Vol. 12(2), pp. 7-16, July-December 2020
DOI: 10.5897/JPVB2020.0381
Article Number: 6CAF04264188
ISSN: 2141-2510

http://www.academicjournals.org/JPVB

ACADEMIC JOURNALS
expand your knowledge

Journal of Parasitology and Vector Biology

Full Length Research Paper

Effect of diminazene diaceturate (Sequzene®) on serum biochemistry and associated histopathological changes of New-Zealand rabbits (*Oryctolagus cuniculus*) experimentally infected with *Trypanosoma brucei brucei*

Abwari Bwala David[1]*, Adamu Inuwa[1], Samson Anjikwi Malgwi[2], Albert Wulari Mbaya[2], Hadiza Abdulsalam[1] and Dennis Ogbonna Okoro[2]

[1]Department of Biological Sciences, Faculty of Science, University of Maiduguri, Maiduguri, Nigeria.
[2]Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Nigeria.

Received 27 January, 2020; Accepted 27 May, 2020

This study was conducted to determine the efficacy of diminazene diaceturate (Sequzene®) on serum biochemistry and associated histopathological changes of New-Zealand white rabbits experimentally infected with *Trypanosoma brucei brucei*. A total of thirty New-Zealand white rabbits weighing between 0.1 and 0.7 kg of both sexes were divided into six groups A to F of five rabbits each. Group A was infected with *T. brucei brucei* only but untreated while Group B was uninfected untreated control. Group C was infected with *T. brucei brucei* but treated with Sequzene® at 3.5 mg/kg, while Group D was infected with *T. brucei brucei* and treated with Sequzene® at 7.0 mg/kg. Group E was uninfected but treated with Sequzene® at 3.5 mg/kg while group F was uninfected and treated with 7.0 mg/kg of Sequzene®. Physical signs were monitored daily and blood samples were taken every 7 days and analyzed for serum biochemical and associated histopathological changes according to standard laboratory techniques. Physical signs manifested were dullness, weakness, anorexia, weight loss, increased respiration, starry hairs with corneal opacity. Rabbits in infected untreated group (A) showed nervous disorder (convulsion) at the point of death. There were significant ($p<0.05$) elevations of serum aspartate amino transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), serum urea and creatinine levels and a significant decline ($p<0.05$) in serum glucose concentration. The mean values for groups B, E and F remained fairly constant ($p>0.05$) throughout the study. The mean values of parameters evaluated in treated groups (C and D) were completely restored to their pre-infection values by day 21 post infection. Histopathological changes include thickened interstitium infiltrated by leucocytes and an inflamed bronchiole in the lungs, degenerative and necrotic renal tubules in the renal interstitium in the kidney, degenerative hepatocytes in the liver and congested blood vessels and neuronal cells in the brain. It is therefore recommended that diminazene diaceturate be used for effective treatment of *T. brucei brucei* in animals suffering from trypanosomosis.

Key words: Serum biochemistry, diminazene diaceturate, *Trypanosoma brucei brucei*, histopathological changes, New-Zealand rabbits.

INTRODUCTION

Trypanosomosis, affects both human and livestock populations in sub-Saharan Africa, which results in measurable socio-economic and public health impacts, especially in poor rural communities where endemicity has been reported during the 20th century (Anon, 2018; Mwiinde et al., 2017; Simwango et al., 2017; WHO, 2015a). This disease has left the health of most afflicted societies precarious and their economies impoverished. It is therefore important to develop a better understanding of the disease process, as control for the benefit of both human and animals. Studies on induced laboratory animal models of the disease have been widely undertaken thus, generating a large quantity of information. The pathology and pathogenesis of the disease has extensively been evaluated by several workers (Anosa and Kaneko, 1983; Bal et al., 2012; Mbaya et al., 2012). Control of trypanosomosis is directly focused on the parasite's destruction in the host with chemical drugs (Mbaya et al., 2012; Sumbria and Singla 2017) or by medicinal plants (Johnson and Omoniwa, 2014; Wurochekke et al., 2014; Lifongo et al., 2014, Ibrahim et al., 2014; Mbaya et al., 2009c; Tekle, 2014; Tadesse et al., 2015; Tesfaye et al., 2015). An excellent evaluation of the chemotherapy of trypanosomosis from an ancient perspective has been made by Onyeyili and Egwuin (1995). Atoxyl, an arsenical and fore runner of tryparsamide was the first clinically productive trypanocide and made its inception in 1905 when African sleeping sickness plague occurred in Equitorial and East Africa (Onyeyili and Egwu, 1995). In the 1940s pentamidine, a diamidine becomes available. It is effective in the treatment of early infections. Right now, the treatment of late stage human trypanosomosis depends on the parenteral administration of organic arsenicals, melosaprol (Onyeyili and Egwu, 1995). This drug has been in use since 1940. Between 1943 and 1945, phenidium was introduced but it was later stopped because of drug resistance by the parasite and adverse effect on the host (Atawodie et al., 2003). Antrycide (Quinapyramine) (Ashcroft, 1959) came into use in 1950 in the appearance of prosalt. Elhidium (homodium bromide), a less harmful version of diminazene aceturate (Berenil®) was introduced and has been widely used in the treatment of animal trypanosomosis because of its high therapeutic index and low incidence of drug resistance (Parashar and Singla, 2019). It has also been used in the initial stage of trypanosomosis (Hudson, 1944).

Following the initiation of isometamidium in 1961 (Desowitz, 1960), little progress has been made in the development of animal trypanocides. The unavailability of drug occurrence coupled with the establishment of parasite resistance and relapse parasitaemia has led to deadlock in the chemotherapy of trypanosomosis (Onyeyili and Egwu, 1995).

So far, little has been achieved in terms of understanding the variation in pre-patency period, course of infection, and clinico-pathology of the disease in various laboratory animal species especially as it relates to *Trypanosoma brucei brucei* and the effect of diminazene diaceturate (Sequzene®) on the experimental *T. brucei brucei* infection changes that might ensue in the course of the infection.

