Scientific Research Publishing

Advances in Infectious Diseases, 2020, 10, 111-129
https://www.scirp.org/journal/aid
ISSN Online: 2164-2656
ISSN Print: 2164-2648

Recorded Marked Changes in the Haematological and Immune Responses of Two Non-Transgenic Rodents Inoculated Orally and Intraperitoneally with *Trypanosoma brucei brucei*

O. N. Goselle[1*], S. S. Udoh[1], C. O. Ejete[1], I. A. Iruobe[1], S. Idoko[1], A. D. Gyang[1], Y. M. Ahmadu[1], G. Y. Ajiji[1], J. T. Sunday[1,2], H. O. Awobode[3], G. N. Imandeh[4], B. M. Matur[1]

[1]Applied Entomology and Parasitology Unit, Department of Zoology, University of Jos, Jos, Nigeria
[2]Department of Zoology, Federal University of Agriculture, Makurdi, Nigeria
[3]Health and Development Support Programme, Dutse, Nigeria
[4]Department of Zoology, University of Ibadan, Ibadan, Nigeria
Email: *obeto247@yahoo.com, *goselleon@unijos.edu.ng

How to cite this paper: Goselle, O.N., Udoh, S.S., Ejete, C.O., Iruobe, I.A., Idoko, S., Gyang, A.D., Ahmadu, Y.M., Ajiji, G.Y., Sunday, J.T., Awobode, H.O., Imandeh, G.N. and Matur, B.M. (2020) Recorded Marked Changes in the Haematological and Immune Responses of Two Non-Transgenic Rodents Inoculated Orally and Intraperitoneally with *Trypanosoma brucei brucei*. *Advances in Infectious Diseases*, **10**, 111-129.
https://doi.org/10.4236/aid.2020.102009

Received: April 10, 2020
Accepted: May 26, 2020
Published: May 29, 2020

Abstract

Objectives: The digestive track of mice and humans has always been an integral part of the pathogenesis of the Trypanosomes but is constantly overlooked. This realization opens up completely new strategies for the development of trypanosomes vaccines, allowing approaches that parenteral delivery forms would not permit. The target of the study was to compare the haematological changes and immunological responses of trypanosomiasis model systems (mice and rats) inoculated orally and intraperitoneally and to observe the afterward effect of a controlled drug [Isometamidium chloride (ISM)] in the restoration of these initial parameters. **Methods:** To achieve this, a total of 40 rodents (20 rats and 20 mice) were purchased, then grouped into two [sixteen younger (1 - 5 weeks) and older (7 - 15 weeks) groups each]. They were further sub-grouped into five each. Body weights, Parasitaemia and Packed Cell Volume (PCV) were taken before, after inoculation and after treatment with ISM at 4 mg/kg. **Results:** Based on presumptive clinical diagnosis, all rodents inoculated intraperitoneally showed clinical signs of fluctuations in weight, PCV and parasitaemia levels before, after inoculations and after treatment compared to those inoculated orally with a significant difference ($P < 0.05$) observed. Both young and older rodents also responded differently to the inoculants and to the different methods of inoculation. But more deaths were recorded among the mice when compared to the rats. **Conclusion:** Although

these non-transgenic models would not have offered a completely new methods to vaccine development, their differences in response to various methods of inoculations is an indication of an exciting research processes and could offer desired results, particularly where transgenic rodents are employed.

Keywords

Trypanosoma, Inoculation, Non-Transgenic Rodents

1. Introduction

Human and Animal Trypanosomiasis are caused by species of Trypanosome and transmitted by Tse-tseflies and recognised as the cause of morbidity and mortality to human and livestock throughout sub-Saharan Africa, Nigeria inclusive [1]. Approximately, 60 million people and 48 million cattle's are at risk for this disease in area of 10 million square kilometre, and is responsible for 5000 human and 3 million livestock death annually [1] [2] [3] [4]. Similar to other microbes and parasite, trypanosomes challenge the immune system and induce a host response. This parasite-host interaction can produce either a poor immune response, with a consequent devastating hyper-infection, or an exaggerated life threatening immune response, also with overwhelming consequences. To be effective, the parasite needs to sangfroid its behaviour between these two extremes, avoiding indiscriminate killing of the host and still escaping destruction by the immune system activation borne out of time constraints and periods shared with humans over their evolution for many million years [5] [6].

However, information regarding the biochemical parameters and cytokine profiles associated with natural infections are limited and/or at aberrations and variability. The digestive track of mice and humans has always been an integral part of the pathogenesis of the *Trypanosoma* parasite Trypanosomes but until of rent has been constantly overlooked. This realization opens up completely new strategies for the development of trypanosomes vaccines, allowing approaches that parenteral delivery forms would not permit. To this end, the aim of the study was to determine the changes in weight; the parasitaemia level (as a measure of immune response) and the haematological changes of rats and mice inoculated orally and intraperitoneally with *Trypanosoma brucei brucei*.

2. Materials and Methods

2.1. Ethical Approval

All Experimental protocol were approved and conducted with strict adherence to guidelines of the institutional animal care and use committee of the University of Jos Plateau state, Nigeria, which are in accordance with the Principle of Laboratory Animal Care Of the Canadian Council on Animal Care Guide (CACC) 2nd edited vol 1 1993.

2.2. Experimental Animal

A total of 20 rats and 20 mice were used for the experiment. The animals were obtained from the Animal experimental unit Department of Pharmacology, Faculty of Pharmaceutical Sciences University of Jos. They Animals were allowed to acclimatise for approximately 2 weeks in the Animal house of the Faculty of Pharmaceutical Sciences, University of Jos, Nigeria. The rats and mice were placed under standard hygienic conditions in plastic cages with steal cover. Wood shavings were used as bedding which were changed every week. They were fed *ad libitum* with standard rodent feeds.

2.3. Trypanosomes Parasite

Trypanosoma brucei brucei used for the study was obtained from Nigerian institute for *Trypanosoma*isis Research Vom Plateau state, Nigeria. The parasite was maintained by serial passage in donor rats. Parasitaemia was maintained daily by preparing wet mount and viewing under a light microscope at 40×.

2.4. Experimental Design

A total of forty rodents (20 rats and 20 mice) were used (Figure 1). The Mice and Rats were grouped base on the age group of 1 - 5 weeks and 8 - 15 weeks with each age group comprising of 10 mice and 10 rats and were further sub-divided into 2 groups of 5 each. The first group of rodents each comprised of younger age group mice and rats had 2 males: 2 Females and 1 control were inoculated orally with the parasite strain *Trypanosoma brucei brucei*. Whereas the second group which also comprised of 2 males: 2 Females and 1 control were however inoculated with the parasite intraperitoneally. The older rodents (7 - 15 weeks) of a group of 10 were also sub-divided into 2 groups of 5 each. The older rodents were also inoculated in the fashion of the younger ones *i.e.* orally and intraperitonealy with the parasite strain *Trypanosoma brucei brucei* parasite. Trypanocidal drugs (Isometamidium chloride) were administered to rodents that have confirmed established parasitaemia (particularly those infected intraperitonealy).

