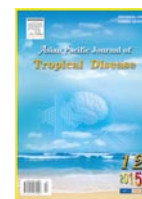




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Comparative biochemical and pathological changes in some laboratory animals experimentally infected with *Trypanosoma brucei* and their responses to diminazene diaceturate (Veriben®) therapy

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ABSTRACT

Objective: To compare the biochemical and pathological changes of *Trypanosoma brucei* infected mice, rats and rabbits, and study the chemotherapeutic effects according to standard criteria.

Methods: A total of 20 Balb/c Albino mice, 20 Wister Albino rats and 20 New Zealand rabbits, all adults and of both sexes were used in the study. Each rodent group was divided into four groups (A, B, C and D) of five animals each. Animals in Groups A and C were individually infected with 0.5 mL of blood from donor rats containing 1.5×10^6 *Trypanosoma brucei*, while Groups B and D remained uninfected. The animals were later treated like this: Group A (infected and untreated control), Group B (uninfected and untreated control), Group C (infected and treated) and Group D (uninfected and treated). The treatment was administered on Day 12 after infection.

Results: The prepatent period for mice and rats was 4 days while that for rabbits was 8 days. There was a significant ($P < 0.05$) increase in levels of liver enzymes and serum metabolites which were more marked among the mice and rats than rabbits. These changes were modulated to their preinfection values in the infected treated animals. At necropsy, all animals showed splenomegaly, hepatomegaly and nephritis. However, cardiomegaly was exclusive to the rabbits. Histopathologically, degenerative changes were observed in both the liver and kidneys which were more severe among mice and rats and moderate in rabbits. Spleen of mice showed giant macrophages, while that of the rats showed epithelioid giant cells. However, spleen of rabbits showed haemosiderosis and eosinophilic infiltrations. Hydrophobic degeneration, necrosis and mononuclear cell infiltrations in the myocardium were observed only among rabbits.

Conclusions: Diminazene diaceturate was able to ameliorate the various biochemical and pathological changes which were suggestive of severe liver and kidney dysfunctions with greater intensity occurring among mice and rats as compared to rabbits. Therefore, it suggested that mice and rats could be better animal models for studying the disease than rabbits.

1. Introduction

Trypanosomosis is a disease of man, domestic[1,2] and wild animals[3-7]. It is transmitted by tsetse flies (*Glossina* spp.) and characterized by anaemia, oedema, cachexia, intermittent fever and death[8]. In other regions of the world including Asia and South America, it is transmitted by several biting flies like *Tabanus*, *Hippobosca*, *Stomoxys*[2,9]. The disease is controlled through

chemotherapy[10,11] or through folkloric administration of medicinal plants with trypanocidal[12-14] or trypanostatic activities[15].

In the course of trypanosomosis, essential biochemical profiles are altered in the host. Subsequently, severe liver and kidney dysfunctions often occur which are usually investigated through biochemical tests. The liver dysfunction following trypanosomosis is accompanied by decreases in serum albumin and cholinesterase levels, increased serum alanine, aspartate aminotransferase and lactic dehydrogenase[16]. Kidney dysfunction is often accompanied by increases in blood urea nitrogen and serum creatinine concentrations[16]. Available literatures suggest that most of biochemical changes due to trypanosomosis are either in laboratory

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or domestic animals. However, little has been achieved in terms of understanding the variations in the biochemical and pathological changes that might occur in various laboratory animal species under the same experimental conditions as it relates to *Trypanosoma brucei brucei* (*T. brucei brucei*), the most commonly studied trypanosome species. Furthermore, the drug of choice, diminazene aceturate (Berenil®) commonly used for the treatment of the disease is currently no longer available in the market. It is against this backdrop that this study seeks to produce a comparative study of the variations in biochemical profiles and associated pathological changes in mice, rats and rabbits infected with *T. brucei brucei* under the same experimental conditions. This is with a view of identifying the most suitable animal model for studying the disease and the effect of diminazene diacetate (Veriben®) in modulating the biochemical and pathological changes that might occur in the course of the experiment.

2. Materials and methods

2.1. Experimental animals

A total of 20 adult Balb/c mice, 20 Wister Albino rats and 20 New Zealand rabbits of both sexes were used for this study. They were obtained from the National Veterinary Research Institute, Vom, Nigeria. They were routinely screened for blood, intestinal and external arthropod parasites using standard criteria as described by Luka *et al.*[17]. The animals were fed pelleted commercial feed (Vital Feeds, Nigeria Ltd.) while water was provided *ad libitum*. They were allowed to acclimatize to their new environment for 14 days before the commencement of the experiment. The animals were handled in accordance with international ethics for the use of animals for biomedical research[18].