MATERIALS AND METHODS

Experimental animals

A total of thirty healthy New Zealand rabbits weighing between 0.1 and 0.7 kg of both sexes were purchased commercially from rabbit farmers within Maiduguri. The rabbits on arrival were kept in the rabbit cage at large Animal Clinic, Veterinary Teaching Hospital, University of Maiduguri, Nigeria. These rabbits were routinely screened for blood, intestinal and external parasites using standard criteria (Soulsby, 1982; Gupta and Singla, 2012). The animals were fed pelleted commercial feed (Vital Feeds LTD, Jos, Nigeria) and fresh green vegetables. Water was provided *ad libitum*. The rabbits were allowed 14 days to acclimatize to their new environment before commencement of the research. The experiment carried out is in accordance to International Guidelines for the Use of Animals for Biomedical Research and Welfare (Murray et al., 1983; Ochei and Kolhatkar, 2000).

Trypanosomes

T. brucei brucei, Federe strain used for this study was obtained from the Nigeria Institute for Trypanosomosis and Onchocerciasis Research (NITOR) in Jos, Nigeria. The stabilates were passaged twice in donor rats. Tail blood from the donor rats was diluted with phosphate buffered saline glucose (PBSG) pH 7.2. The rabbits were infected intra-peritoneally with blood from the donor rats containing 1.5 $\times 10^6$ trypanosomes. The parasitaemia was initially detected by the wet mount and haematocrit buffy-coat methods (Murray and Jennings, 1982) after collection of 2 ml of blood sample via the ear vein. The level of parasitaemia was measured by the rapid matching method (Herbert and Lumsden, 1976).

In vivo experimental design

The 30 rabbits were weighed and randomly separated into 6 groups A, B, C, D, E, and F. The groups were infected and treated as follows:

Group A: Infected and untreated control (negative control).
Group B: Uninfected and untreated control (normal).

*Corresponding author. E-mail: abwaridavidbwala@gmail.com.

Table 1. Mean levels of serum urea (mmol/L) of New Zealand rabbits experimentally infected with *Trypanosoma brucei brucei* and treated with diminazene diaceturate and their controls.

Group (n=5)	PP Tb	Day of treatment		Days post-infection	
	0	7	10	14	21
A	5.55 ±0.40[a]	8.77 ±0.17[b]	-	10.57 ±0.26[c]	15.35 ±0.52[d]
B	5.55 ±0.31[a]	5.37 ±0.33[a]	-	6.07 ±0.09[a]	5.25 ±0.43[a]
C	5.27 ±0.22[a]	7.05 ±0.12[b]	-	6.62 ±0.15[c]	5.55 ±0.42[a]
D	5.20 ±0.14[a]	7.30 ±0.42[b]	-	6.62 ±0.15[c]	5.45 ±0.12[a]
E	5.20 ±0.18[a]	5.40 ±0.18[a]	-	5.27 ±0.22[a]	5.57 ±0.45[a]
F	5.30 ±0.21[a]	5.32 ±0.22[a]	-	5.27±0.25[a]	5.22 ±0.26[a]

Mean values with different superscripts in rows and in columns differed significantly ($p<0.05$). A: Infected/Untreated control; B: Uninfected/Untreated control; C: Infected/Treated with 3.5 mg/kg of diminazene diaceturate; D: Infected/Treated with 7.0 mg/kg of diminazene diaceturate; E: Uninfected/Treatedwith3.5 mg/kg of diminazene diaceturate; F: Uninfected/Treated with 7.0 mg/kg of diminazene diaceturate; PP Tb= Pre-patent period for *Trypanosoma brucei brucei*.

Group C: Infected and treated with a single standard dose of diminazene diaceturate (Sequzene®) at 3.5 mg/kg by day 10.
Group D: Infected and treated with single standard dose of diminazene diaceturate (Sequzene®) at 7.0 mg/kg by day 10.
Group E: Uninfected and treated with a single standard dose of diminazene diaceturate (Sequzene®) at 3.5 mg/kg by day 10.
Group F: Uninfected and treated with single standard dose of diminazene diaceturate (Sequzene®) at 7.0 mg/kg by day 10.

Statistical analysis

Data generated from the study were expressed as mean ± standard deviations (S.D) using 2-way analysis of variance (ANOVA) and $p < 0.05$ was considered significant (GraphPad Instat, 2009).

RESULTS

The mean levels of serum urea of the New Zealand rabbits infected with *T. brucei brucei* with their controls are shown in Table 1. In group A, infected with *T. brucei brucei* but untreated control, the pre-infection value of 5.55±0.40 increased significantly ($p<0.05$) from day 7 with 8.8±0.2 (P.I) to 15.4±0.5 by day 21 (P.I). All infected rabbits in the group died by day 22 post infection. In group B (uninfected/untreated control), group E (uninfected/treated with diminazene diaceturate at 3.5 mg/kg) and group F (uninfected/treated with 7.0 mg/kg of diminazene diaceturate), the pre-infection values of 5.6±0.3, 5.2±0.2, and 5.3±0.2 remained fairly constant throughout the study. In group C (infected/treated with diminazene diaceturate at 3.5 mg/kg) and group D (infected/treated with diminazene diaceturate at 7.0 mg/kg), the pre-infection values of 5.3±0.2 and 5.2±0.14, respectively increased significantly by day 7 to 7.1±0.1 and 7.3±0.4, respectively, while there was a significant decline by day 14 post infection (P.I) or day 4 post treatment (P.T) 6.6±0.2 and 6.6±0.2. Pre-infection values were attained by day 21 post infection 5.6±0.4 and 5.5±0.1 or day 11 post treatment.

