have been reported to deplete blood glucose due to aerobic glycolysis (Igbokwe, 1994). The organism metabolizes glucose to produce 4-hydroxyl-4-methyl alpha-ketogluterate, which is a toxic catabolite inhibitory to the tricarboxylic acid cycle in the mitochondria. The altered mitochondrial function and the resultant gluconeogenesis lead to severe energy deficit in the host (Igbokwe, 1994). This might have caused the profound body weakness experienced by the baboons during the course of the study. The observed weakness, which persisted despite the effect of both drugs, might have been further compounded by the relapse parasitaemia encountered with the Berenil® and DFMO treatments, which led to further depletion of the remaining glucose reserve by the parasites. Relapse trypanosomosis have been reported in Berenil® therapy due none penetration of the drug into central nervous system (Mbaya *et al.*, 2007). This is because the organism often evades the action of trypanocidal agents, when the drug molecules are too large to cross the blood brain barrier in sufficient quantity to exert a curative effect (Jennings, 1990).

The infection was also associated with increased levels of ALT, AST and total serum bilirubin. The two trypanocides (Berenil® and DFMO) effectively modulated these effects to almost pre-infection levels. The increased level of these hepatic enzymes is indicative of hepatic damage (Teitz, 1994). Increased serum levels of ALT and AST due to experimental trypanosomosis in goats (Igbokwe, 1994) and red fronted gazelles (*Gazella rufifrons*) infected with *T. brucei* (Mbaya, 2007) have been reported. Similarly, an experimental *T. brucei* infection of Red Sokoto goats was associated with marked elevation of total blood plasma bilirubin concentration in goats (Igbokwe and Mohammed, 1992) and in red fronted gazelles (*Gazella rufifrons*) (Mbaya *et al.*, 2007). These effects were, however, modulated to pre-infection value after treatment with DFMO. The reason why Berenil® could not do so is not clear, but such setbacks have led to the preference of DFMO over Berenil® in the treatment of human sleeping sickness (Radomski *et al.*, 1995).

Creatinine is produced in the body in proportion to muscle mass (Teitz, 1994). The high level of serum creatinine in the baboons following infection may be due to the muscle wasting observed or failure of their kidney to excrete creatinine due to renal failure. These values were effectively modulated to pre-infection levels following treatment with the two trypanocides.

The infected but treated baboons did not show any significant changes in the alkaline phosphatase levels, which is suggestive that treatments prevented the infection from causing any significant damage to bones of the primates.

CONCLUSION

In conclusion, it is evident from this study that baboons (*Papio anubis*) can be a good primate model in the study of biochemical changes following infections with *T. b. gambiense*. Similarly, DFMO from the present study may be a better drug of choice than Berenil® in treating the infection.

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What is your diagnosis?

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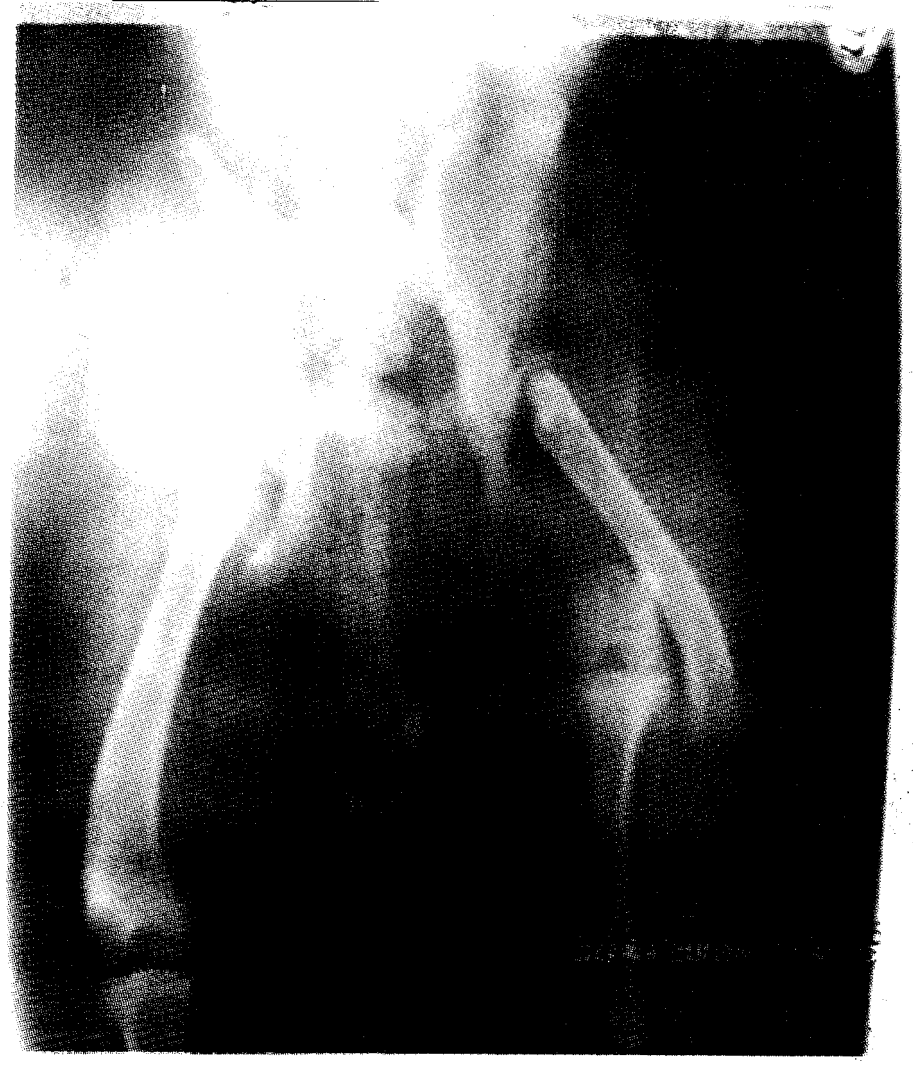

Plate 1: The radiograph of a five year -old female terrier presented to the Small Animal Clinic of Ahmadu Bello University 2 months following a road traffic accident (RTA)
a). What is your diagnosis? b). What are the treatment options for the case?

(Find Answers and Explanations on page 68)