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Effects of Diminazene Diaceturate (veriben®) on Serum and Clinico-pathological Changes in Guinea Pigs (*Cavia porcellus*) Experimentally Infected with *Trypanosoma brucei brucei*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study was designed to determine effects of diminazene diaceturate (veriben®) on the serum and clinico-pathological changes in guinea pigs experimentally infected with *Trypanosoma brucei brucei* (*T. brucei brucei*). Thirty apparently healthy unsexed guinea pig weighing between 5-10 kg were used for the study. *Trypanosoma brucei brucei* and 4 Albino rats used as donorswere obtained from NAITOR (Nigerian Institute of Trypanosome and Onchcerciasis) Kaduna State Nigeria. Guinea Pigs were randomly allocated into 6 groups designated as A, B, C, D, E and F. All the infected (A,B and

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C) had prepatent period of 16 days with similar level of parasitaemia of 45.7 ± 3.38 and clinical sign observed are pyrexia, pale feet, snout, anaemia and emaciation. Mean chloride, bicarbonate, sodium, calcium, potassium and magnesium serum ion concentration level decreased significantly following establishment of parasitemia. These changes reverted to their pre-infection values by day 28 in all the affected animals following treatment with diminazine diaceturate (veriben®) at 3.5 mg/kg and 7.0 mg/kg BW. Liver, kidney, lungs, and spleen samples were taking from humanly sacrificed guinea pig and fixed in 10% formalin. The histopathological examination from positive control animals showed no visible area of lesions but in contrast with the infected negative control group revealed gross distortion of tissue architecture. Observations from the treated animals showed less distortions of tissue architecture this might have been aided by administered drugs at 7.0 mg/kg. In conclusion, evidence has shown that the administration of Veriben® at the dose rate of 3.5 mg/kg and 7.0 mg/kg have the potentials of modulating the state of anaemia, immunosuppression and serum electrolytes levels, gross and histopathological changes in trypanosome-infected guinea pigs in a dose dependent manner.

Keywords: Diaminazenediaceturate (veriben ®); dosage; vital organs; Trypanosoma brucei brucei; Guinea pigs; histopathological changes.

1. INTRODUCTION

Trypanosome is an important haemoparasite of man and animals especially in Africa particularly in South Sahara and Sudan region [1]. African trypanosomosis is caused by protozoan parasite of the genus *Trypanosoma*. Trypanosomosis affect almost all vertebrates including man and his domestic animals. Wild animals such as bovidae and suidae, specifically act as asymptomatic carriers. These organisms are transmitted by tsetse fly and have high morbidity and mortality among livestock. The trypanosomes group of *T. brucei* (*T. brucei brucei,, T. b. gambiense, T. b. rhodensiense*) and *T. evansi* are more tissue invading (humoral) whereas, *T. congolense*, *T. vivax* and *T. cruzi*are restricted to the blood circulation (haemic) as reported by Igbokwe, [2] and Mbayaand Ibrahim [3].

Guinea pigs (*Cavia porcellus*), also known as the cavy, belong to rodents species of the family *Caviidae* and the genus *Cavia*, they are universally kept as pets and often used as experimental animals [4-6]. These rodents are of different colours, with no visible tail and short-eared. Apart from their common name, these animals are not in the pig family, nor are they from Guinea. They originated from Andes, and from initial studies based on biochemistry and hybridisation studies it was concluded that they are domesticated descendants of a closely related species of cavy such as *Cavia aperea*, *Cavia fulgida*, or *Cavia tschudii*, and therefore, do not exist naturally in the wild [4- 6].

Despite of vast information on trypanosomosis and effect of diminazene diaceturate (veriben®) on Serum and Clinico-pathological changes among laboratory animals, little or no such information exists on the effects of this drug on clinic-pathological changes and serum electrolyte levels in guinea pigs experimentally infected with *T. brucei brucei.* As such this novel information prompted the need for this research.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Thirty (30) apparently healthy Guinea pigs of both sexes, weighing between 5-10kgwere used for the study. They were purchased from Jos, Plateau State and transported to Maiduguri Borno state. Thereafter, the animals were kept in clean and well ventilated cages at the Large Animal Clinic of the Veterinary Teaching Hospital, University of Maiduguri, Nigeria. They were fed with fresh vegetables and pelleted commercial grower feed (Vital® Feeds, PLC, Nigeria), and water was provided *ad libitum.* They were allowed to acclimatise for two weeks prior to the commencement of the experiment, during which they were routinely screened for parasites using standard methods as described by Soulsby [7]. The experimental procedure was done in accordance with the regulations of the Ethical Committee of the Faculty of Veterinary Medicine, University of Maiduguri.

2.2 Source of *Trypanosoma brucei brucei*

The trypanosoma parasites used for the study was "*Federe*" strain of *Trypanosoma brucei brucei*, with strain number which was obtained from NITOR (Nigerian Institute of Trypanosome and Onchocerciasis) Kaduna State, Nigeria. The

organism was isolated from an outbreak of bovine trypanosomosis in Nassarawa State of Nigeria. It was identified based on its morphology as described by Soulsby [7] and negative blood inhibition and infectivity test [8] and was stabilised by four passage in rats before storage in liquid nitrogen (LN). Four donor rats were used to multiply the parasites and transported by road from Kaduna to the Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Borno State Nigeria. The parasites were then maintained in Albino rats by serial passage until used.

2.3 Experimental Infection

Four (4) adult albino rats were used as donors of *T. brucei brucei*, the rats were purchased from NITOR, Kaduna State. They were screened for internal and external parasites using standard methods [7]. They were inoculated intraperitoneally with 0.5 ml of *T. brucei brucei* parasiteamic blood to multiply the parasites as described by Trindade et al. [9]. At 4 days post inoculation, parasiteamia was established and became patent. The donor rats were bled via the tail vein into a petri dish, the blood was diluted with phosphate buffered saline glucose (PBSG) (pH 7.4). Each Guinea pig in groups A, B and C was inoculated through intraperitoneal route with 0.5 ml of blood containing 1.0×10^6 *Trypanosoma brucei brucei* as quantified using serial dilution as reported by Herbert and Lumsden [10].