2.2. Source of trypanosomes

T. brucei brucei, Federe strain used in this study was obtained from the Nigeria Institute of Trypanosomosis and Onchocerciasis Research in Jos, Nigeria. The organism was first isolated in 2006 from N'Dama and Muturu breeds of cattle. It was identified as *T. brucei brucei* based on morphology and negative blood inhibition and infectivity test and stabilized by serial passage in rats before storage in liquid nitrogen. The organisms obtained from Nigeria Institute of Trypanosomosis and Onchocerciasis Research were passaged twice in donor rats. The blood of tail from the donor rats was diluted with phosphate buffered saline and glucose, pH 7.2. The mice, rats and rabbits were infected intraperitoneally with 0.5 mL of infected blood from the donor rats containing 1.5×10^6 *T. brucei brucei*. Initial detection of parasitemia was by the wet mount and haematocrit buffy coat methods[19], while the degree of parasitemia was estimated by the rapid matching method[20].

2.3. Experimental design

The mice, rats and rabbits were weighed and randomly separated into four groups (A, B, C and D) of five each. The groups were infected and treated as follows, Group A: infected and untreated

control; Group B: uninfected and untreated control; Group C: infected and treated intraperitoneally with a single standard dose of diminazene diacetate (Veriben®, B12 LA® Ceva, Sante Animale-La Balastiere, France) at 3.5 mg/kg body weight by Day 12 post-infection at the peak of parasitemia, while Group D served as uninfected and treated intraperitoneally with a single standard dose of diminazene diacetate (Veriben®) at 3.5 mg/kg body weight by Day 12 post-infection.

2.4. Estimation of parasitemia

The parasite counts were determined every four days using the rapid matching technique of Herbert and Lumsden[20].

2.5. Determination of serum biochemical parameters

Sera obtained from blood of the animals collected without anticoagulant every four days from the orbital venous sinus in rats and mice and by catheterization of the auricular vein in the rabbits[21] were subjected to biochemical tests. Alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase activity levels were determined using commercial kits (Randox Laboratories, Ltd., Ardmore, UK) as described by Reitman and Frankel[22]. The levels of serum creatinine and urea were measured using commercial kits (Biotechnical, Varginha, Minas Gerais, Brazil) as earlier described[23].

2.6. Histopathological examinations

The carcasses of the animals that died and those routinely sacrificed at the end of the experiment were subjected to detailed postmortem examination for gross pathology as described by Igbokwe and Mohammed[24]. Samples of the spleen, liver, heart, lungs, lymph node and kidneys were taken and fixed in 10% formal saline. They were embedded in paraffin wax, sectioned at 5 μ m thickness and stained with haematoxylin and eosin (H&E)[25]. Tissues were examined under the light microscope (Olympus, Japan) for the presence of lesions.

2.7. Statistical analysis

Data collected from the study were summarized as means $\pm$ SD and differences between the means were determined at 5% level of significance using ANOVA[26].

3. Results

3.1. Comparative changes in parasitemia

The mean parasitemia ($\times 10^3/\mu$ L) of *T. brucei* infected mice, rats and rabbits treated with Veriben® and their controls are presented in Figure 1. In mice and rats, parasitemia became patent by Day 4 post-infection while in rabbits by Day 8 post-infection. The clinical signs observed were pallor of mucous membranes, lethargy and starry hair coat, which were observed mainly among mice and rats, while rabbits had mainly mild pallor of the ocular mucous membranes. The parasitemia in the infected control (Group A) reached their