2.5. Determination of Parasitaemia

Blood films were made from the caudal vein of each rat after sterilization. Trypanosome count was determined by examination of the wet mount microscopically

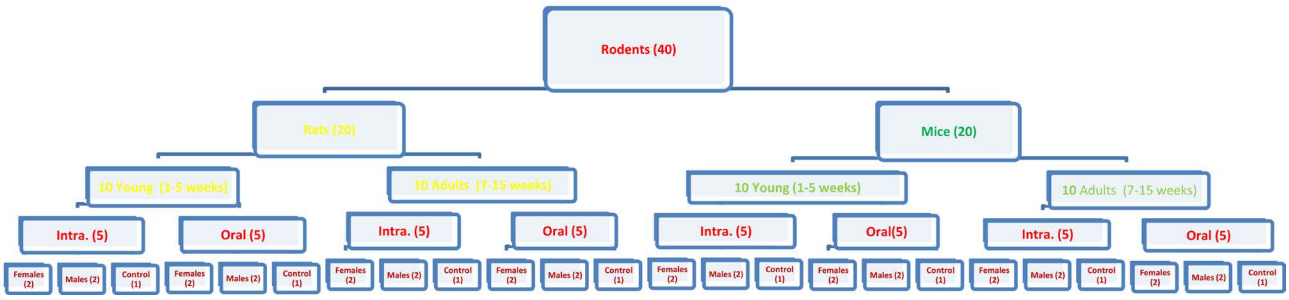


Figure 1. Schematic representation of the schedule for Experimental Animals (Goselle *et al.*, 2020).

at ×40 magnification using the "Rapid Matching" method. Briefly, this method involves microscopic counting of parasites per field in pure blood or blood appropriately diluted with PBS (pH 7.2).

2.6. Preparation of Inoculum

Inoculum was prepared from infected whole blood collected from infected or donor rat. Blood sample was collected from donor rat when parasitaemia was between 20% to 30% in the blood. The total volume obtained separated into two each in an anti-coagulant tube. Blood smear was prepared in order to estimate the number of Trypanosomes per millilitre of blood present in each dose of inoculum.

Oral Inoculum

Phosphate Buffer Saline (PBS) was used to dilute the oral inoculum. Phosphate Buffer Saline served as a neutraliser to the high acidic content of the stomach of the mice and rats respectively. Blood sample was collected from a donor passaging rat and put into an anti-coagulant tube. The blood was rinsed three times by centrifuging at 6000 revolutions per minutes (rpm) by discharging the supernatant and adding the Phosphate Buffer Saline. At the last washing step infected Red Blood Cells was gently Re-suspended with a specific volume of Phosphate Buffer Saline so as to produce an approximate concentration of 1×10^8 Red blood Cells per inoculum. The volume of blood and PBS needed to create a concentration of approximately 1×10^8 red blood cells per inoculum was calculated using the formula:

$$\text{RBC} = \frac{\text{Average RBC} \times \text{Estimated Parasitaemia}}{100} \quad [7]$$

Oral Route of Inoculation

Oral Inoculation was achieved with the use of a cannula inoculums gently deposited into the oral cavity and animal were allowed to swallow. This method was preferred so as to avoid any potential damage to the oral epithelium the gavage could cause. Controls were inoculated with stride Phosphate Buffer Solution (PBS).

2.7. Weighing the Animals

Weighing of the animals was done using electronic weighing balance. These were carried out 3 times with the first taken two weeks before inoculation; the second was taken 3 days after pre-patent period of infection and the last 3 days after treatment.

2.8. Pack Cell Volume Count

The Pack Cell Volume Count was determined using the micro-haematocrit reader. Blood samples were obtained from the tail of both rodents using a capillary tube and then transferred to the micro-haematocrit centrifuge and then

spinned at 15,000 revolutions per minute. Measurements were carried out 3 times with the first taken two weeks before inoculation; the second was taken 3 days after pre-patent period of infection and the last 3 days after treatment.

2.9. Parasitaemia Count

Thick and thin films were prepared from each subject's blood sample. The thin film was fixed with absolute methanol and both thick and thin films were stained with Leishman's stain and were then examined microscopically with oil immersion (×100) objective. The parasite counting was done using the thick blood film and the thin blood films on wet mounts in accordance to the method describe by WHO [8] [9] [10]. The parasite counts in relation to Red Blood Cell count were converted to parasite per microliter of blood using mathematical formula:

$$\text{Parasitaemia}\left(\mu\text{l}\right) = \frac{\text{No. Of parasite}}{200} \times 8000$$

where 8000 = putative means of Red Blood Cell.

The numbers of parasites were counted against 200 Red Blood Cell using laboratory counter (N.B. Once started, a field is always counted to the end; therefore, it is usual that the final red blood count will be over 200).

2.10. Standard Drug Used

Isomethamedium chloride was our drug of choice. Trypanocidal drug treatments were given for those inoculated intraperitoneally after observation of peak parasitaemia by wet film examination [11]. Drug injections were given intraperitonealy with each rodent receiving Isomethamedium chloride at a dose of 4 mg/kg base on their body weight.

3. Results

3.1. Comparative Changes in Weight of Rats (Young and Older Age Group) Inoculated Intraperitoneally with *Trypanosoma brucei brucei* and Treated with Isomethmidium Chloride

The initial mean weight of the younger rats taken 2 weeks before inoculation was at 39 g; 4 days after inoculation was at 62.13 g and 2 days after treatment was at 104.65 g. The statistical analysis before and after inoculation showed there was a significant difference ($P < 0.05$) and this also applies to after inoculation and after treatments where there was a significant difference ($P < 0.05$) as seen in **Table 1**. Similarly, the initial mean weight of the older rats which was taken 2 weeks before inoculation was at 111.43 g; four (4) days after inoculation was at 132 g and 2 days after treatment was at 104.65 g. The statistics analysis indicates that before and after inoculation there was a significant difference ($P < 0.05$) and this also applies to after inoculation and after treatments where there was a significant change ($P < 0.05$) as seen on **Table 1** based on one way ANOVA.

Table 1 also shows the mean weight (g) for younger rats inoculated orally having an initial mean weight taken 2 weeks before inoculation at 40.16 g; four

(4) days after inoculation at 68.16 g and 2 days after treatment at 68.06 g. The statistics analysis indicates that before and after inoculation there was a significant difference ($P < 0.05$); but after inoculation and after treatments there was no significant difference at $P > 0.05$. The same pattern was observed between the older rats' age group with an initial weight of 112.71 g taken 2 weeks before inoculation and 137.05 g taken 4 days after inoculation and 137.4 g 2 days after treatment. Though the statistics indicates that before and after inoculation there was a significant difference ($P < 0.05$); but after inoculation and after treatments there was no significant difference ($P > 0.05$) based on one way ANOVA.

Comparative study based on inoculation and treatment as seen in Table 1 indicates that the weight of both younger and older rats inoculated orally were higher in weight than those inoculated intraperitoneally. Statistical analysis using ANOVA to compare routes of inoculation indicate that $P < 0.05$ at 95% confidence interval. In all, fewer deaths were recorded among the rats especially among the younger female rats (1 - 5 weeks) as compared to their male counterpart.

3.2. Comparative Changes in Weight of Mice Inoculated Intraperitoneally with *Trypanosoma brucei brucei* and Treated with Isomethamidium Chloride

Table 1 shows the mean weight (g) for younger mice inoculated intraperitoneally having an initial mean weight taken 2 weeks before inoculation at 20.20 g; 4 days after inoculation was at 24.20 g and 2 days after treatment at 14.86 g. The statistics analysis using ANOVA indicates that before and after inoculation there was a significant difference at $P < 0.05$; but after inoculation and after treatments there was a significant difference ($P < 0.05$). Similarly, Table 1 shows the mean weight (g) for younger mice inoculated orally having an initial mean weight taken 2 weeks before inoculation at 20.6 g; 4 days after inoculation at 23.18 g and 2 days after treatment at 19.3 g. The statistical analysis using one way ANOVA indicates that before and after inoculation there was a significant difference at $P < 0.05$; but after inoculation and after treatments, there was no significant difference at $P > 0.05$.