2.4 *In vivo* Experimental Protocol

Thirty Guinea pigs were randomly separated into six groups (A, B, C, D, E and F) of five each. At day zero, all the animals were bled for serum biochemistry to establish a baseline data. Thereafter, physical signs, general body conditions and body weights of all the animals were evaluated. Groups A, B and C were intraperitoneally (IP) inoculated. Blood sample were collected for serum electrolytes at interval of 4 days from day zero until the end of the experiment. Group A was infected with 0.5 ml of *Trypanosoma brucei brucei* (untreated control), Group B was infected with 0.5 ml of *Trypanosoma brucei brucei* but treated with *diaminazene diaceturate* (veriben®) at a dose rate of 7.0 mg/kg (single dose) IP by day 28 post infection. Group C was infected with 0.5 ml of *Trypanosoma brucei brucei* but treated with diaminazene diaceturate (Veriben®) at a dose rate of 3.5 mg/kg (single dose) IP by day 28 post infection. Group D was uninfected/untreated control. Group E was uninfected but treated with diaminazene diaceturate (Verben®) at a dose rate of 7.0 mg/kg (single dose) IP by day 28 post infection. Group F was uninfected but treated with diaminazene diaceturate (Veriben®) at a dose rate of 3.5 mg/kg (single dose) IP by day 28 post infection.

2.5 Post Infection Evaluation of Guinea Pigs

All the Guinea pigs were observed daily for the manifestation of clinical signs of trypanosomosis, which include morbidity and mortality. Meanwhile, detection of parasitaemia was done every 4 days post day zero and the degree of parasitaemia was projected by the rapid matching technique as described by Herbert and Lumsden [10].

2.6 Blood Collection

Blood for serum biochemistry examination were aseptically collected at 4 days interval preliminary from day 0 to day 64 post infection. The Guinea pigs were bled using 2 ml syringe through cardiac puncture 1.5 ml of the blood was transferred into plain tubes for serum biochemistry. Serum biochemistry l values were determined as described by Henry et al. [11] and Tietz [12].

2.7 Detection of Parasitaemia in Guinea Pig

To establish parasitaemia following inoculation of the respective groups with *Trypanosoma brucei brucei*, blood samples obtained from the cardiac puncture of the infected Guinea pigs were examined using wet mount, and haematocrit buffy coat method, while degree of parasitaemia was established by the "rapid matching technique" [10] every four days throughout the experimental period.

2.8 Histopathological Examinations of Guinea Pigs

All the guinea pigs that died or humanely sacrificed at the end of the study were subjected to detailed necropsy. Samples were taken from liver, kidney, lungs, spleen and were fixed in 10% formalin prior to fixation and subsequent stained with haematoxylin and eosin (H & E) according to the methods described by Drury and Wellington, Drury et al. [13,14]. Lesions were observed using

light microscope (Olympus Japan) at magnification ranging from ×100-400. The lesions observed under microscope were photographed using canon digital camera power short (A470).

2.9 Statistical Analysis

Data generated were expressed as mean ± standard deviation (SD). Two-way analysis of variance was used to compare the data between groups and value $p < 0.05$ was considered significant [15].

3. RESULTS

All the infected groups (A, B, and C) had a pre-patent period of 16 days with similar levels of parasitaemia of 45.7±3.38. The observed clinical signs in the infected groups (A, B and C) were pyrexia, pale feet, snout, pinae and mucous membrane, anaemia, dullness, emaciation and weight loss.

The mean chloride ion concentrations of the Guinea pigs (*C. porcellus*) continually decreased following establishment of parasitaemia by day 16 in all the treated groups at different days, similar findings was noticed on the mean bicarbonate ion concentrations of the Guinea pigs (*C. porcellus*) at day 16 post infection and mean serum sodium levels of Guinea pigs (*C. porcellus*) the serum sodium levels decreased significantly following establishment of parasitaemia by day 16 post infection in all the infected groups as show in (Fig. 1 - 3).

The mean serum calcium ion concentration, mean serum potassium levels and magnesium ion concentration of Guinea pigs (*C. porcellus*) experimentally infected with *T. brucei brucei* were continually decreased following establishment of parasitaemia by day 16 post infection in all the treated groups at different interval as presented in (Fig. 4 - 6).

In histopathology, the photomicrograph of the lungs of infected/untreated Guinea pigs (*C. porcellus*) in Group A, revealed multifocal aggregation of lymphoid cells (LGC) around the enlarged congested blood vessel (BV) and fibrous connective tissue as indicated by arrows on Plate I while the photomicrograph of lungs of Guinea pig (*C. porcellus*) infected and treated with 3.5 mg/Kg of veriben® in Group C, showed interstitial haemorrhage as indicated by (arrows), enlarged congested blood vessel (BV) and fibrous connective tissue (FB) (Plate II). The photomicrograph of the liver of the infected/untreated Guinea pigs (*C. porcellus*) in Group A, showed portal traidoedema (OD) congestion (AX) and interstitial inflammatory cells indicated by arrows on Plate III. The photomicrograph of the liver of Guinea pig (*C. porcellus*) infected and treated with 3.5 mg/Kg of veriben® in Group C, showed severe cirrhosis as indicated by (yellow arrow) and surrounded with interstitial inflammatory cellular infiltration on (Plate IV).

The photomicrograph of the kidney of infected/untreated Guinea pig (*C. porcellus*), showed focal interstitial oedema in the medulla as indicated by arrows on Plate V. The photomicrograph of the kidneys of Guinea pigs (*C. porcellus*) infected and treated with 3.5 mg/Kg of veriben®, showed a zone of inflammatory cells (X), glomerular degeneration (MX) and interstitial haemorrhage in the cortex as indicated by arrows on Plate VI.

The photomicrograph of the spleen of the infected/untreated Guinea pigs (*C. porcellus*) in Group A, showed white pulp hyperplasia and mild trabeculae as indicated by arrows on Plate VII. The photomicrograph of the spleen of the Guinea pigs (*C. porcellus*) infected and treated with 3.5 mg/Kg of veriben® in Group C, revealed white pulp hyperplasia, brown patches of haemosiderin and mild trabeculae as indicated by arrows (Plate VIII). While no histopathologically changes seen in the lungs, liver, kidneys and spleen of Groups B, D, E and F.

4. DISCUSSION

All the infected Guinea pigs became parasitaemic by day 16 post infection. This is contrary to the findings of Umar et al. [16] and Omer et al. [17] who observed a prepatent period of 4 days in rats infected with *Trypanosoma brucei brucei*. The major clinical signs observed were those of respiratory distress, pale ocular mucous membrane, raised hair coat, dullness, anorexia and emaciation. The experimental Guinea pigs, contrary to control animals, experienced weight loss after a little less than two weeks of infection. This may be due to the lack of appetite and drop in feed intake, observed amongst the infected guinea pigs. Similar findings have been reported in rats infected with *T. brucei brucei* [18,19] as well as in rats infected with *T. brucei gambiense* [20] and in rabbits infected with *T. brucei brucei* [21].