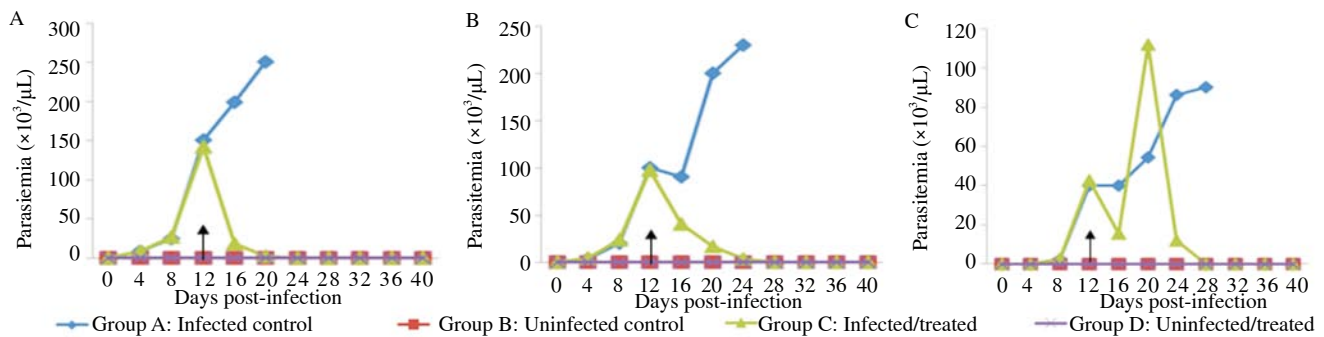


Figure 1. Comparative changes of parasitemia ($\times 10^3/\mu\text{L}$) in *T. brucei* infected mice (A), rats (B) and rabbits (C) treated with Veriben® and controls. Arrow: Day of treatment.

respective peaks in mice, rats and rabbits on Days 20, 24 and 28 of the infection, when all the rodents ($n = 5$) died of the infection. No death or parasitemia was recorded in Groups B and D. In Group C, mice, rats and rabbits showed peak parasitemia by Days 12, 12 and 20, respectively. This however declined significantly ($P < 0.05$) by Days 16, 20 and 24 in mice, rats and rabbits, respectively. Complete disappearance of parasites from peripheral circulation was observed by Day 24 post-infection or Day 12 post treatment in mice, Day 28 post-infection or by Day 16 post treatment in rats and Day 28 post-infection or by Day 16 post treatment in rabbits.

3.2. Comparative effect of diminazene diaceturate on serum alkaline phosphatase changes

The mean alkaline phosphatase activity of *T. brucei* infected mice, rats and rabbits treated with Veriben® and their controls are present in Figure 2. Among all rodents, alkaline phosphatase activity of the

infected control (Group A), reaching their respective peaks on Days 20, 24 and 28 in mice, rats and rabbits. Among animals in Groups B and D, the values ranged between (17.90 ± 0.53) and (28.30 ± 0.66) IU/L were remained fairly constant throughout the study ($P > 0.05$). However in Group C, the values increased significantly ($P < 0.05$), reaching their respective peaks on Day 12 in mice and rats. These values subsequently declined significantly ($P < 0.05$) after treatment till Day 40 post-infection or by Day 28 post treatment in all the rodents.

3.3. Comparative effect of diminazene diaceturate on serum alanine aminotransferase changes

The mean alanine aminotransferase activity of the *T. brucei* infection in mice rats and rabbits and treated with Veriben® and their controls are present in Figure 3. Mean preinfection values of the infected control (Group A) mice, rats and rabbits appreciated

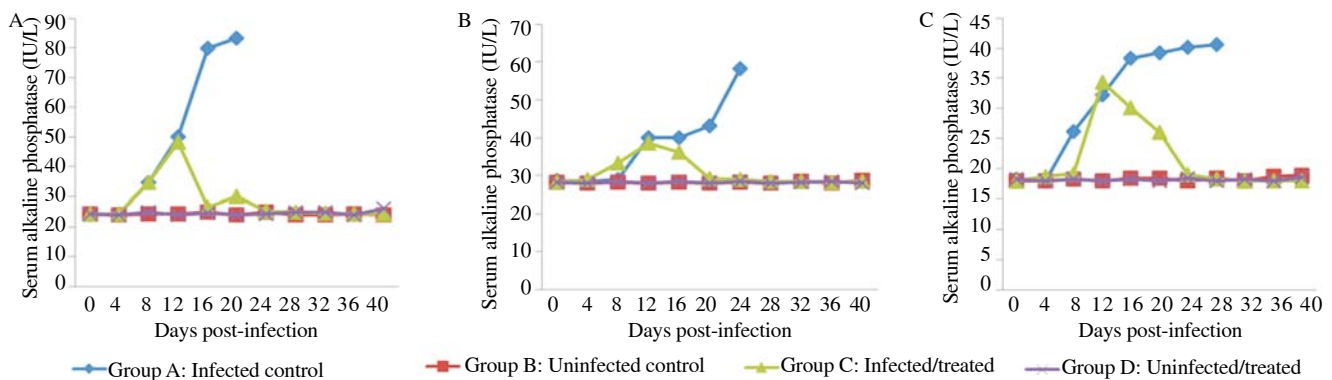


Figure 2. Comparative changes of serum alkaline phosphatase in *T. brucei* infected mice (A), rats (B) and rabbits (C) treated with Veriben® and their controls.