Table 1 also shows the mean weight (g) for older mice inoculated intraperitoneally having an initial mean weight taken 2 weeks before inoculation at 23.36 g; 4 days after inoculation at 26.85 g and 2 days after treatment at 14.46 g. The statistics analysis using one way ANOVA indicates that before and after inoculation there was a significant difference ($P < 0.05$), and after inoculation and after treatments there was also an observed significant difference at $P < 0.05$. Similarly, Table 1 shows the mean weight (g) for older mice inoculated orally having an initial mean weight taken 2 weeks before inoculation at 24.26 g; 4 days after inoculation at 31.00 g and 2 days after treatment at 30.86 g. The statistics analysis using one way ANOVA indicates that before and after inoculation there was a significant difference at $P < 0.05$; but after inoculation and after treatments there was no significant difference at $P > 0.05$.

Table 1. Weights (grams) of rodents.

Treatments/ Age/Species	Before Inoculation								After Inoculation								After Treatment							
1 - 5 weeks	Rats				Mice				Rats				Mice				Rats				Mice			
	Female		Male		Female		Male		Female		Male		Female		Male		Female		Male		Female		Male	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Oral	43.2	39.5	37.7	39.4	18.9	18.5	20.3	22.3	76.3	70.4	61.6	63.5	21.4	22.4	24.4	27.9	76.5	70.4	61.4	63.4	21.7	22.5	24.5	28.9
Intraperitoneal	34.1	40.2	37.7	40.2	18.3	17.5	20.3	21.9	56.9	58.3	53.4	55.3	22.2	21.4	26.9	24.9	56.0	Dead	50.4	49.5	20.0	18.0	Dead	Dead
Control	43.2		38.2		17.1		24.4		72.7		59.3		20.6		28.5		72.1		59.4		20.6		28.9	
7 - 15 weeks	Rats				Mice				Rats				Mice				Rats				Mice			
	Female		Male		Female		Male		Female		Male		Female		Male		Female		Male		Female		Male	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Oral	91.0	112.0	129.3	114.0	20.0	23.0	29.8	23.0	107.2	127.3	164.8	151.4	28.9	29.5	34.2	31.8	107.2	128.0	165.0	151.5	29.0	29.5	33.2	31.9
Intraperitoneal	115.1	101.0	113.1	127.4	20.8	20.4	20.8	20.4	128.7	112.4	131.0	148.1	26.4	25.9	20.6	27.0	125.0	127.6	125.0	144.9	Dead	25.5	Dead	Dead
Control	104.0		125.0		24.2		24.2		106.8		164.6		29.2		31.4		106.7		164.7		29.2		31.4	

Statistical analysis: Mean weight of young and old RATS and MICE inoculated both orally to intraperitoneally (ANOVA: $P < 0.05$).

Table 1 is also based on the inoculation routes, where we observed that the weight of both younger and older rats inoculated orally were higher than those inoculated intraperitoneally with statistical analysis using ANOVA to compare routes of inoculation indicating that $P < 0.05$ at 95% confidence interval. In all, more deaths were recorded among the mice especially among the male mice as compared to their female counterpart. The deaths were seen in both adults and younger mice.

3.3. Comparative Changes in the PCV of Rats and Mice Inoculated Intraperitoneally with *Trypanosoma brucei brucei* and Treated with Isomethamidium Chloride

Figure 2 shows the mean PCV range for younger rats inoculated orally having an initial mean PCV % taken 2 weeks before inoculation at 42.93%; 4 days after inoculation at 43.83% and 2 days after treatment at 37.85%. The statistical analysis indicates that before and after inoculation there was no significant difference at $P > 0.05$ and also after inoculation and after treatments there was no significant difference at $P > 0.05$ when one way ANOVA was used. Similarly, Figure 3(a) shows the mean PCV range for younger rats inoculated intraperitoneally having an initial mean PCV % taken 2 weeks before inoculation at 42.85%; 4 days after inoculation at 29.26%; and 2 days after treatment at 18.95%. The statistical analysis indicates that before and after inoculation there was a significant difference at $P < 0.05$; and after inoculation and after treatments there was also a significant difference at $P < 0.05$ based on one way ANOVA.

Figure 2 based on the inoculation routes, it was observed that the PCV range for younger and older rats inoculated orally were higher than those inoculated

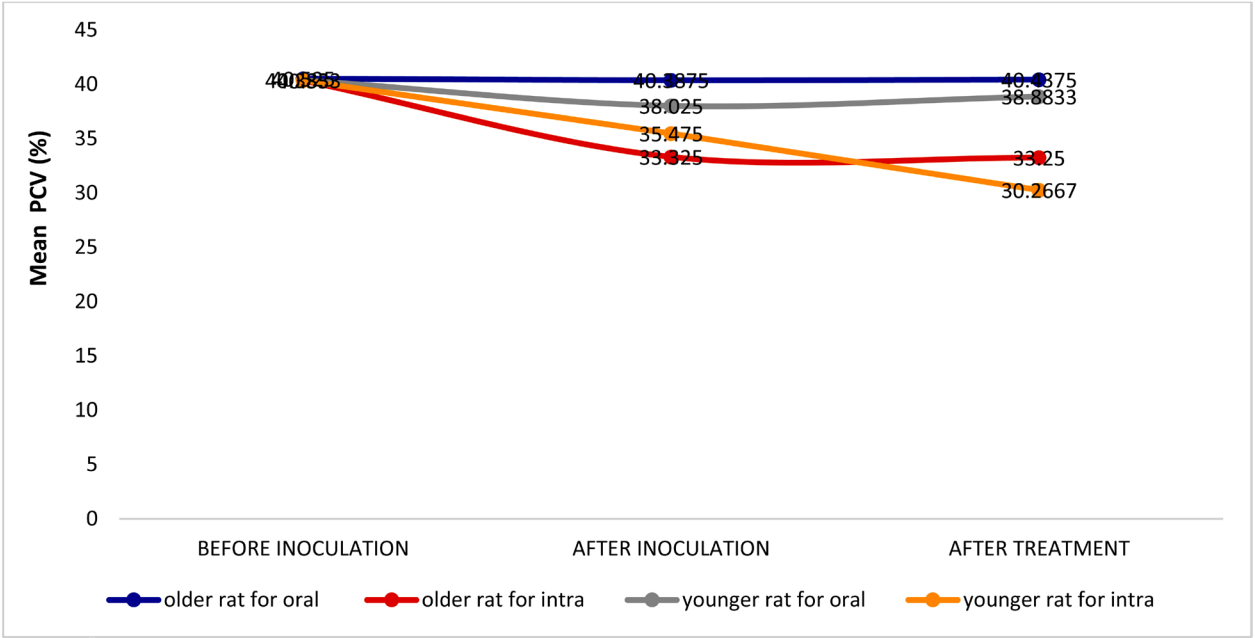


Figure 2. Polygonal curve showing comparison in PCV of Younger and Older Rats inoculated intraperitoneally and Orally [$P < 0.05$].