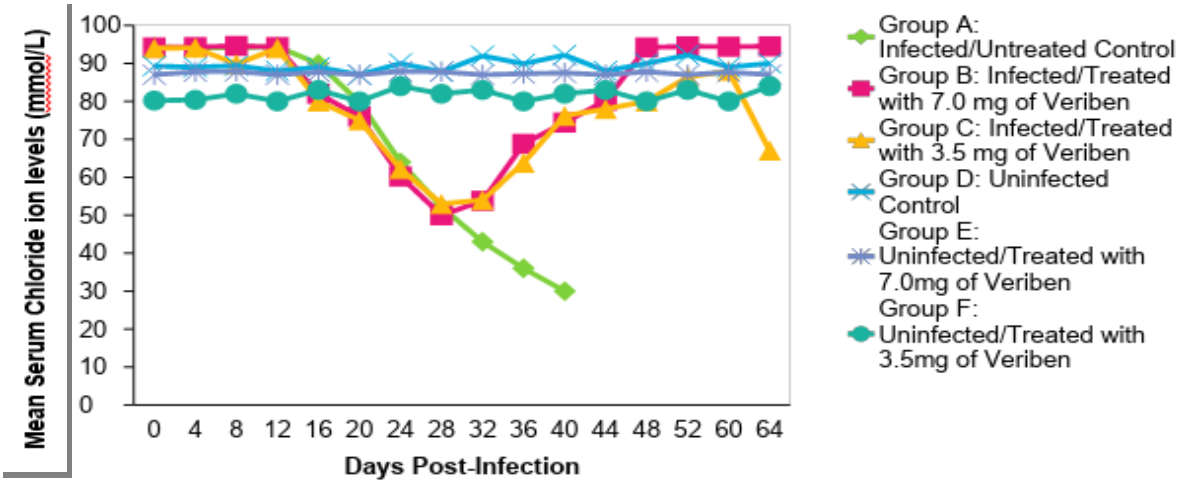

Fig. 1. Effect of diminazene diaceturate (Veriben®) on the mean serum chloride ion concentration (mmol/L) of Guinea pigs (*C. porcellus*) experimentally infected with *T. brucei brucei* and their controls

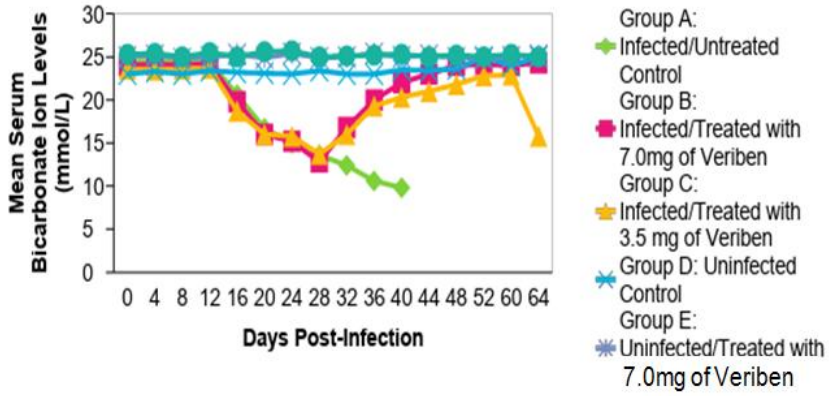

Fig. 2. Effect of diminazene diaceturate (Veriben®) on the mean serum bicarbonate ion levels (mmol/L) of Guinea pigs (*C. porcellus*) experimentally infected with *T. brucei brucei* and their controls

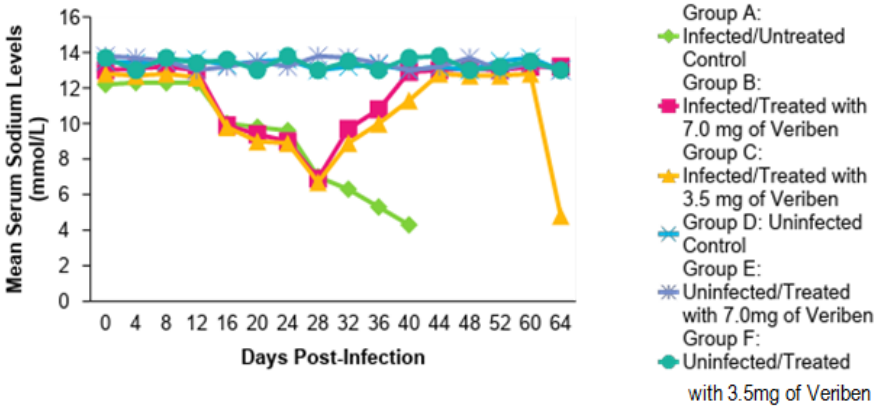

Fig. 3. Effect of diminazenediaceturate (Veriben®) on the mean serum sodium levels (mmol/L) of Guinea pigs (*C. porcellus*) experimentally infected with *T. brucei brucei* and their controls

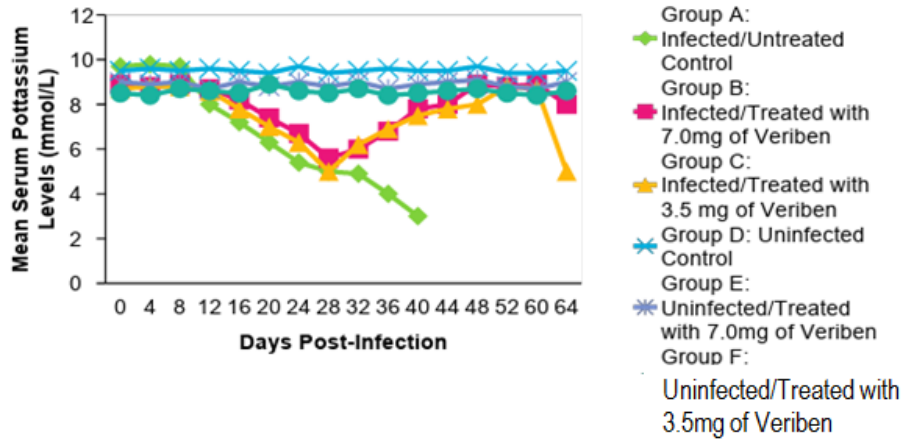

Fig. 4. Effect of diminazenediaceturate (Veriben®) on the mean serum potassium levels (mmol/L) of Guinea pigs (*C. porcellus*) experimentally infected with *T. bruceibrucei* and their controls