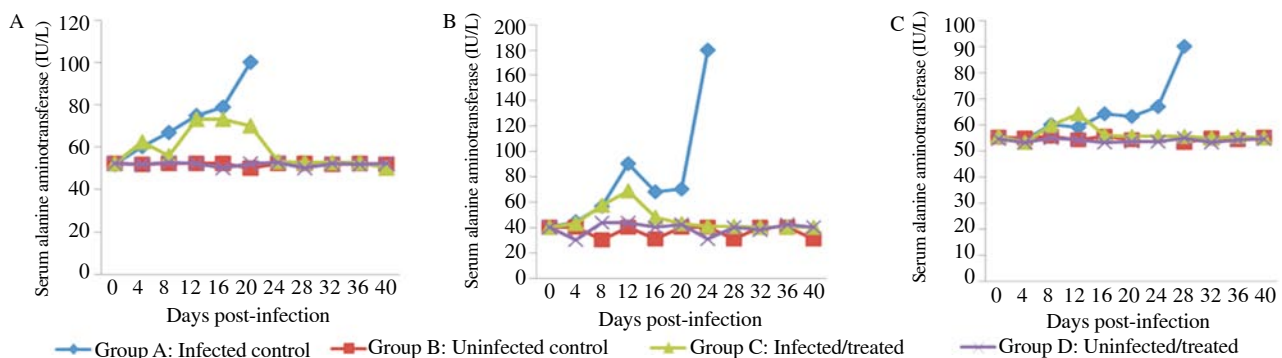


Figure 3. Comparative changes of alanine aminotransferase in *T. brucei* infected mice (A), rats (B) and rabbits (C) treated with Veriben® and their controls.

significantly ($P < 0.05$) by Days 20, 24 and 28 post-infection, while in Groups B and D, the values ranged between (40.20 ± 0.79) and (55.40 ± 0.93) IU/L were remained fairly constant ($P > 0.05$) throughout the study. In Group C, treated with 3.5 mg/kg of Veriben®, the preinfection value in mice attained peak by Day 12 post-infection and declined significantly ($P < 0.05$) to its preinfection value by Day 40 post-infection or by Day 28 post treatment. Similarly, in rats and rabbits, the preinfection values appreciated significantly ($P < 0.05$) to (68.90 ± 1.04) and (64.20 ± 1.00) IU/L, respectively before declining to their respective preinfection states by Day 40 post-infection or by Day 28 post treatment.

3.4. Comparative effect of diminazene diaceturate on serum aspartate aminotransferase changes

The mean serum aspartate of *T. brucei* infected mice, rats and rabbits treated with Veriben® and their controls are present in Figure 4. In the infected control mice, rats and rabbits (Group A), the preinfection values appreciated significantly ($P < 0.05$) by Days 20, 24 and 28 post-infection, respectively. In Groups B and D, the values remained fairly constant ($P > 0.05$) in the mice, rats and rabbits. In Group C infected but treated with 3.5 mg/kg of Veriben®, the preinfection values of all rodents appreciated before declining significantly ($P < 0.05$) by Day 40 post-infection or by Day 28 post treatment.

3.5. Comparative effect of diminazene diaceturate on serum creatinine changes

The mean serum creatinine levels of *T. brucei* infected mice, rats

and rabbits treated with Veriben® and their controls are present in Figure 5. In the infected control (Group A) mice, rats and rabbits, the pre-infection values reached their respective peaks ($P < 0.05$) on Days 20, 24 and 28 post-infection respectively. In Groups B and D, the values ranged between (0.20 ± 0.06) and (0.08 ± 0.11) mmol/L were remained fairly constant ($P > 0.05$) without any appreciable increase for all rodents. However, in the infected mice, rats and rabbits treated with Veriben® (Group C), the pre-infection values appreciated to (0.70 ± 0.10) and (1.00 ± 0.13) mmol/L by Day 12 post-infection in mice and rats respectively. These later declined significantly ($P < 0.05$) to its preinfection value by Day 40 post-infection or by Day 28 post treatment in mice, rats and rabbits.

3.6. Comparative effect of diminazene diaceturate on blood urea nitrogen changes

The mean blood urea nitrogen of *T. brucei* infected mice, rats and rabbits treated with Veriben® and their control are present in Figure 6. In the infected control (Group A) mice, rats and rabbits, the preinfection values appreciated significantly ($P < 0.05$) by Days 20, 24 and 28 post-infection respectively. In Groups B and D, the values remained fairly constant ($P > 0.05$) throughout the experiment. Meanwhile in the mice, rats and rabbits infected but treated with 3.5 mg/kg of Veriben® (Group C), the preinfection values appreciated to (28.70 ± 0.67), (22.20 ± 0.59) and (30.80 ± 0.69) mmol/L by Day 12 post-infection. These values later declined significantly ($P < 0.05$) to their preinfection values by Day 40 post-infection or by Day 28 post treatment.