The mean levels of serum creatinine of the New Zealand rabbits experimentally infected with *T. brucei brucei* with their controls are shown in Table 2. In group A, infected with *T. brucei brucei* but untreated control, the pre-infection value of 101±1 increased significantly ($p<0.05$) from day 7 with 208±7 (P.I) to 278±9 by day 21 (P.I). All infected rabbits in the group died by day 22 post infection. In group B (uninfected/untreated control), group E (uninfected/treated with diminazene diaceturate at 3.5 mg/kg) and group F (uninfected/treated with 7.0 mg/kg of diminazene diaceturate), the pre-infection values of 102±3, 102±2, and 102±2 remained fairly constant ($p>0.05$) throughout the study. In group C (infected/treated with diminazene diaceturate at 3.5 mg/kg) and group D (infected/treated with diminazene diaceturate at 7.0 mg/kg), the pre-infection values of 102±3 and 103±3, respectively increased significantly by day 7 to 201±13 and 196±8, respectively, while there was a significant decline by day 14 post infection (P.I) or day 4 post treatment (P.T) 140±18 and 133±15. Pre-infection values were attained by day 21 post infection 105±5 and 103±5 or day 11 post treatment.

The mean levels of alanine aminotransferase of the New Zealand rabbits experimentally infected with *T. brucei brucei* with their controls are shown in Table 3. In group A, infected with *T. brucei brucei* but untreated control, the pre-infection value of 50±1 increased significantly ($p<0.05$) from day 7 with 101±5 (P.I) to 186±9 by day 21 (P.I). All infected rabbits in the group died by day 22 post infection. In group B (uninfected/untreated control), group E (uninfected/treated with diminazene diaceturate at 3.5 mg/kg) and group F (uninfected/treated with 7.0 mg/kg of diminazene diaceturate), the pre-infection values of 49±2, 51±7, and 52±5 remained fairly constant ($p>0.05$) throughout the study. In group C (infected/treated with diminazene diaceturate at 3.5 mg/kg) and group D (infected/treated with diminazene diaceturate at 7.0 mg/kg), the pre-infection values of 49±2 and 49±2, respectively increased significantly by day 7 to 103±5 and 101±5, respectively, while there was a significant decline

Table 2. Mean levels of serum creatinine (mmol/L) of New Zealand rabbits experimentally infected with *Trypanosoma bruceibrucei* and treated with diminazene diaceturate and their controls.

Group (n=5)	PP Tb	Day of treatment		Days post-infection	
	0	7	10	14	21
A	100.75 ±0.95[a]	207.50±7.14[b]	-	243.25 ±5.37[c]	278.00 ±9.09[d]
B	101.50 ±2.64[a]	100.75±2.21[a]	-	104.50 ±2.38[a]	101.50±2.64[a]
C	102.25 ±2.63[a]	201.25±13.20[b]	-	140.00±18.25[c]	105.00±4.76[a]
D	103.25 ±3.30[a]	196.50±8.34[b]	-	132.50 ±15.00[c]	103.25±4.71[a]
E	102.25 ±2.21[a]	103.75±3.30[a]	-	103.25 ±3.40[a]	103.00±2.94[a]
F	101.75 ±1.70[a]	103.75±3.50[a]	-	103.00 ±2.44[a]	102.50±3.31[a]

Mean values with different superscripts in rows and in columns differed significantly ($p<0.05$). A: Infected/Untreated control; B: Uninfected/Untreated control; C: Infected/Treated with 3.5 mg/kg of diminazene diaceturate; D: Infected/Treated with 7.0 mg/kg of diminazene diaceturate; E: Uninfected/Treatedwith3.5 mg/kg of diminazene diaceturate; F: Uninfected/Treated with 7.0 mg/kg of diminazene diaceturate; PP Tb= Pre-patent period for *Trypanosoma brucei brucei.*

Table 3. Mean levels of alanine aminotransferase (u/L) of New Zealand rabbits experimentally infected with *Trypanosoma brucei brucei* and treated with diminazene diaceturate and their controls.

Group (n=5)	PP Tb	Day of treatment		Days post-infection	
	0	7	10	14	21
A	49.50 ±1.29[a]	100.50±5.26[b]	-	136.25 ±11.08[c]	186.25 ±9.46[d]
B	49.25 ±2.21[a]	49.50±2.64[a]	-	51.75 ±4.99[a]	49.25±2.21[a]
C	48.75 ±1.50[a]	102.50±5.26[b]	-	82.00±6.27[c]	51.75±5.37[a]
D	49.25 ±1.70[a]	101.00±5.03[b]	-	83.75 ±2.98[c]	52.25±3.86[a]
E	50.50 ±7.04[a]	50.75±6.50[a]	-	53.00 ±7.65[a]	53.00±8.08[a]
F	52.25 ±4.57[a]	51.75±2.75[a]	-	53.25 ±3.77[a]	50.00±1.82[a]

Mean values with different superscripts in rows and in columns differed significantly ($p<0.05$). A: Infected/Untreated control; B: Uninfected/Untreated control; C: Infected/Treated with 3.5 mg/kg of diminazene diaceturate; D: Infected/Treated with 7.0 mg/kg of diminazene diaceturate; E: Uninfected/Treatedwith3.5 mg/kg of diminazene diaceturate; F: Uninfected/Treated with 7.0 mg/kg of diminazene diaceturate; PP Tb= Pre-patent period for *Trypanosoma brucei brucei.*

by day 14 post infection (P.I) or day 4 post treatment (P.T) 82±6 and 84±3. Pre-infection values were attained by day 21 post infection 52±5 and 52±4 or day 11 post treatment.