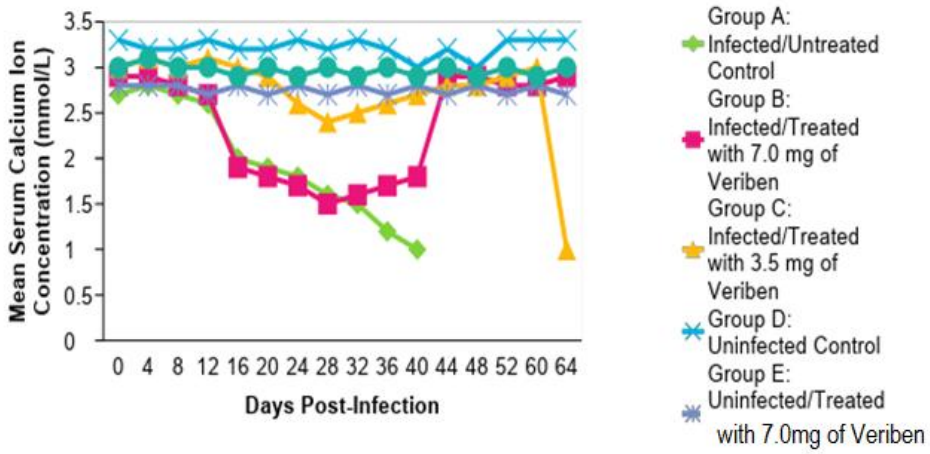

Fig. 5. Effect of diminazenediaceturate (Veriben®) on the mean serum calcium ion concentrations (mmol/L) of Guinea pigs (*C. porcellus*) experimentally infected with *T. brucei brucei* and their controls

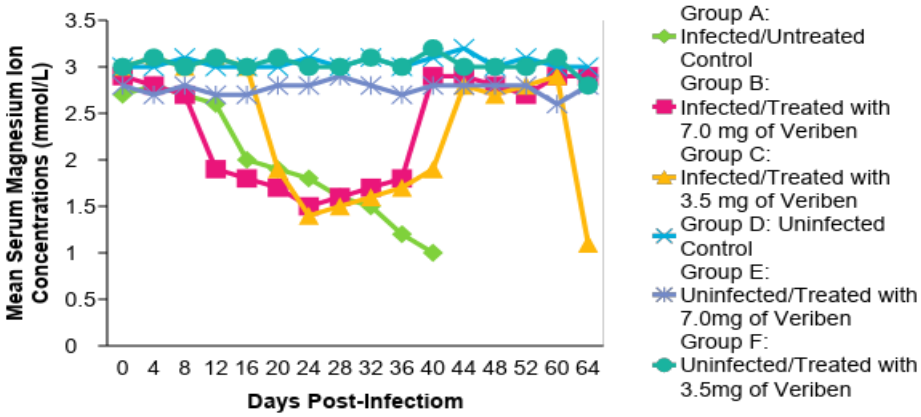

Fig. 6. Effect of diminazene diaceturate (Veriben®) on the mean serum magnesium ion concentrations (mmol/L) of Guinea pigs (*C. porcellus*) experimentally infected with *T. brucei brucei* and their controls

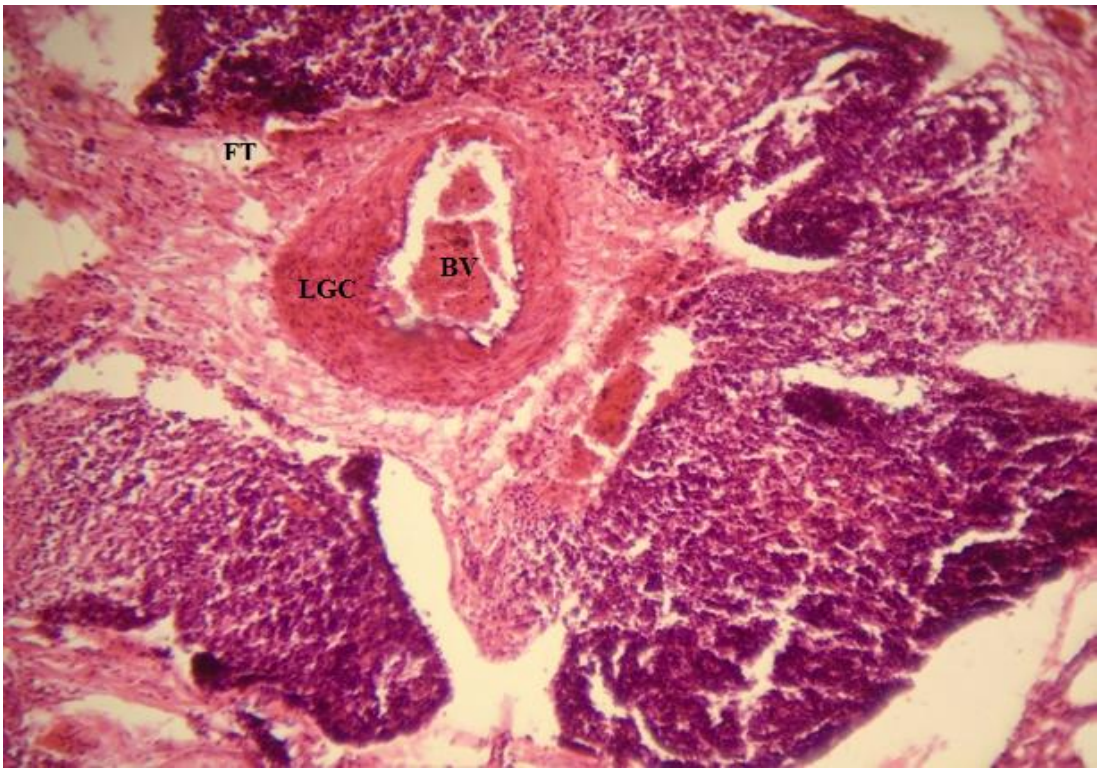

Plate I. Photomicrograph of lungs of infected/untreated Guinea pig (*C. porcellus*) showing multifocal aggregation of lymphoid cells (LGC) around the enlarged congested blood vessel (BV) and fibrous connective tissue (FT) H&E x100