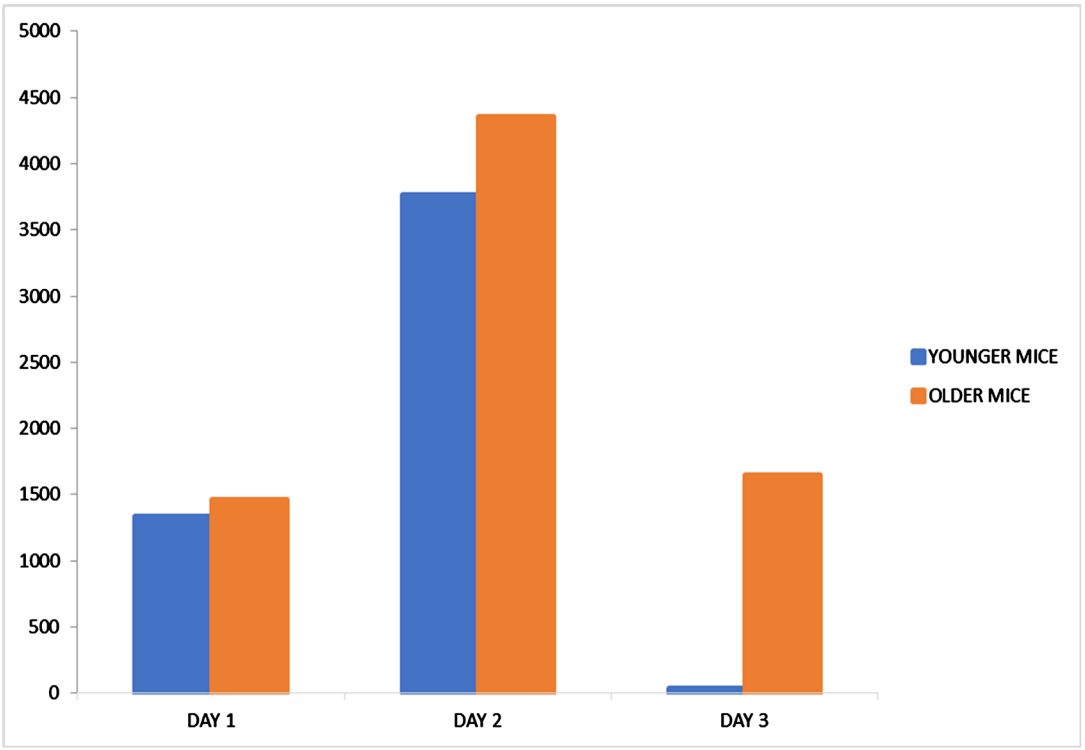
intraperitoneally. Statistical analysis using ANOVA to compare routes of inoculation indicates that P<0.05 at 95% confidence interval.

Figure 2 also shows the mean PCV range for younger mice inoculated orally having an initial mean PCV % taken 2 weeks before inoculation at 47.97%; 4 days after inoculation at 46.72% and 2 days after treatment at 46.82%. The statistical analysis indicates that before and after inoculation there was no significant difference at P > 0.05; and after inoculation and after treatments there was also no significant difference at P > 0.05 based on one way ANOVA. Similarly, Figure 2 shows the mean PCV range for older mice inoculated intraperitoneally having an initial mean PCV % taken 2 weeks before inoculation at 47.96%; 4 days after inoculation at 33.38% and 2 days after treatment at 43.27%. The statistical analysis indicates that before and after inoculation there was a significant difference at P < 0.05; and after inoculation and after treatments there was also a significant difference at P < 0.05 based on one way ANOVA.

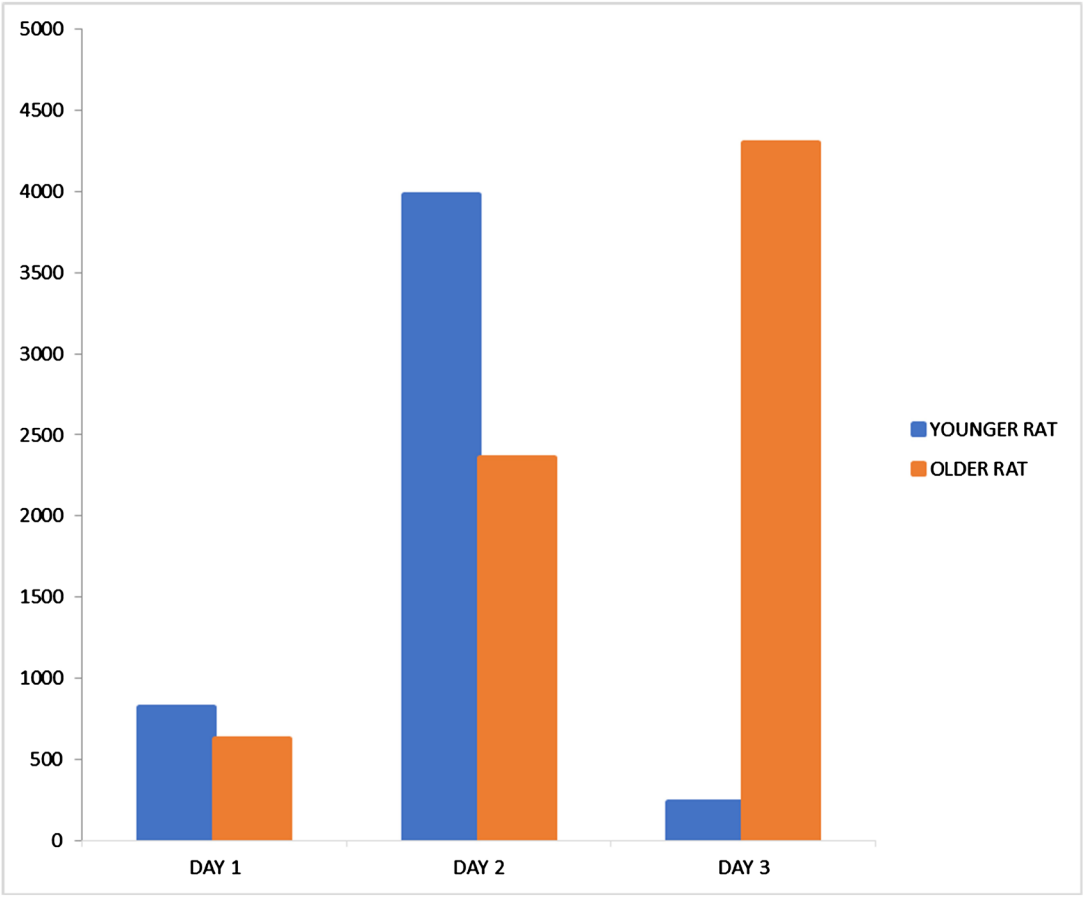
3.4. Comparative Changes in the Mean Parasitaemia Level of Rodents Inoculated Intraperitoneally with *Trypanosoma brucei brucei* and Treated with Isomethamidium Chloride

Figure 3(a) shows the mean parasitaemia count for younger mice inoculated intraperitoneally having an initial mean parasitaemia count taken 4 days after inoculation at 1466; 5 days after inoculation at 43,538 and 2 days after treatment at 1650. The statistical analysis indicates that before and after inoculation there was a significant difference at P < 0.05; and after inoculation and after treatments there was a significant difference (P < 0.05) based on one way ANOVA. Similarly, the second curve in Figure 3(a) shows the mean parasitaemia count for older mice inoculated intraperitoneally having an initial mean weight taken 2 weeks before inoculation at 1338; 4 days after inoculation at 3766 and 2 days after treatment at 40. The statistics analysis indicates that before and after inoculation there was a significant difference (P < 0.05); and after inoculation and after treatments there was a significant difference (P < 0.05) based on one way ANOVA.