The mean levels of aspartate aminotransferase of the New Zealand rabbits experimentally infected with *T. brucei brucei* with their controls are shown in Table 4. In group A, infected with *T. brucei brucei* but untreated control, the pre-infection value of 53±3 increased significantly ($p<0.05$) from day 7 with 94±5 (P.I) to 235±13 by day 21 (P.I). All infected rabbits in the group died by day 22 post infection. In group B (uninfected/untreated control), group E (uninfected/treated with diminazene diaceturate at 3.5 mg/kg) and group F (uninfected/treated with 7.0 mg/kg of diminazene diaceturate), the pre-infection values of 54±4, 52±2, and 52±1 remained fairly constant ($p>0.05$) throughout the study.In group C (Infected/treated with diminazene diaceturate at 3.5 mg/kg) and group D (Infected/treated with diminazene diaceturate at 7.0 mg/kg), the pre-infection values of 54±2 and 54±3, respectively increased significantly by day 7 to 96±5 and 100±2, respectively, while there was a significant decline by day 14 post infection (P.I) or day 4 post treatment (P.T) 70±5 and 72±6. Pre-infection values were attained by day 21 post infection 55±4 and 54±3 or day 11 post treatment.

The mean levels of alkaline phosphatase of the New Zealand rabbits experimentally infected with *T. brucei brucei* with their controls are shwn in Table 5. In group A, infected with *T. brucei brucei* but untreated control, the pre-infection value of 11±2 increased significantly ($p<0.05$) from day 7 with 24±1 (P.I) to 61±2 by day 21 (P.I). All infected rabbits in the group died by day 22 post infection. In group B (uninfected/untreated control), group E (uninfected/treated with diminazene diaceturate at 3.5 mg/kg) and group F (uninfected/treated with 7.0 mg/kg of diminazene diaceturate), the pre-infection values of 10±1, 10±2, and 10±2 remained fairly constant ($p>0.05$) throughout the study. In group C (infected/treated with diminazene diaceturate at 3.5 mg/kg) and group D (infected/treated with diminazene diaceturate at 7.0 mg/kg),

Table 4. Mean levels of aspartate aminotransferase (u/L) of New Zealand rabbits experimentally infected with *Trypanosoma. bruceibrucei* and treated with diminazene diaceturate and their controls.

Groups (n=5)	PP Tb	Day of Treatment		Days post-infection	
	0	7	10	14	21
A	53.00 ±2.58[a]	94.25 ±5.12[b]	-	165.00 ±12.91[c]	235.00±12.91[d]
B	53.50 ±3.69[a]	53.75 ±3.50[a]	-	53.25 ±1.25[a]	53.25 ±4.42[a]
C	53.50±2.38[a]	96.25 ±4.99[b]	-	70.00 ±5.09[c]	54.75 ±3.86[a]
D	53.75±2.75[a]	99.75 ±1.70[b]	-	71.75 ±6.18[c]	53.75 ±2.75[a]
E	51.50 ±2.38[a]	53.00 ±0.81[a]	-	51.50±2.38[a]	52.00±2.82[a]
F	51.50 ±1.29[a]	53.00 ±0.81[a]	-	52.50 ±2.38[a]	52.25 ±2.63[a]

Mean values with different superscripts in rows and in columns differed significantly ($p<0.05$). A: Infected/Untreated control; B: Uninfected/Untreated control; C: Infected/Treated with 3.5 mg/kg of diminazene diaceturate; D: Infected/Treated with 7.0 mg/kg of diminazene diaceturate; E: Uninfected/Treated with 3.5 mg/kg of diminazene diaceturate; F: Uninfected/Treated with 7.0 mg/kg of diminazene diaceturate; PP Tb= Pre-patent period for *Trypanosoma brucei brucei.*

Table 5. Mean levels of alkaline phosphatase (u/L) of New Zealand rabbits experimentally infected with *Trypanosoma brucei brucei* and treated with diminazene diaceturate and their controls.

Group (n=5)	PP Tb	Day of treatment		Days post-infection	
	0	7	10	14	21
A	10.50 ±2.08[a]	23.50 ±1.29[b]		39.75±1.70[c]	61.00±1.82[d]
B	9.50 ±1.29[a]	10.50 ±1.29[a]		10.00±0.81[a]	9.75 ±0.50[a]
C	9.75±0.95[a]	20.75 ±2.21[b]		13.00 ±0.81[a]	11.25 ±0.95[a]
D	9.50±1.29[a]	23.75 ±1.50[b]		14.00 ±0.81[a]	12.50 ±0.57[a]
E	10.00 ±1.82[a]	10.25 ±2.75[a]		9.25±2.63[a]	11.00±2.16[a]
F	9.50 ±2.38[a]	10.25 ±2.75[a]		9.00 ±2.16[a]	9.75 ±2.06[a]

Mean values with different superscripts in rows and in columns differed significantly ($p<0.05$). A: Infected/Untreated control; B: Uninfected/Untreated control; C: Infected/Treated with 3.5 mg/kg of diminazene diaceturate; D: Infected/Treated with 7.0 mg/kg of diminazene diaceturate; E: Uninfected/Treatedwith3.5 mg/kg of diminazene diaceturate; F: Uninfected/Treated with 7.0 mg/kg of diminazene diaceturate; PP Tb= Pre-patent period for *Trypanosoma brucei brucei.*

the pre-infection values of 10±1 and 10±1, respectively increased significantly by day 7 to 21±2 and 24±2, respectively, while there was a significant decline by day 14 post infection (P.I) or day 4 post treatment (P.T) 13±1 and 14±1. Pre-infection values were attained by day 21 post infection 11±1 and.12.5±06 or day 11 post treatment.

The mean levels of serum glucose of the New Zealand rabbits experimentally infected with *T. brucei brucei* with their controls are shown in Table 6. In group A, infected with *T. brucei brucei* but untreated control, the pre-infection value of 98±3 declined significantly ($p<0.05$) by day 7 with 74±6 and continued so without abating (P.I) up to 25±5 by day 21 (P.I). In group B (uninfected/untreated control), group E (uninfected/treated with diminazene diaceturate at 3.5 mg/kg) and group F (uninfected/treated with 7.0 mg/kg of diminazene diaceturate), the pre-infection values of 100±3, 100±2, and 99±3 remained fairly constant ($p>0.05$) throughout the study. In group C (infected/treated with diminazene diaceturate at 3.5 mg/kg) and group D (infected/treated with diminazene diaceturate at 7.0 mg/kg), the pre-infection values of 99±2 and 100±2, respectively decreased significantly ($p<0.05$) to 78±9 and 78±9 by day 7, respectively, while the value increased by day 14 P.I to 84±1 and 85±3, respectively which was not significant enough to attain the pre-infective value. Pre-infection values were attained by day 21 post infection 99±0.8 and 98±1 or day 11 post treatment.