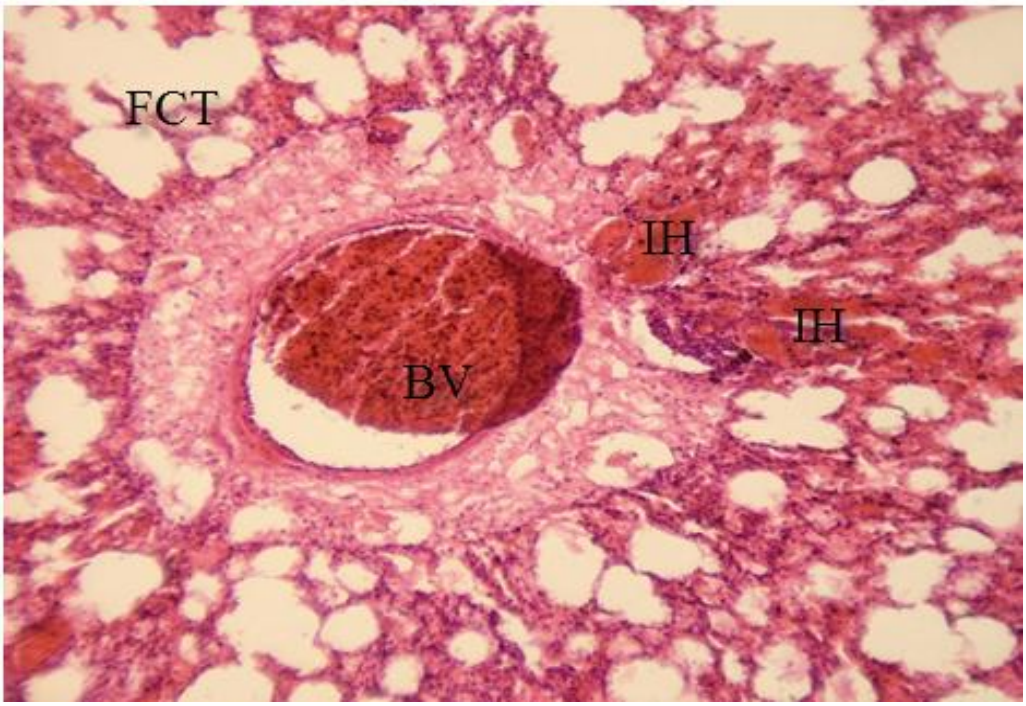

Plate II. Photomicrograph of lungs of Guinea pig (*C. porcellus*) infected and treated with 3.5 mg/Kg of Veriben® showing interstitial haemorrhage (IH) enlarged congested blood vessel (BV) and fibrous connective tissue (FCT) H&E x100.

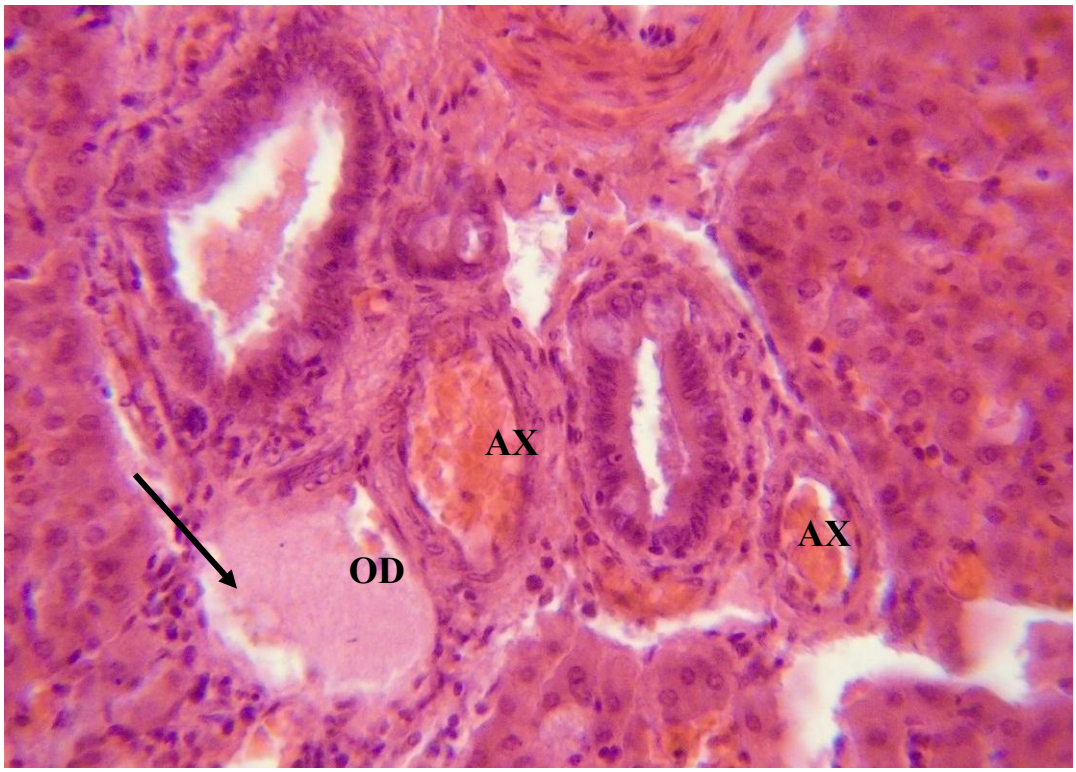

Plate III. Photomicrograph of liver of infected/untreated Guinea pig (*C. porcellus*) showing portal traidoedema (OD), congestion (AX) and interstitial inflammatory cells (arrows) H&E x400