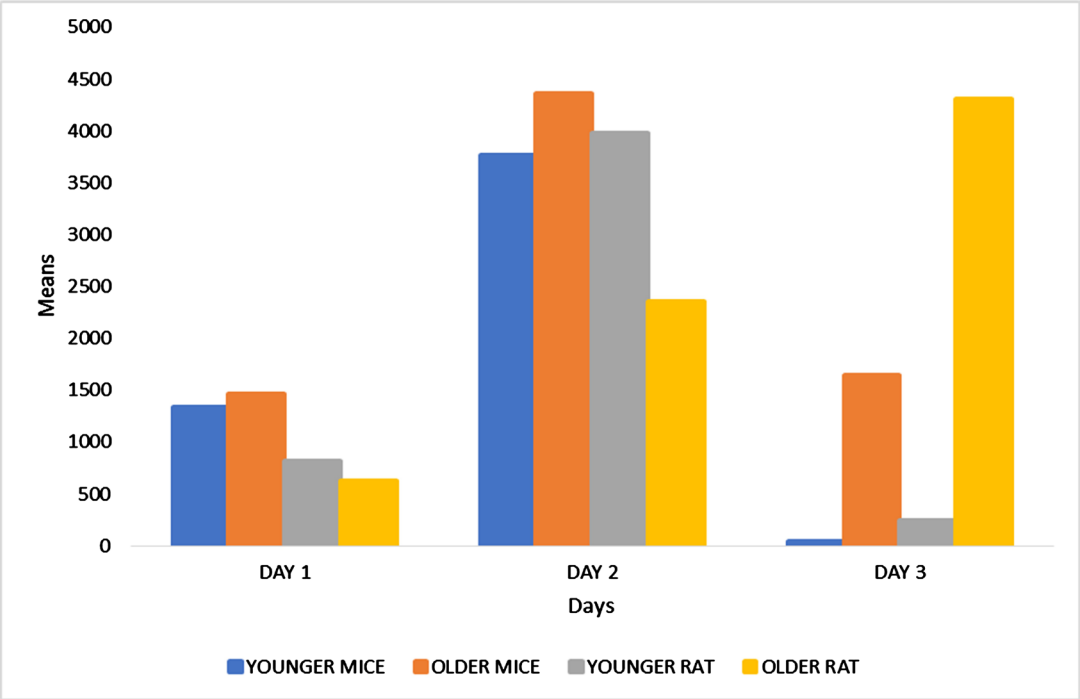
Figure 3(b) shows the mean parasitaemia count for younger rats inoculated intraperitoneally having an initial mean parasitaemia count taken 4 days after inoculation at 820; 5 days after inoculation was at 3983 and 2 days after treatment at 200. The statistical analysis indicates that 4 days after inoculation and 5 days after inoculation, there was a significant difference (P < 0.05); and 4 days after inoculation and 2 days after treatment there was also a significant difference (P < 0.05) based on one way ANOVA. Similarly, Figure 3(b) shows the mean parasitaemia count for older rats inoculated intraperitoneally having an initial mean parasitaemia count taken 4 days after inoculation at 624; 5 days after inoculation at 2358 and 2 days after treatment at 4305. The statistical analysis indicates that 4 days after inoculation and 5 days after inoculation there was a significant difference (P < 0.05); and 4 days after inoculation and 2 days after treatment there was a significant difference (P < 0.05) using one way ANOVA. Based on inoculation and treatment as seen in Figure 3(c), we observed that the


5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
DAY 1
DAY 2
DAY 3
YOUNGER MICE
OLDER MICE

(a)


5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
DAY 1
DAY 2
DAY 3
YOUNGER RAT
OLDER RAT

(b)



(c)

Figure 3. (a) Bar chart showing comparison in Parasitaemia between older and younger mice infected intraperitoneally [$P < 0.05$]; (b) Bar chart showing comparison in Parasitaemia between older and younger rats infected intraperitoneally [$P < 0.05$]; (c) Bar chart showing comparison in Parasitaemia between older and younger rodents infected intraperitoneally [$P < 0.05$].

Parasitaemia number of both younger and older rodents inoculated intraperitoneally showed a significant difference ($P < 0.05$) and also statistical analysis using ANOVA to compare the two (2) species of rats and mice indicate that ($P < 0.05$) at 95% confidence interval. The stained thin film of parasitaemia for the younger and older rodents are shown in Figures 4-7.

4. Discussion

In this study, typical clinical signs of parasitaemia such as pallor of mucous membranes, lethargy and starry hair coat were observed among the rodents. This is in contrast to the works of Anosa [12] and Losos and Ikede [13] who reported severe clinical signs due to *Trypanosoma brucei brucei* infection in rats and mice. The contrast might probably be associated with strain variation and individual infectivity or host susceptibility. Successive waves of parasitaemia were recorded among the infected mice, rats inoculated intraperitoneally.

The mean weight gain between the two methods of inoculation indicates that a remarkable change were observed among those inoculated orally as compared to the intraperitoneally. The significant difference in mean weight was observed within and between groups of rodents and also based on treatments ($P < 0.05$) before, after inoculation and after treatment. An undocumented wave of Anorexia

could therefore be suspected to have probably occurred in the intraperitoneally infected rodents as compared to the orally infected rodents. Which could agree with the report of Ezeokonkwo *et al.* [14], who reported that the presence of anorexia is an indication of the loss of apetite which could be attributed to the acute nature of the infection of Trypanosomes.

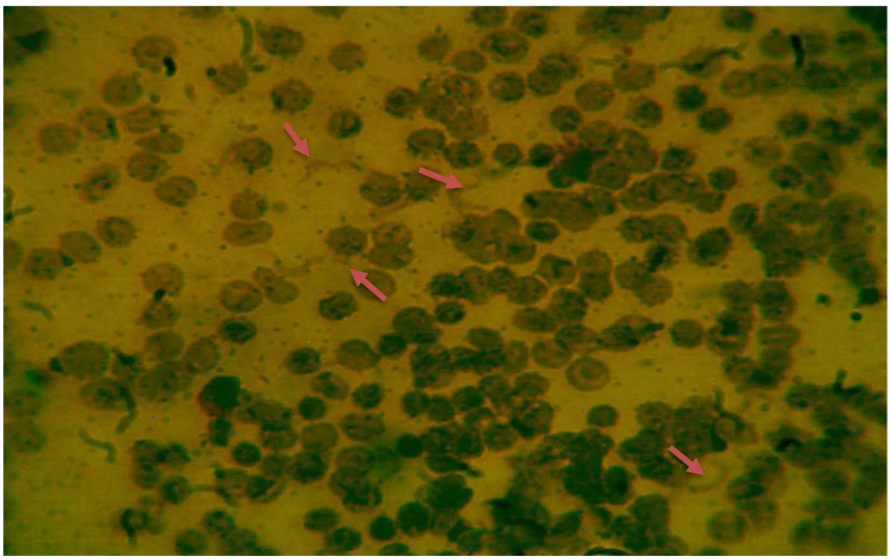


Figure 4. Stained thin Film with arrows pointing to *Trypanosoma brucei brucei* obtained from infected younger rats.