DISCUSSION

This study showed that the infected groups (A, C, and D) presented physical signs characterized by pyrexia, weakness, anorexia, emaciation, increased respiration,

Table 6. Mean levels of serum glucose (mmol/l) of New Zealand rabbits experimentally infected with *Trypanosoma brucei brucei* and treated with diminazene diaceturate and their controls.

Group (n=5)	PP Tb	Day of treatment		Days post-infection	
	0	7	10	14	21
A	97.50 ±3.00[a]	73.50 ±6.35[b]		54.50±3.41[c]	24.75±4.99[d]
B	100.00 ±2.82[a]	99.75 ±3.30[a]		99.75±2.63[a]	98.75 ±1.50[a]
C	98.50±1.91[a]	78.00 ±8.90[b]		83.50 ±1.29[c]	99.00 ±0.81[a]
D	99.50±1.91[a]	78.00 ±8.67[b]		84.50 ±3.10[c]	98.00 ±1.41[a]
E	99.75 ±1.70[a]	99.50 ±2.64[a]		99.50±2.64[a]	99.00±6.21[a]
F	98.50 ±3.41[a]	99.50 ±2.64[a]		97.00 ±2.58[a]	98.00 ±3.65[a]

Mean values with different superscripts in rows and in columns differed significantly ($p<0.05$). A: Infected/Untreated control; B: Uninfected/Untreated control; C: Infected/Treated with 3.5 mg/kg of diminazene diaceturate; D: Infected/Treated with 7.0 mg/kg of diminazene diaceturate; E: Uninfected/Treatedwith3.5 mg/kg of diminazene diaceturate; F: Uninfected/Treated with 7.0 mg/kg of diminazene diaceturate; PP Tb= Pre-patent period for *Trypanosoma brucei brucei.*

alopecia and corneal opacity. However, following treatment in group C (infected/treated with diminazene diaceturateat 3.5 mg/kg) and group D (infected/treated with diminazene diaceturate at 7.0 mg/kg), these signs reduced or even reversed, suggesting an attempt by the administered drug to restore cellular functions to its pre-infection status. This change can be ascribed to the potency of diminazene diaceturate in the treatment of trypanosomosis (Anosa, 1988; Mbaya et al., 2009e). In this study, standard doses of the inocula were administered and uniform pre-patent periods were encountered, which showed that the initial parasite replication rate were similar irrespective of the host susceptibility. The infection of *T. brucei brucei* have been observed and reported in dogs (Nwosu and Ikeme, 1992), in red fronted gazelles (*Garrulax rufifrons*) (Mbaya et al., 2009a, 2010) and *T. brucei gambiense* infection in Baboons (*Papio anubis*) (Mbaya et al., 2009b). All rabbits infected became parasitaemic 7 days after infection, believed to have a prepatent period of 5 to 10 days for the parasite (Sorden and Andreasen, 2008). Similarly, the prepatent period of 7 days observed among rabbits is in agreement with the report of Anosa (1988a).

The serum enzymes, aspartate aminotransferase, alkaline phosphatase and alanine aminotransferase evaluated in this study showed a significant increase ($p<0.05$) in all the infected groups, which were later restored to their pre-infection values in groups treated with diminazene diaceturate (Groups C and D). These increases were suggestive of liver damage. This was observed more classically in the control animals (Group A), where the liver cells experienced degenerative changes, thereby releasing the liver enzymes in circulation. The infection advanced without abating as the enzymes appreciated (Kagwa et al., 1984). Similar reports have been reported in *T. evansi* infection of dogs (Aquino et al., 2002) and equines (Cardioli et al., 2006; Parashar et al., 2018). *T. brucei brucei* infection of Gazelles (Mbaya et al., 2008b) and in *T. brucei gambiense* infection of Baboons (*P. anubis*) (Mbaya et al., 2009a). The rise in enzyme levels may also be an outcome from the effect of trypanosomelyses resulting from the host defense mechanism (Gray, 1969).

Serum metabolites of glucose and urea were also assessed in this study. Serum glucose levels decreased significantly in all the groups infected (A, C, and D) and were later increased or restored completely to their pre-infection values in groups treated with Sequzene®. Sequel to this, weakness in the rabbits was encountered. This may likely be associated with the fact that the high energy demand in the rabbits during high parasitaemia impaired glucose release from the gluconeogenic pathways as well as the fact that trypanosomes during high parasitaemia consumed large quantities of the host glucose (Moon et al., 1968; Welled et al., 1974; Anosa, 1988b). During aerobic glycolysis, it has been sited that trypanosomes consume blood glucose (Igbokwe, 1994). Trypanosomes metabolize glucose to produce 4 hydroxyl 1-4 methylα-ketogluterate which is inhibitory to the tricarboxylic acid cycle (TCA) in the mitochondria, leading to acute energy deficiency in the host (Asham and Seed, 1973; Igbokwe, 1994; Mbaya et al., 2008c). Consequently, this indicates that the TCA cycle and oxidative phosphorylation might have been inhibited, leading to its total failure to produce energy from energy rich compounds (Igbokwe, 1994; Mbaya et al., 2008c). Ninety percent of the energy available in glucose is released when pyruvate is oxidized to CO_2 and H_2O through the TCA cycle and electron transport chain (Conn and Stumpf, 1996). The rise in blood urea nitrogen (BUN) faced in this study could be due to increased red blood cell and other tissue destruction or insufficient renal tubular. It may also be due to increased haemolysis (Igbokwe et al., 1994; Mbaya et al., 2008c).