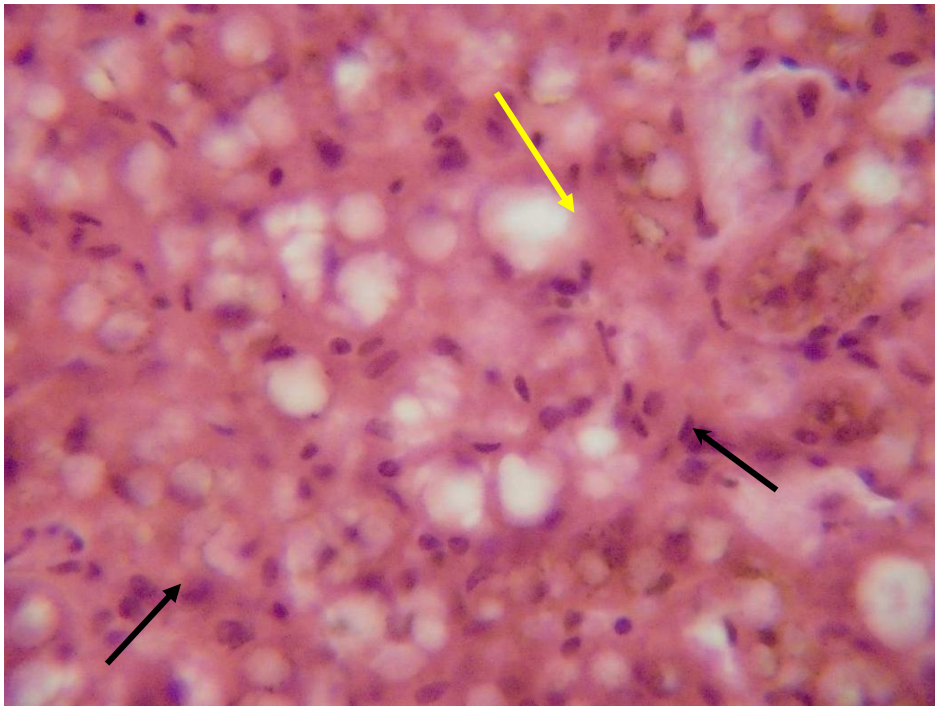

Plate IV. Photomicrograph of liver of Guinea pig (*C. porcellus*) infected and treated with 3.5 mg/Kg of Veriben® showing severe cirrhosis (yellow arrow) and interstitial inflammatory cellular infiltration (arrows) H&E x 200

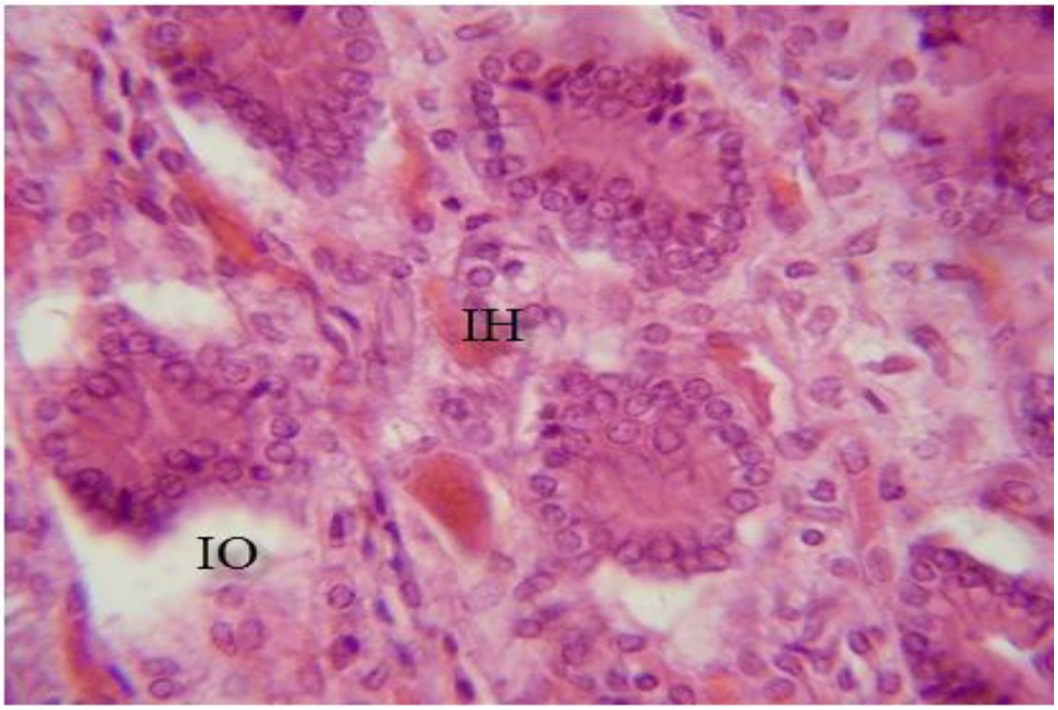

Plate V. Photomicrograph of kidney of infected/untreated Guinea pig (*C. porcellus*) showing focal interstitial oedema in the medulla (IO) and interstitial haemorrhage (IH) H&E x 400

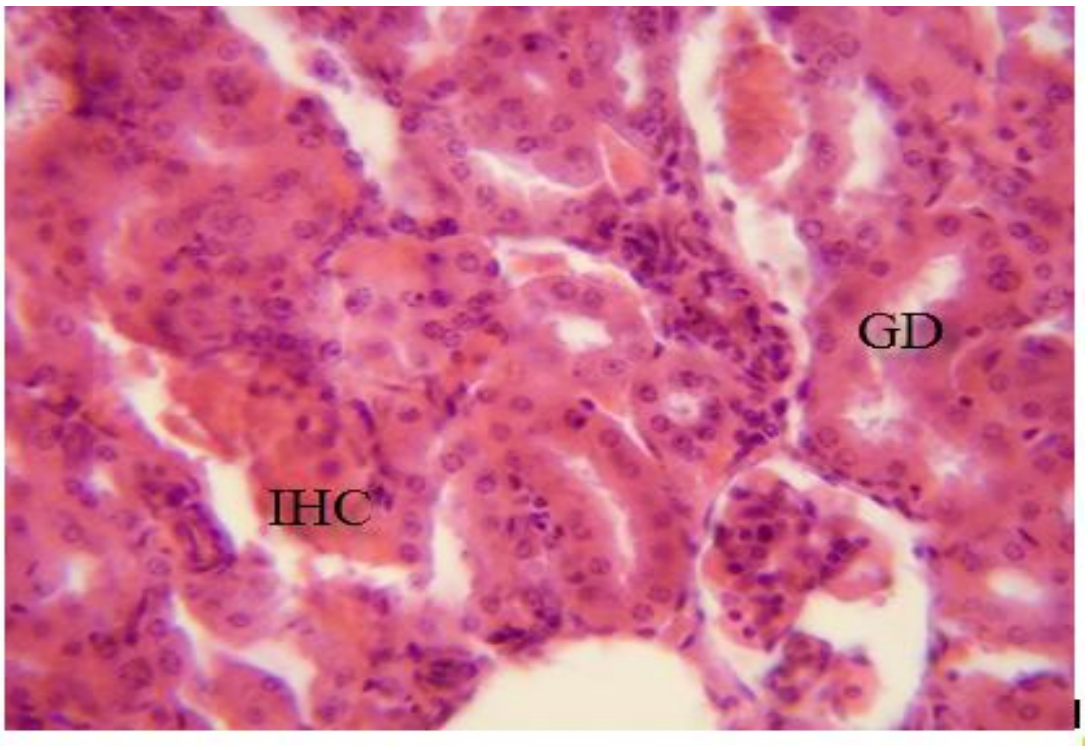

Plate VI. Photomicrograph of kidney of Guinea pig (*C. porcellus*) infected and treated with 3.5 mg/Kg of Veriben® showing zone of inflammatory cells (X), glomerular degeneration (MX) and interstitial haemorrhage in the cortex (arrows) H&E x 400