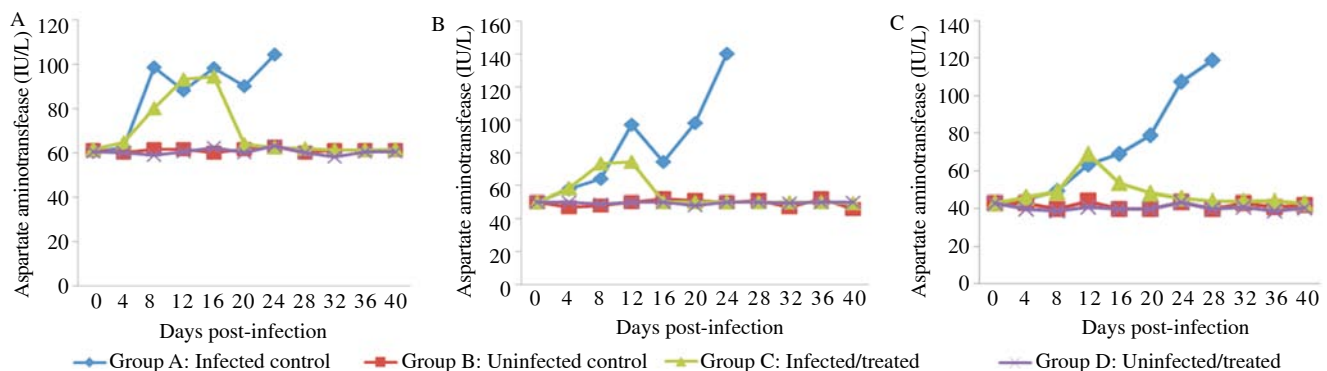


Figure 4. Comparative changes of aspartate aminotransferase in *T. brucei* infected mice (A), rats (B) and rabbits (C) treated with Veriben® and their controls.

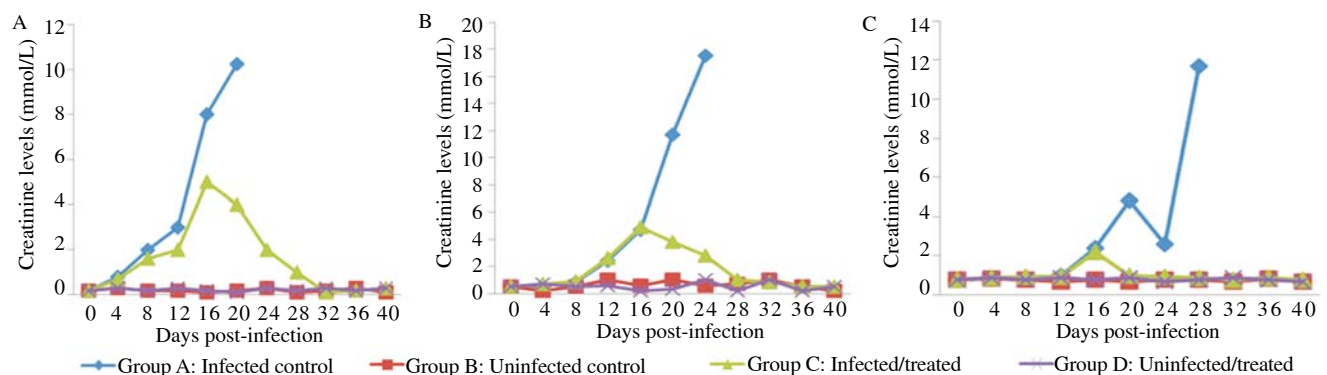


Figure 5. Comparative changes of creatinine changes in *T. brucei* infected mice (A), rats (B) and rabbits (C) treated with Veriben® and their controls.