The elevated creatinine levels in this study could be associated to acute kidney disorder as disclosed by lesions seen in histopathology of infected rabbits which was also described by Arowolo et al. (1988) and Mbaya

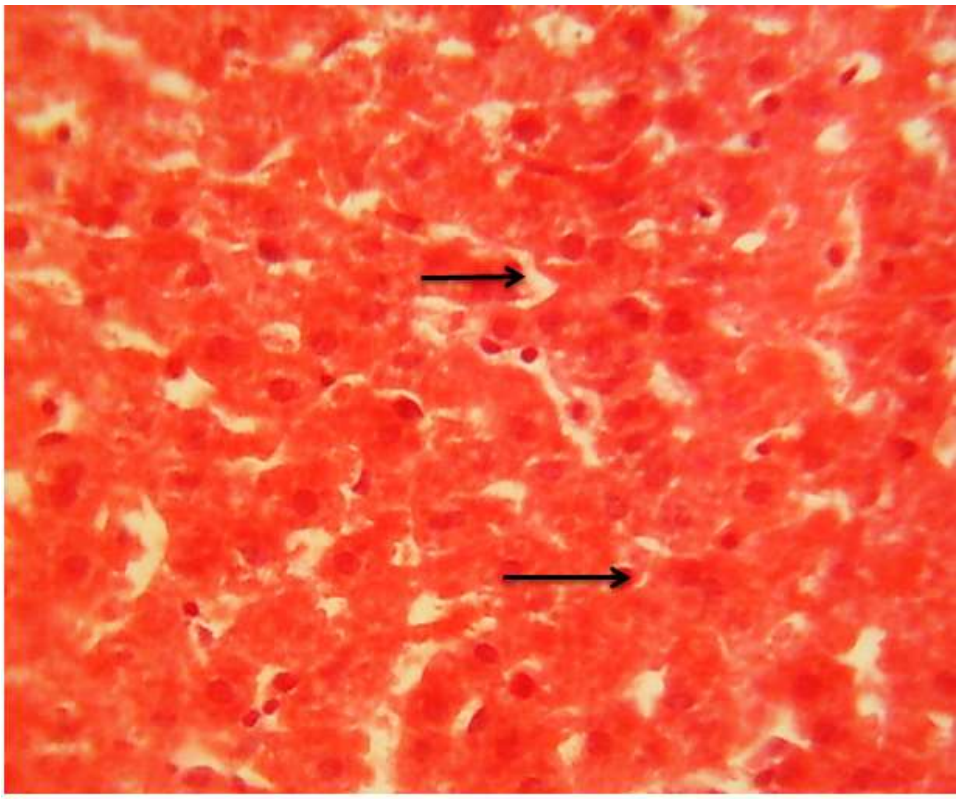

Plate 1. Photomicrograph of the liver of a New Zealand rabbit (Group A) showing a few degenerated hepatocytes (arrows). H&E, x200.

et al. (2007). The retention of creatinine in the body showed that the kidneys were severely affected, thereby, failing to excrete these catabolic products. Similarly, high levels of creatinine particularly in the infected but untreated rabbits might also be attributed to the body weight loss and muscle wasting. However, it is important to note that, all parameters evaluated during the course of this study remained fairly constant for the uninfected Groups B (uninfected/untreated group), Group E (uninfected/treated with diminazene diaceturate at 3.5 mg/kg and Group F (uninfected/treated with diminazene diaceturateat 7.0 mg/kg). The hispathological changes observed in (Plates 1 to 4) may help to explain further the significant changes in the activities of enzymes evaluated. The liver which showed a degenerative hepatocyte and thickened interstitium infiltrated by leucocytes and an inflamed bronchiole of the lungs with a congested blood vessels and neuronal cells in the brain. Increased plasmal tissues enzymes have been associated with organ damage, particularly the kidney and liver and other organs, and this would explain the increased enzyme concentrations in the plasma of the infected rabbits in this study.

Distortion of the tissue architecture, with the loss of cellular morphology, inflammatory change and extensive mononuclear cellular infiltration in the tissues observed in this study may be due to the effect of the *T. brucei brucei* infection, which is known to infiltrate organs and tissues.

Conclusion

Based on the results from this study, New Zealand rabbits were found to be highly susceptible to *T. brucei brucei* infection. The infection in rabbits had effects on their serum biochemistry, tissues and organs. It was also found that a single dose of diminazene diaceturate at 3.5 and 7.0 mg/kg worked to ameliorate the deleterious effects of *T. brucei brucei* on serum biochemistry and in tissues of *T. brucei brucei* infected rabbits.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

ACKNOWLEDGEMENTS

The authors thank the laboratory staff who provided technical support and assistance during the course of the research.