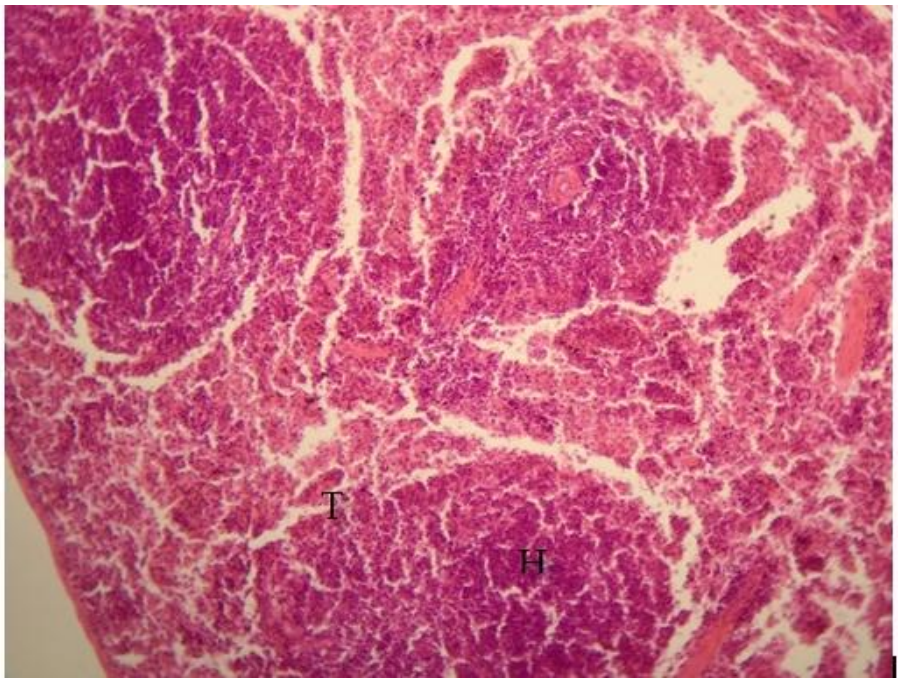

Plate VII. Photomicrograph of spleen of infected/untreated Guinea pig (*C. porcellus*) showing white pulp hyperplasia (H) and mild trabeculae (T) H&E x100.

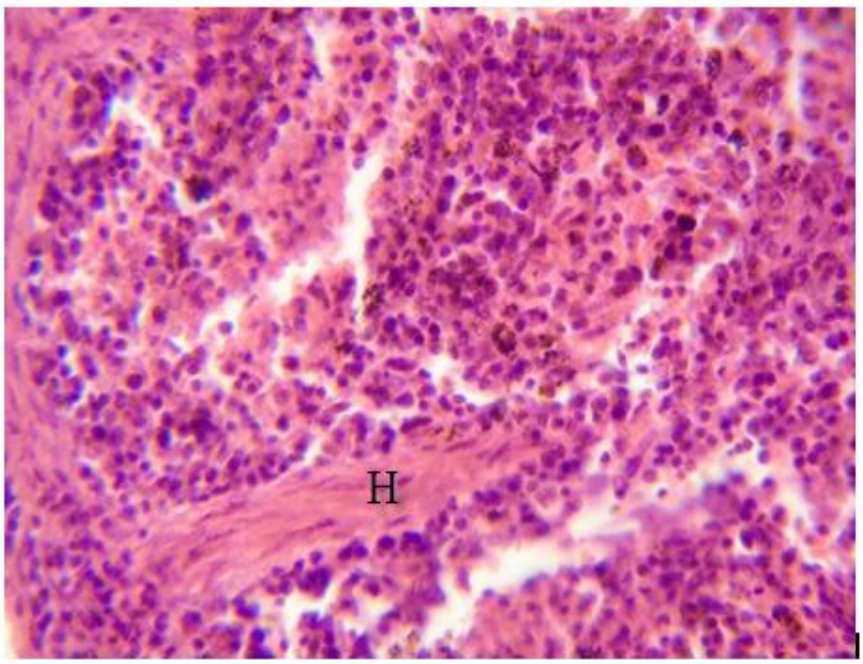

Plate VIII. Photomicrograph of spleen of Guinea pig (*C. porcellus*) infected and treated with 3.5 mg/Kg of Veriben® showing white pulp hyperplasia with brown patches of haemosiderin and mild trabeculae (H) H&E x100

The *T. brucei brucei* infected Guinea pigs were observed to be associated with marked reduction in serum sodium and chloride ion levels, and this might have been due to renal tubular damage of the kidneys.

The decreased level of serum potassium observed in the current study was probably due to dehydration associated with tissue hypoxia [12]. Reduction in bicarbonate ion (HCO_3) levels may be probably due to acidosis. The reduction

may also be due to decreased alveolar ventilation and tissues hypoxia similar findings was reported by Ogunsanmi et al. [22] in sheep infected by trypanosome.

The low bicarbonate levels can also be attributed to the massive leakages of some electrolytes from cells and tissues damage. However, the intermittent increase, low level and subsequent return of these electrolytes to pre-infection levels suggest the efficacy of the therapies, otherwise it might be as a result of massive cell and tissue damage at the terminal phase of this single infection. The decrease in the levels of calcium that was observed in this study agrees with the findings reported in cattle infected with *T. congolense* [23] and sheep infected with *T. brucei brucei* [22]. This is said to be due to the deficiency in the parathyroid hormone as a consequence of the destruction of the parathyroid glands or a decrease in serum carriers, which in this case happens to be albumin. The drop in the level of serum magnesium concentration noted among the *T. brucei brucei* infected Guinea pigs observed in this study does not tally with the findings of Sow et al. [24] among donkeys in Burkina Faso and that of Chaudhary and Iqbal [25] among camels in Pakistan. This may be as a result of the difference in the species of animals used. The drop in magnesium concentration in blood observed in this study might be due to lowered dietary intake due to the infections. Biochemical evaluation of the body fluids gives an indication of the functional state of the various body organs and biochemical changes in body fluids that result from infections depend on the species of the parasite and its virulence [26].