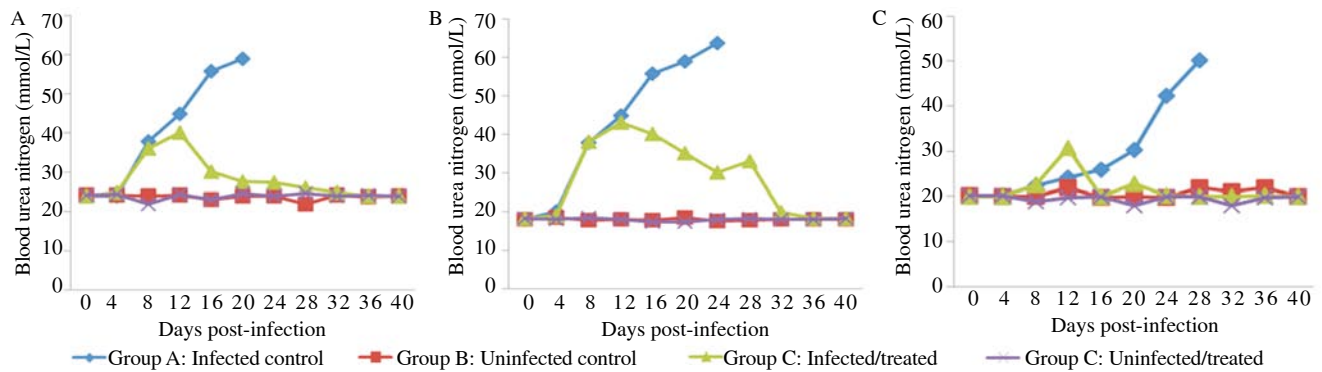


Figure 6. Comparative changes of blood urea nitrogen in *T. brucei* infected mice (A), rats (B) and rabbits (C) treated with Veriben® and their controls.

3.7. Comparative effect of diminazene diaceturate on gross and histopathological changes

At postmortem, the animals (mice, rats and rabbits) that died of the infection all showed splenomegaly, hepatomegaly and nephritis, however cardiomegaly was exclusive to the rabbits. At histopathology however, common to all species were degenerative changes in both liver and kidneys which were more severe in mice and rats and moderate in rabbits. The spleen of the mice showed giant macrophages (Figure 7) that of the rats showed epithelioid giant cells (Figure 8), while those of the rabbits showed haemosiderosis and eosinophilic infiltrations (Figure 9). However, exclusive to the rabbits there was hydrophobic degeneration, necrosis and mononuclear cellular infiltrations in the myocardium (Figure 10). Meanwhile, the liver of the mice showed central vascular congestion with sheathed artery (Figure 11A), that of the rats (Figure 11B) showed sinusoidal haemorrhages and nuclear vacuolation while that of the rabbits (Figure 11C) showed very mild, but widespread vacuolar degeneration of hepatocytes with periportal mononuclear cellular aggregations and presence of scattered megalocytes. The kidneys of the mice (Figure 12A) infected with *T. brucei* showed interstitial haemorrhages and necrosis of the tubular epithelial cells, that of rats (Figure 12B) showed multi-focal glomerular degenerations and tubular ballooning in the cortex while that of rabbits (Figure 12C) showed mild but diffuse interstitial mononuclear cell infiltration especially at the corticomedullary junction.

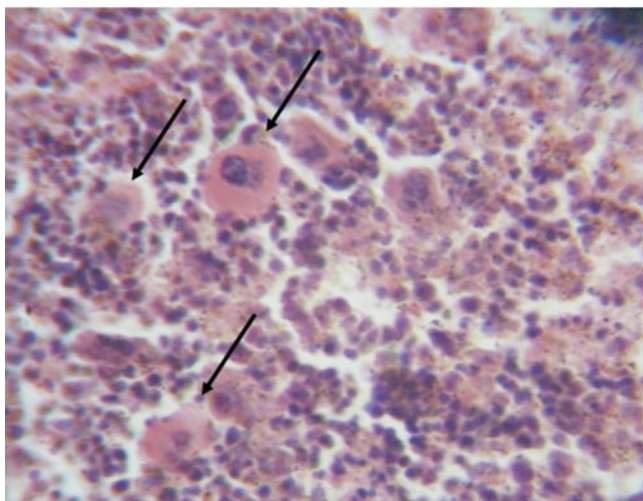


Figure 7. Photomicrograph of the spleen of *T. brucei* infected but untreated mouse showed macrophages (arrows) (H&E 1320×).