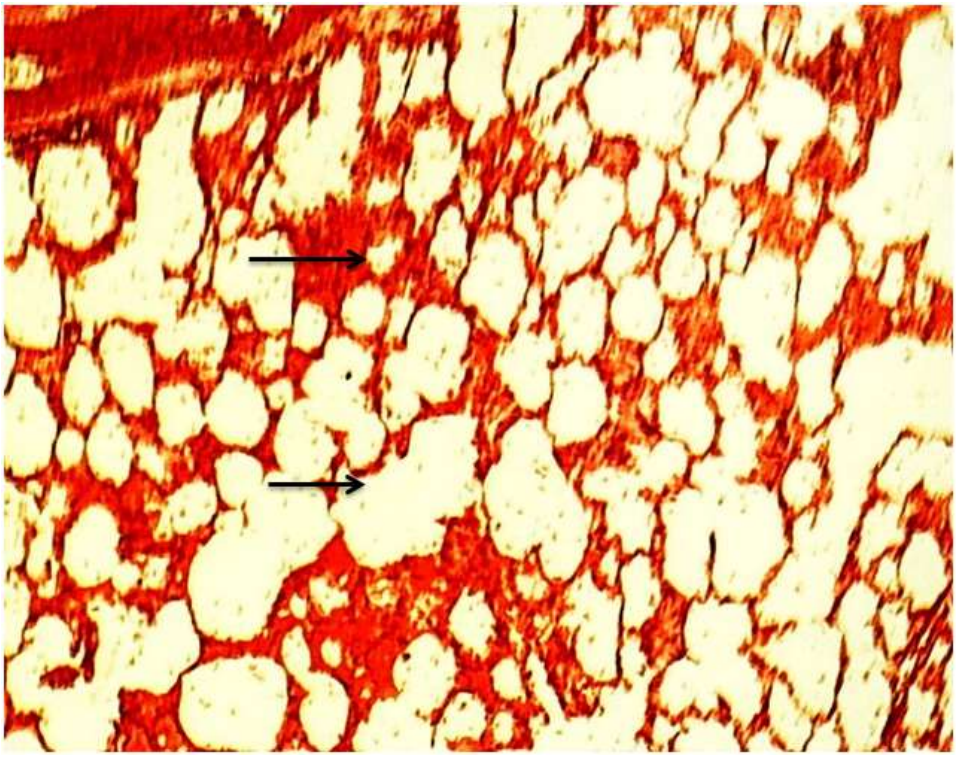

Plate 2. Photomicrograph of the lungs of a New Zealand rabbit (Group A) showing thickened interstitium infiltrated by leucocytes and an inflamed bronchiole (arrows). H&E, x200.

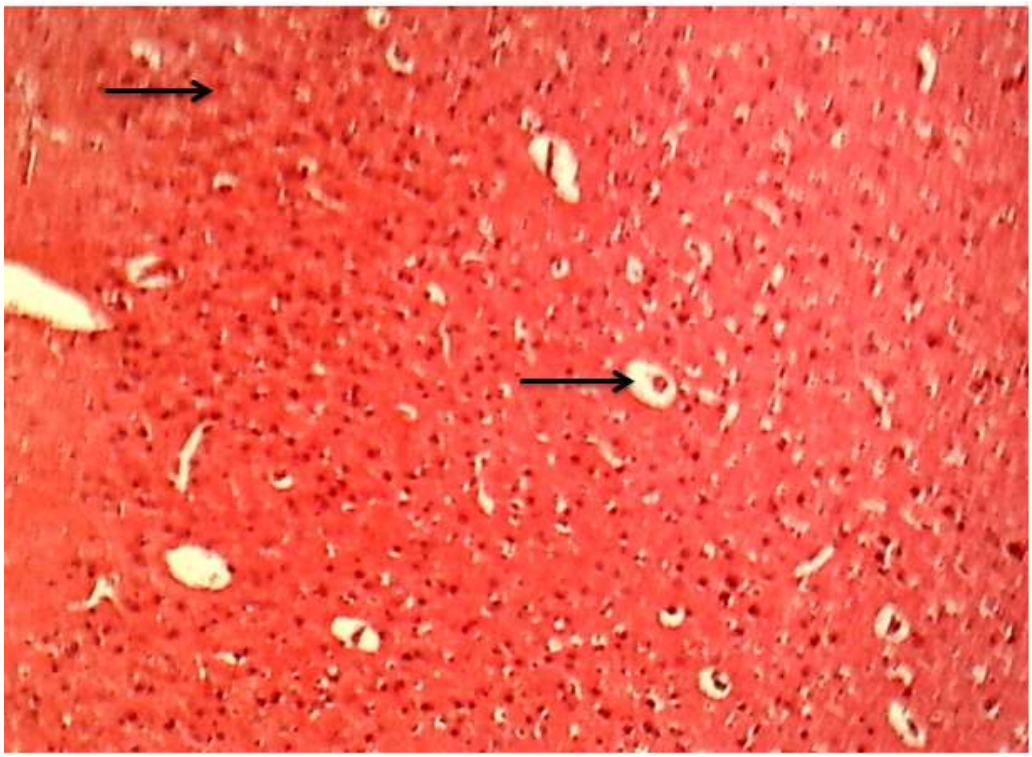

Plate 3. Photomicrograph of the brain of a New Zealand rabbit (Group A) showing congested blood vessels and neuronal cells (arrows). H&E, x200.

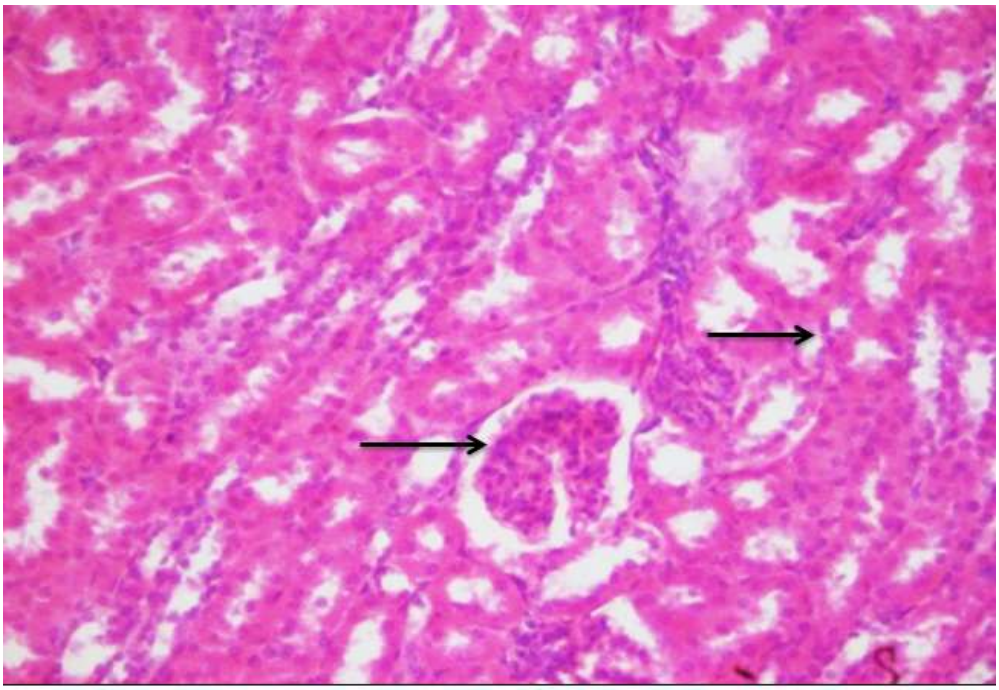

Plate 4. Photomicrograph of the kidney of New Zealand rabbit (Group A) showing degenerative and necrotic renal tubules in the renal interstitium (arrows). H&E, x200.