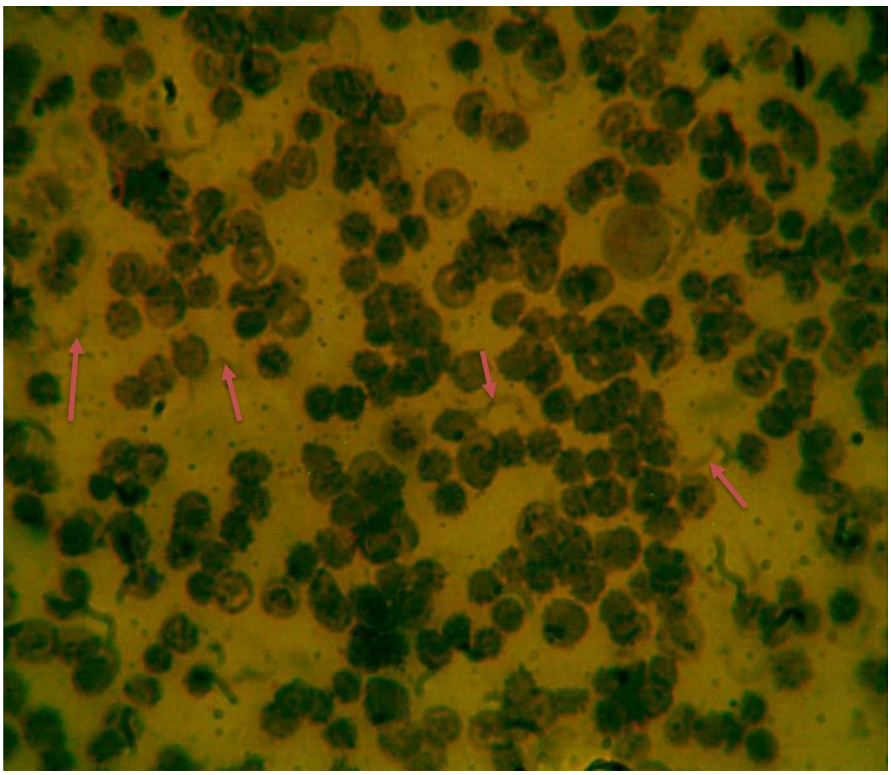


Figure 5. Stained thin Film with arrows pointing to *Trypanosoma brucei brucei* obtained from infected younger mice.

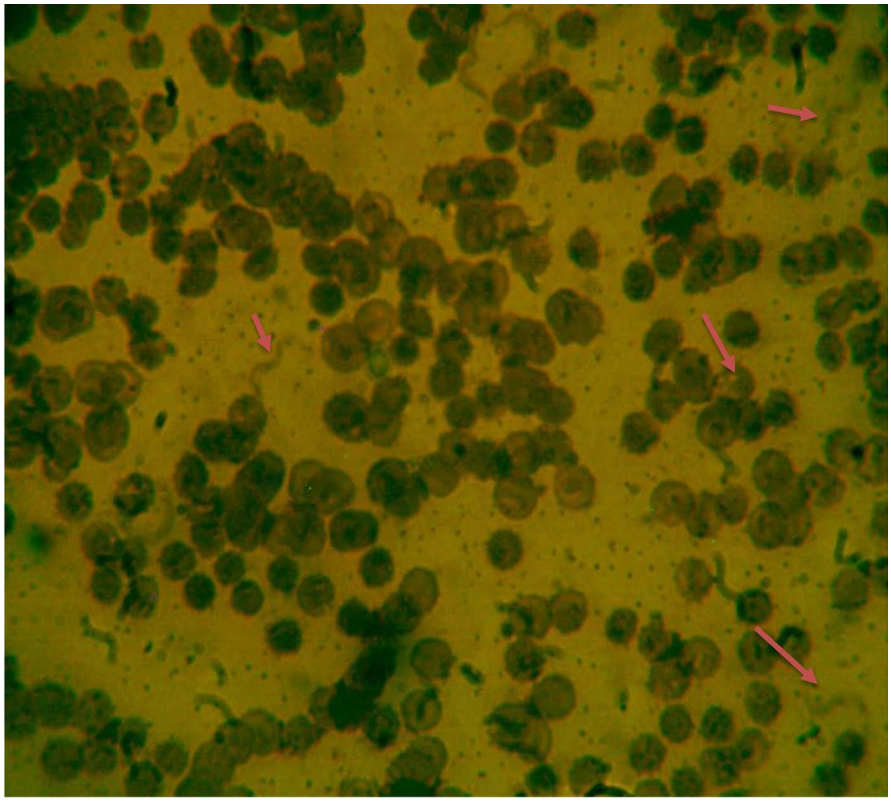


Figure 6. Stained thin Film with arrows pointing to *Trypanosoma brucei brucei* obtained from infected younger rats.

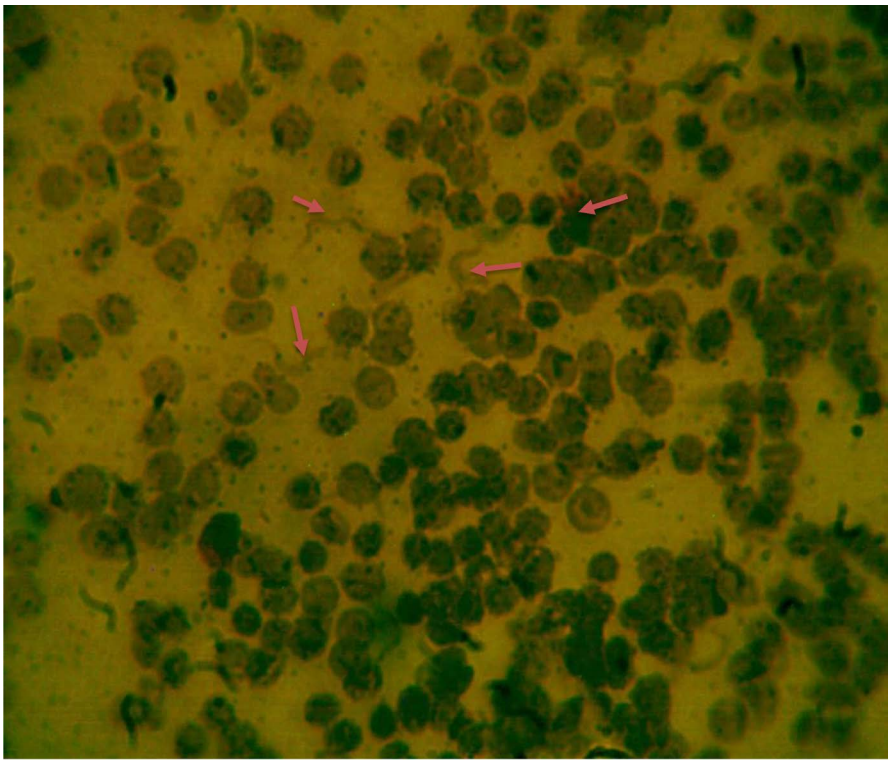


Figure 7. Stained thin Film with arrows pointing to *Trypanosoma brucei brucei* obtained from infected older mice.

Based on the Packed Cell Volume (PCV), it was observed that in both rodents inoculated intraperitoneally, there was a significant difference ($P < 0.05$) in mean PCV whereas those inoculated orally showed no significant differences ($P > 0.05$). The decline in these parameters which started at the onset of parasitaemia was indicative of anaemia which was not observed in rodents inoculated orally. However, variation in prepatent period among the different rodent species showed that the prepatent period was an average of (3 - 5 days) in both mice and rats. Similarly, the severity of the anaemia was more noticeable in mice than in rats. This agrees with several reports that the prepatent period in mice and rats was 4 days and the anaemia which is often haemolytic in nature began at the 1st wave of parasitaemia [12] [15] [16] [17]. In a related development, the severity of infections were pronounced in older rodents as compared to the younger rodents with the PCV showing a significant difference a $P < 0.05$. Animal trypanosomiasis generally involves fever and lethargy leading to anaemia, which can be fatal if left untreated [18]. Anaemia following trypanosome infection is typically diagnosed by the presence of a low 'packed cell volume' of erythrocytes. The mechanisms of anaemia are thought to arise due to symptoms from the infection, induced by the innate immune response, leading to haemolysis, or from haemolysins released by trypanosomes [19]. The onset of anaemia occurs in the early stages of infection when parasitaemia is at its height.