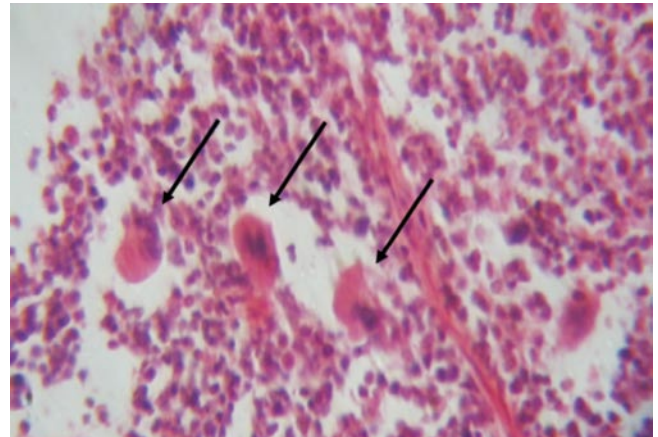


Figure 8. Photomicrograph of the spleen of *T. brucei* infected untreated Albino rat showed epithelioid giant cells (arrows) (H&E 1330×).

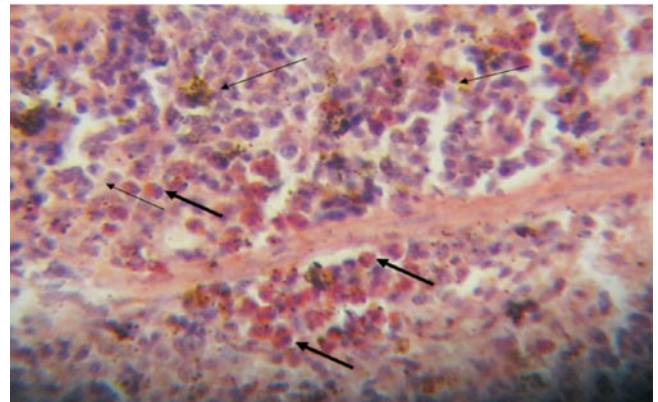


Figure 9. Photomicrograph of the spleen of *T. brucei* infected but untreated New Zealand rabbit showed hemosiderosis (thin arrows) and eosinophilic infiltrations (thick arrows) (H&E 1320×).

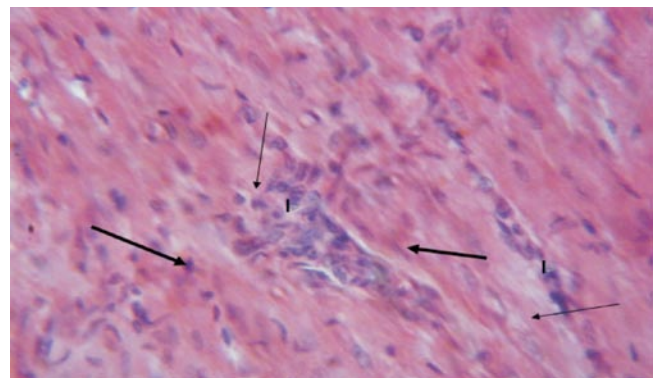


Figure 10. Photomicrograph of the myocardium of *T. brucei* infected but untreated New Zealand rabbit showed hydrophobic degeneration (thin arrows), necrosis (thick arrows) and mononuclear cell infiltration (I) (H&E 330×).

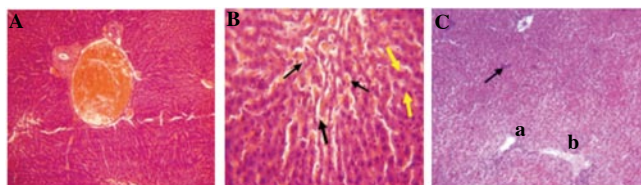


Figure 11. Photomicrograph of the liver of mice (A) infected with *T. brucei* showed central vascular congestion and sheathed artery (H&E 200×), rats (B) showed sinusoidal haemorrhages (black arrows) and nuclear vacuolation (yellow arrows) (H&E 400×) and rabbits (C) showed very mild, but widespread vacuolar degeneration of hepatocytes (a), periportal mononuclear cellular aggregations (b) and presence of scattered megalocytes (arrow) (H&E 350×).

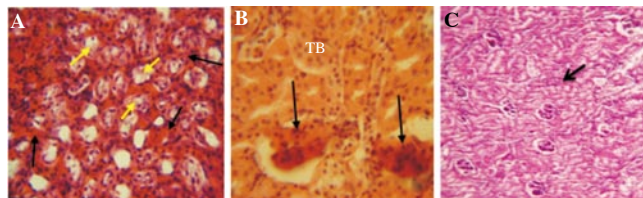


Figure 12. Photomicrograph of the kidney of mouse (A) infected with *T. brucei* showed interstitial haemorrhages (black arrows) and necrosis of the tubular epithelial cells (yellow arrows) (H&E 200×), rat (B) showed multifocal glomerular degenerations and tubular ballooning (TB) in the cortex (arrows) (H&E 400×) and rabbits (C) showed mild but diffuse interstitial mononuclear cell infiltration especially at the corticomedullary junction (arrowed) (H&E 350×).