The histopathological examination is used to explain the details information of the histology of the concern tissues. The tissue sections from positive control animals showed no visible area of lesions but in contrast sections of the tissues from infected negative control animals group revealed gross distortion of tissue architecture with complete loss of cellular morphology accompanying with pronounced inflammatory changes associated with multifocal aggregation of lymphoid cells around enlarged congested blood vessels and fibrous connective tissues. Interstitial haemorrhages, severe cirrhosis and interstitial inflammatory cellular infiltration, interstitial oedema in the medulla showed a zone of inflammatory cells, glomerular degeneration and interstitial haemorrhage in the cortex, white pulp hyperplasia, mild trabeculae and brown patches of haemosiderindue to lysis of blood cells. Observations of tissue sections in treated animals showed less distortions of tissue architecture suggesting attempts by the host at restoration of cellular morphology; this might have been aided by administered drugs.

The lungs of the infected and untreated Guinea pigs (*C. porcellus*) showed multifocal aggregation of lymphoid cells around enlarged congested blood vessels and fibrous connective tissues. Biswas et al. [27] and Biswas et al. [28] also detected similar type of changes in the lungs of rats experimentally infected with *T. evansi*. In contrast, .Nagle et al. [29] observed no clinical changes in the lungs of *T. rhodesiense* infected rabbits. while the photomicrograph of lungs of Guinea pig (*C. porcellus*) infected and treated with 3.5 mg/Kg of Veriben® in Group C, showed interstitial haemorrhages, enlarged and congested blood vessel and fibrous connective tissue which is contrary to the findings reported by Takeet and Fagbemi [30], Rehamand Magdi [31], Sivajothi et al. [32] in which they observed oedema, congestion and multifocal alveolar emphysema. The congestion and oedema in the lungs were primarily due to the inflammatory response to the parasite resulting in vasodilatation and exudation in the focal areas. Atelectasis, augmented cellularity of the alveolar wall, hyperplasia of the peri-bronchiolar lymphoid tissues and perivascular infiltration of lymphocytes around small blood vessels and haemorrhages. Correlated type of changes were also observed in the lungs of rats experimentally infected with *T. evansi* [27,28]. However, these findings were not in link with those reported by Nagle et al. [29] who reported no changes in the lungs of *T. rhodesiense* infected rabbits. The photomicrograph of the liver of the infected and untreated Guinea pigs (*C. porcellus*) in Group A, showed portal traid oedema, congestion, and interstitial inflammatory cells. Meanwhile, the photomicrograph of the liver of the Guinea pigs (*C. porcellus*) infected and treated with 3.5 mg/Kg of Veriben® in Group C, showed severe cirrhosis and interstitial inflammatory cellular infiltration. This finding agrees with the report of [33,34] that observed hepatic lesions in *T. brucei brucei* infected deer mice (*C. P. maniculatus*) and in red fronted gazelles (*Gazella rufifrons*) which consist of necrosis of few hepatocytes, proliferation and hypertrophy of Kupffer's cells which exhibited increased phagocytosis--particularly of erythrocytes, as well as perivascular cuffs consisting of lymphocytes, plasma cells and macrophages which agrees

with the findings of Omotainse and Anosa [1] observed hyperplasia of the red pulp, congestion of the sinuses, enlargement of the lymphoid nodules, increased erythrophagocytosis, haemosiderosis and proliferation of plasma cells were the lesions seen in the spleen in acute *T. congolense, T. vivax* and *T. brucei* infections.

The histopathology of the kidneys of Guinea pig (*C. porcellus*) infected with *T. brucei brucei*, but not treated showed focal interstitial oedema in the medulla meanwhile, the photomicrograph of the kidney of Guinea pig (*C. porcellus*) infected and treated with 3.5 mg/Kg of Veriben® in Group C, showed a zone of inflammatory cells, glomerular degeneration and interstitial haemorrhage in the cortex which agreed with the result reported by Bal et al. [35] and Sivajothi et al. [32] in the rats infected with *T. evansi* and also similar with the result reported by Onah et al. [36] and Auduo et al. [37]. It has been reported that changes in the kidneys are mainly due to the toxins produced by the parasite and the accumulation of immune complexes which impair the structure and function of the kidney [38,39].

This is similar to the observations of Anosa and Kaneko [33] that deer mice and in red fronted gazelles [34] infected with *T. brucei brucei* showed renal lesions consistent with severe glomerulonephritis characterised by deposition of electron dense material along the basement membrane and in the mesangium of the glomerular tufts, and less frequently beneath the basement membrane and visceral epithelium of the Bowman's capsule and within the peritubular vessels. This is in part, associated with the fact that, neutrophils with fewer macrophages and lymphocytes invaded the glomeruli. The photomicrograph of the spleen of the infected/untreated Guinea pig *(C. porcellus*) in Group A, showed white pulp hyperplasia and mild trabeculae. The spleen of Guinea pig (*C. porcellus*) infected and treated with 3.5 mg/Kg of Veriben®, showed white pulp hyperplasia, brown patches of haemosiderin granules was evident in most of the sections of spleen which agrees with the findings of Reham and Magdi [31]. as well as with the findings reported by Bal et al. [35]. In rats infected with *T. evansi* and mild trabeculae. The formation and location of haemosiderin coincides with and place with destruction of blood leading to haemoglobin breakdown and subsequent splitting of the iron from the hematin hemosidering [40]. However, there were no histopathological changes seen in the lungs, liver, kidneys and spleen in the groups infected with *T. brucei brucei,* but treated with 7.0 mg/kg of Veriben®, the uninfected control group, the uninfected but treated with 7.0 mg/kg of Veriben® and the group that was uninfected but treated with 3.5 mg/kg of Veriben®.

5. CONCLUSION

In conclusion, evidence has shown that the administration of Veriben® at the dose rate of 3.5 mg/kg and 7.0 mg/kg have the potentials of modulating the state of anaemia, immunosuppression, and serum electrolytes levels, gross and histopathological changes in trypanosome-infected guinea pigs in a dose dependent manner. It also provide information on the changes that might occured following *T. brucei brucei* infection in Guinea pigs.

In this findings, we can concluded that the *T. brucei brucei* isolate used in this study was pathogenic to guinea pigs.

6. RECOMMENDATION

It is therefore recommended that Guinea pigs infected with *Trypanosoma brucei brucei* should be treated with a single dose of diaminazene diaceturate (Veriben®) at the rate of 7.0 mg/kg body weight being the more effective of the two doses.

ETHICAL APPROVAL

Ethical committee on animal welfare in University of Maiduguri, Borno state, Nigeria has given approval to go ahead with project considering the importance, relevance and noble concept of the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

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