The significant decrease in PCV of *T. brucei brucei* infected intraperitoneally inoculated rodents is in consonance with earlier reports [20] [21] in trypanosome-infected animals. This has been attributed to the release of haemolytic factors into the animal's blood by dead trypanosomes causing destruction of erythrocytes and hence, reduction in PCV [22] [23]. The observed trypanostatic effect of ISM was accompanied by corresponding increase in PCV. Anaemia is the most outstanding clinical and laboratory feature of African trypanosomiasis [24] and also the primary cause of death [13] [25]. Anaemia as indicated by PCV level is known to worsen with increasing parasitaemia [26]. The prolongation of lives of treated animals may therefore also be associated with the ability of ISM to improve the PCV possibly by reducing the parasite load or neutralizing the toxic metabolites produced by trypanosomes.

Within the different age group of rodents inoculated intraperitoneally, a mean significant difference ($P < 0.05$) was observed between the older and younger age groups before, after inoculation and after treatments with ISM. However, no recorded changes ($P > 0.05$) in parasitaemia was recorded between and within the groups of rodents inoculated orally. It has been reported that successive waves of parasitaemia are known features of trypanosomosis commonly caused by antigenic variation [16] [27]. The ability of host to limit the peak and number of each wave of parasitaemia is however dependent on whether the infection is acute, sub-acute or chronic [28]. And this may explain the reason why parasitaemia in the rats and mice is appreciated but declined following treatment by 2 Days post treatment. It is worthy of note that each infected rodent received the

same amount of infective trypanosome inoculum to ensure the elimination of possible influence of the infective dose on the pre-patent period and subsequent parasitaemia [19]. The intraperitoneally inoculated rodents however showed high parasitaemia, an observation which is supported by the report that *T.brucei brucei* is highly pathogenic to rats [13]. The manifestation of parasitaemia in all the infected groups of rodents (particularly intraperitoneally infected ones), indicates that the breed of rodents used in the study are susceptible to the *T. brucei brucei* (Federe strain) infection. However, the short prepatent period (2 - 5 days) recorded shows the virulence of the strain [29] [30]. The experimental increase in the parasitaemia which led to the death of the entire rats in the untreated group 4 within 6 days agrees with the observation of Eghianruwa and Anika [31] that the survival period of *T. brucei brucei* infection is 1 - 4 weeks.

The level of parasitaemia increased progressively in all the intraperitoneally infected groups to reach a peak 7 days post infection. Treatment however did not directly affect the course of parasitaemia, although the rodents treated with ISM lived beyond Day 9. What this suggests is that the ISM strengthened the host immune defense, which was already activated because of the presence of parasites in circulation in the rodents with established infection. This observation is of particular interest because ISM was earlier reported to boost immune system [32]. It is therefore likely that the ISM was able to elicit the production of antibodies in the presence of parasites in circulation. This, taken together with the longer prepatent period observed in 24 hrs treated animals suggests that the ISM may positively influence the defense capacity of the treated animals which also explains why there was no significant difference in the body weight of the infected animals. It may well be that the ISM enhanced phagocytosis. The net effect of all these was the resultant prolongation of the lives of the treated animals as a consequence of the parasites being kept in check. Because it has been reported that a complete elimination or reduction of motility of parasites when compared to the control could be taken as index of trypanocidal activity [33].

The drug Isomethamidium chloride was very effective in modulating parasitaemia and the haematological changes in all the categories of rodent species. This study, where a standard dose of the inoculum was administered and uniform pre-patent periods were encountered in all infected groups, means that the initial parasite replication rates were similar irrespective of the rodent species or susceptibility both a changes where recorded afterwards. These observations have been reported in *Typanosoma brucei brucei* infection of dogs, red fronted gazelles (*Gazella rufifrons*), giant rats (*Cricetomys gambianus*) and in *Trypanosoma brucei gambiense* infection in vervet monkeys (*Cercopithecus aethiops*) and baboons (*Papio anubis*) [34] [35] [36] [37].

5. Conclusion

It was therefore concluded that infecting rats and mice with experimental *Trypanosoma brucei brucei* via the oral route proved unsuccessful as no record of

infection was observed among, between and within the groups of rodents. But within the groups of rodents inoculated intraperitoneally, older age group were severely affected by the parasites compared to the younger age groups because the older age group rats showed more mean weight lost, greater reduction in PCV (%) and more number of parasitaemia. We therefore conclude that the immune system of younger age group of mice and rats proved stronger in combating *Trypanosome brucei brucei* infection compared to older age group of rodents. However infection among the rodents caused various parasitaemia and haematological changes which were reversed by treatment with Isometamidium chloride. But it was observed that mice were more susceptible to the infection compared to rats.

6. Recommendation

Although these non-transgenic models would not have offered a completely new methods to vaccine development, their differences in response to various methods of inoculations is an indication of an exciting research processes and could offer desired results, particularly where transgenic rodents are employed.

Author Contributions

ONG, Drafted and Supervised project work, wrote manuscript and contributed to laboratory work; SSU, Carried out laboratory work, collected data and contributed to manuscript writing; COE, Carried out laboratory work and collected data; IAI, Carried out laboratory work and collected data; SI, Carried out laboratory work and collected data; ADG, Carried out laboratory work and collected data; YMA, Carried out laboratory work and collected data; GYA, Carried out laboratory work and collected data; JTS, Carried out laboratory work and collected data; HOA, Contributed to the writing of manuscript; GNI, Contributed to draft of project and manuscript; BMM, Contributed to writing manuscript and data analysis.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] WHO (1998) Control and Surveillance of African Trypanosomiasis. Report of a WHO Expert Committee. Technical Report Series No. 881, Geneva.

[2] Kristjanson, P.M., Swallow, B.M., Rowland, G.J., Krusoka, R.L. and Belew, P.P. (1999) Measuring the Cost of Animal African Trypanosomiasis: The Potential Benefit of Control and Retune to Research. *Agricultural Systems*, **59**, 79-98. https://doi.org/10.1016/S0308-521X(98)00086-9

[3] Fèvre, E.M., Wissmann, B.V., Welburn, S.C. and Lutumba, P. (2008) The Burden of Human African Trypanosomiasis. *PLoS Neglected Tropical Diseases*, **2**, e333. https://doi.org/10.1371/journal.pntd.0000333

[4] Abenga, J.N. (2014) A Comparative Pathology of *Trypanosoma brucei* Infections.

Global Advanced Research Journal of Medicine and Medical Sciences, **3**, 390-399.

[5] Steverding, D. (2008) The History of African Trypanosomiasis. *Parasites and Vectors*, **1**, 1-8. https://doi.org/10.1186/1756-3305-1-3

[6] Cnops, J., Magez, S. and De Trez, C. (2015) Escape Mechanisms of African Trypanosomes: Why Trypanosomosis Is Keeping Us Awake. *Parasitology*, **142**, 417-427. https://doi.org/10.1017/S0031182014001838

[7] Roscoe, B.J.M. and Green, E.L. (1975) Biology of the Laboratory Mouse. Dover Publications, London.

[8] WHO (2003) Manual of Basic Technique or a Health Laboratory. Who, Geneva, 172-182.

[9] WHO (1996) Estimation of Parasite Number, in Assessment of Therapeutic Efficacy of Antimalarial on Uncomplicated Falciparum Malaria in Area with Intense Transmission. Geneva, 7-8.

[10] WHO (1995) Method of Counting Malaria Parasite in Thick Blood Film. Bench Aids or Diagnosis of Malaria, 1-8.