4. Discussion

The results of this study showed serious variations in intensities and patterns with a few similarities of the infection in the different animal models examined under the same experimental conditions. Continuous rise with variation in peaks of parasitemia were recorded among the infected mice, rats and rabbits. However, peak parasitemia was lower among the rabbits as compared to that of mice and rats. The ability of the host to limit the peak and the number of the parasites is however dependent on whether the infection is acute, subacute or chronic and this may explain the reason why parasitemia in the rabbits, appreciated but declined following treatment by Day 12 post-infection and by Day 16 post-infection as it maintained a gradual increase and declined again. The drug diminazene diaceturate (Veriben®) long-acting used in this study is a diminazene, a drug of aromatic diamidines family. It was first introduced in 1999, which in this study, proved to be very effective in modulating the parasitemias and the attendant biochemical and pathological changes in all the different animal models back to their preinfection values or states respectively. This drug has been reported lately, to be effective against *Trypanosoma congolense* infection in an Alsatian dog[27]. According to the manufacturers, the mechanism of its action is that, it distorts the helical structure and subsequent destruction of trypanosome DNA and has a higher therapeutic index with an added advantage being in combination with cyanocobalamin, hydroxocobalamin and antipyrine which has the ability to control pyrexia drastically. This is in agreement with Kimeli *et al.*[27] and in consonance with the findings of this study, where parasitemia and pyrexia were drastically reduced following the administration of this drug in all the animal models. In addition, the presence of hydroxocobalamin is a long analogue of cyanocobalamin which gives to sustain high levels of cyanocobalamin, which is necessary for the

synthesis of red blood cells and a quicker recovery of haematocrit indices[27]. In this study, a standard dose of the inoculums was administered, which produced uniform prepatent period among rats and mice (4 days) and different in rabbits (8 days), however, had a similar initial parasite counts, which means that the initial parasite replication rates were similar irrespective of the rodent species or susceptibility. These observations have earlier been observed among *T. brucei* infections in dogs[27], red-fronted gazelles (*Gazella rufifrons*)[5,6,28-30] and giant rats (*Cricetomys gambianus*)[7], and in *Trypanosoma brucei gambiense* infection in vervet monkeys (*Cercopithecus aethiopes*)[3], and in baboons (*Papio anubis*)[4].

On one hand, from Day 4 post-infection, among the mice and rats and Day 8 in rabbits experimentally infected with *T. brucei*, increased liver enzymes (aspartate and alanine aminotransferase) and serum metabolites (creatinine, urea, total and conjugated bilirubin concentrations) occurred thereafter without abating in the infected but untreated controls. The presence of these enzymes and metabolites in sera can be corroborated by the unprecedented tissue damages observed in the liver and kidneys of the laboratory animals in this study. Similar tissue damages and associated biochemical alterations following experimental *T. brucei* infections have been reported in rats[16] and in *Trypanosoma brucei gambiense* infected baboons (*Papio anubis*)[4]. The peak of the various biochemical parameters was however, higher among mice and rats than in rabbits with the exception of aspartate aminotransferase where the peak value was the highest among the mice followed by rabbits then rats. The reason for this change in pattern was not clear, however, variations in host parasite relationship have often accounted for unanticipated algorithms in values in research groups[31]. These biochemical changes were however reversed in the various animal species following the administration of diminazene diaceturate (Veriben®) at 3.5 mg/kg body weight. The elevated levels of aspartate and alanine aminotransferase were suggestive of liver damage. This was also observed more classically among the infected mice and rats, where these enzymes increased as the infection progressed without abating. Elevated levels of creatinine and blood urea nitrogen also accompanied the experimental infection in all the various species of laboratory animals. These enzymes in sera during trypanosomosis have been linked with severe kidney dysfunction[28]. The retention of urea with high levels of creatinine in the body showed that the kidneys were severely disabled as such. They failed to excrete the byproducts of metabolism due to renal failure. It was therefore, concluded that the experimental *T. brucei* infection among the mice, rats and rabbits caused various biochemical changes which were suggestive of severe liver and kidney dysfunctions and were reversed by the treatment with diminazene diaceturate (Veriben®). Secondly, the mice and rats were better animal models for studying the progression of the disease than rabbits.

Conflict of interest statement

We declare that we have no conflict of interest.

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