[11] Murray, M.P.K. and Mcintyre, W.I.M. (1977) An Improved Parasitological Technique for the Diagnosis of African Trypanosomosis. *Transaction of the Society for Tropical Medicine and Hygiene*, **71**, 325-326. https://doi.org/10.1016/0035-9203(77)90110-9

[12] Anosa, V.O. (1988) Haematological and Biochemical Changes in Human and Animal Trypanosomiasis. Part I. *Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux* (*Paris*), **41**, 65-78.

[13] Losos, G.J. and Ikede, B.O. (1972) Review of the Pathology of Domestic and Laboratory Animals Caused by *T. congolense*, *T. vivax*, *T. brucei*, *T. rhodesiense*, and *T. gambiense. Veterinary Pathology*, **9**, 1-71. https://doi.org/10.1177/030098587200901s01

[14] Ezeokonkwo, R.C., Akpa, P.O., Eze, C.A. and Anene, B.M. (2008) Comparative Efficacy Assessment of Pentamidine Isethionate and Diaminazene Aceturate in the Chemotherapy of *Trypanosoma brucei* Infection in Dogs. *Veterinary Parasitology*, **151**, 139-149. https://doi.org/10.1016/j.vetpar.2007.10.024

[15] Mbaya, A.W., Kumshe, H.A. and Nwosu, C.O. (2012) The Mechanisms of Anaemia in Trypanosomosis: A Review. InTech Publishers, London, 270-282. https://doi.org/10.5772/29530

[16] Nwosu, C.O. and Ikeme, M.M. (1992) Parasitaemia and Clinical Manifestations in *Trypanosoma brucei* Infected Dogs. *Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux* (*Paris*), **45**, 273-277.

[17] Igbokwe, I.O. (1995) Nutrition in the Pathogenesis of African Trypanosomiasis. *Protozoology Abstract*, **19**, 797-807.

[18] Reid, S.A. (2002) *Trypanosoma evansi* Control and Containment in Australasia. *Trends in Parasitology*, **18**, 216-224. https://doi.org/10.1016/S1471-4922(02)02250-X

[19] Murray, M. and Dexter, T.M. (1988) Anaemia in Bovine African Trypanosomiasis: A Review. *Acta Tropica*, **45**, 389-432.

[20] Oduola, T., Bello, I., Adeosun, G., Ademosun, A., Raheem, G. and Avwioro, G. (2010) Hepatotoxicity and Nephrotoxicity Evaluation in Wistar Albino Rats Exposed to *Morinda lucida* Leaf Extract. *National American Journal of Medical Science*, **2**, 230-233.

[21] Faremi, A.Y. and Ekanem, J.T. (2011) Haematological Parameters and Enzyme

Studies in *Trypanosoma brucei* Infected Rats Reared on *Nigella sativa* Oil-Based Diet. *Asian Journal of Biochemistry*, **6**, 90-97. https://doi.org/10.3923/ajb.2011.90.97

[22] Atawodi, S.E., Bulus, T. and Mamman, M. (2011) Bioassay Guided Fractionation and Anti-*Trypanosoma*l Effect of Fractions and Crude Aqueous and Methanolic Extracts of *Terminalia avicennioides* (Guill. & Perr.) Parts. *International Journal of Biology*, **3**, 19-30. https://doi.org/10.5539/ijb.v3n3p19

[23] Olukunle, J.O., Abatan, M.O., Soniran, O.T., Takeet, M.I., Idowu, O.A., Akande, F.A., *et al.* (2010) *In Vivo* Anti*trypanosoma*l Evaluation of Some Medicinal Plant Extracts from Ogun State, Nigeria. *Science World Journal*, **5**, 17-19. https://doi.org/10.4314/swj.v5i1.61480

[24] Suliman, H.B. and Fieldman, B.F. (1989) Pathogenesis and Aetiology of Anaemia in Trypanosomiasis with Special Reference to *T. brucei* and *T. evansi*. *Protozoology Abstracts*, **13**, 37-45.

[25] Mamo, E. and Holmes, P. (1975) The Erythrokinetics of Zebu Cattle Chronically Infected with *T. congolense*. *Research in Veterinary Science*, **18**, 105-106. https://doi.org/10.1016/S0034-5288(18)33638-5

[26] Ogbadoyi, E.O., Agwu, I.U. and Elizabeth, K. (1999) Anemia in Experimental African Trypanosomiasis. *The Journal of Protozoology Research*, **9**, 55-63.

[27] Mbaya, A.W., Nwosu, C.O. and Onyeyili, P.A. (2007) Toxicity and Anti-Trypanosomal Effects of Ethanolic Extract of *Butyrospermum paradoxum* (Sapotaceae) Stem Bark in Rats Infected with *Trypanosoma brucei* and *Trypanosoma congolense*. *Journal of Ethnopharmacology*, **111**, 526-530. https://doi.org/10.1016/j.jep.2006.12.020

[28] Soulsby, E.J.L. (1982) Helminths, Arthropods and Protozoa of Domesticated Animals. Bailliere Tindall, London, 46-49.

[29] Ezeokonkwo, R.C. and Agu, W.E. (2004) Experimental Infections of Domestic Rabbits (*Oryctolagus cuniculus*) with *Trypanosoma brucei* and *Trypanosoma congolense*: A Comparative Study. *Nigerian Journal of Animal Production*, **31**, 100-111.

[30] Ajayi, O.O., Toshak, L.E., Obaloto, O.B., Iliyasu, B., Igweh, C.A., Dadah, J.A. and Idehen, O.C. (2013) Comparative Efficacy of Berenil and Samorin in Albino Rats Experimentally Infected with Current Field Isolates of *Trypanosoma brucei brucei*. *International Journal of Biological and Chemical Science*, **7**, 1452-1460. https://doi.org/10.4314/ijbcs.v7i4.3

[31] Eghianruwa, K.I. and Anika, S.M. (2012) Effects of DMSO on Diminazine Efficacy in Experimental *T. brucei* Infection. *International Journal of Animal Veterinary Advances*, **4**, 2041-2908.

[32] Pakia, M. (2005) African Traditional Plant Knowledge Today: An Ethnobotanical Study of the Digo at the Kenya Coast. Doctorate Thesis, University of Bayreuth, Bayreuth.

[33] Atawodi, S.E. and Ogunbusola, F. (2009) Evaluation of Anti-*Trypanosoma*l Properties of Four Extract of Leaves, Stem and Root Barks of *Prosopis* African in Laboratory Animals. *Biokemistri*, **21**, 101-108. https://doi.org/10.4314/biokem.v21i2.56478

[34] Abenga, J.N. and Anosa, V.O. (2006) Clinical Studies on Experimental Gambian Trypanosomosis in Vervet Monkeys. *Veternarski Arhiv*, **76**, 11-18.

[35] Mbaya, A.W., Aliyu, M.M., Nwosu, C.O. and Ibrahim, U.I. (2009) Effect of DL α-Difluoromethylornithine on Biochemical Changes in Baboons (*Papio anubis*) Experimentally Infected with *Trypanosoma brucei gambiense*. *Nigerian Veterinary Journal*, **31**, 34-44.

[36] Herbert, W.J. and Lumsden, W.H. (1976) *Trypanosoma brucei brucei*: A Rapid

"Matching" Method for Estimating the Host's Parasitaemia. *Experimental Parasitology*, **40**, 427-431. https://doi.org/10.1016/0014-4894(76)90110-7

[37] Mbaya, A.W., Aliyu, M.M., Nwosu, C.O. and Ibrahim, U.I. (2008) Effect of Berenil® or Cymelarsan® on the Alteration of Biochemical Parameters in Red-Fronted Gazelles (*Gazella rufifrons*) Experimentally Infected with *Trypanosoma brucei*. *Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux* (*Paris*), **61**, 169-175. https://doi.org/10.19182/remvt.9984