REFERENCES

Anon (2018). Report of the second WHO stakeholders meeting on gambiense human African trypanosomiasis elimination Geneva, 21-23.

Anosa VO (1988a). Haemotological and Biochemical Changes in Human and Animal Trypanosomosis II. Revenue Elevage Medicine Veterinary Des Pays Tropicaux, p. 41.

Anosa VO (1988b). Pathology of experimental Trypanosoma vivax infection of sheep and goats. Veterinary Medicine 80:685-700.

Anosa VO, Kaneko JJ (1983). Pathogenesis of Trypanosoma brucei infection in deer mice (Cp. manuculatus). Light electron microscopic studies on erythrolytes pathological changes and phagocytosis. Annual Journal of Veterinary Research 44:645-651.

Anosa VO (1988a). Haemotological and Biochemical Changes in Human and Animal Trypanosomosis II.Revenue ElevageMedicine Veterinary Des Pays Tropicaux, p. 41.

Aquino LP, MachadoRZ, Alessi AC, SantanaAE, Castro MB, Marques LC, Malhero EB (2002). Haematological, biochemical and anatormorphological aspects of the experimental infection with Trypanosoma evansi in Dogs. Arquino Brasileiro Medicinal Veterinaria Zootecnia 54:8-18.

Arowolo ROA, Elhassan EO, Amure BO (1988). Revued' Elavageet Medicine de pays Tropicaux 41:277-279.

Asham PV, Seed JR (1973) Biochemical studies in the vole (Microtus monatus) II. The effects of Trypanosoma brucei gambiense infection in the diurnal variation of hepatic glucose-6-phosphate and liver glycogen. Comparative Biochemistry and Physiology 451:379-392.

Ashcroft MJ (1959). The Importance of African Wildlife as Reservoirs of Trypanosomosis. West Africa Medical Journal 36:289–297.

Atawodie SE, Bulus T, Ibrahim S, Ameh DA, Nok AJ, Mamman M, Galadima M (2003). In vitro trypanocidal effect of methanolic extract of some Nigerian Savannah plants. African Journal of Biotechnology, 2(9):317-321.

Bal MS, Singla LD, Kumar H, Ashuma, Gupta K, Juyal PD (2012). Pathological studies on experimental Trypanosoma evansi infection in Swiss albino mice. Journal of parasitic Disease 36:260-264.

Cardioli FA, Marques LC, Machado RZ, Elessi AC, Aquino LPT, Barnabe PA (2006). Experimental Trypanosoma evansi infection in donkeys: Haemotological, biochemical and histopathological changes. Arquino Brasileiro de Mediciba Veterinaria Zootecnia 58: 749-756.

Conn EE, Stumf PK (1996). Outline of Biochemistry. John Wiley, New York pp. 110-115.

Desowitz RS (1960). Studies on Immunity and Host-parasite Relationships: The Immunological Response of Resistance and Susceptible Breeds of Cattle on Trypanosome Challenge. Annals of Tropical Medicine and Parasitology 2:293-313.

Graphpad Instat® (2009). Version 3.00 for windows, Graphpad software, San Diego, C.A, U.S.A. www. Graphpad.com.

Gray AR (1969). Serum transaminase level in cattle and sheep infected with Trypanosoma vivax. Experimental Pathology 14:374-381.

Gupta SK, Singla LD (2012). Diagnostic trends in parasitic diseases of animals. In: Veterinary Diagnostics: Current Trends. Gupta RP, Garg SR, Nehra V and Lather D (Eds), Satish Serial Publishing House, Delhi, pp. 81-112.

Herbert WJ, Lumsden WHR (1976). Trypanosoma brucei; A rapid matching method for estimating the hosts parasitaemia. Experimental Parasitology 40:427-432.

Hudson JR (1944). Acute and sub-acute trypanosomosis in cattle caused by Trypanosoma vivax. Journal of Comparative Pathology 54:108-119.

Ibrahim MA, Mohammed A, Isah MB, Aliyu AB (2014). Anti-trypanosomal activity of African medicinal plants: A review update. Journal of Ethnopharmacology 154: 26-54.

Igbokwe IO (1994). Mechanisms of Cellular Injury in African trypanosomosis. Veterinary Bulletin 64:611-620.

Johnson TO, Omoniwa BP (2014). In vivo trypanocidal activity of ethanolic crude extract and phytochemical fractions of Garcinia kola Seeds. Annual Research and Review in Biology 4:212–222.

Kagwa E, Mungua WK, Mugera GM (1984). The Prevalence of trypanosome in sheep and goats at slaughter. Israel Journal of Veterinary Medicine 50(2):67-71.

Lifongo LL, Simoben VC, Ntie-Kang F, Babiaka BS, Judson NP (2014).

A bioactivity versus ethnobotanical survey of medicinal plants from Nigeria, West Africa. Natural Products and Bioprospecting 4:1-19.
Mbaya AW (2010). Effect of cortisolaemia on the pathogenesis of T. brucei infected in Red fronted Gazelles (*G. rufirens*). International Journal of Parasitology 78: 45-50.
Mbaya AW, Ahmed MI, Adamu M, Gyang SN (2009d). Toxicity and combined therapeutic activity of Armtemether and Lumefantrine in Trypanosoma brucei infected rats. Nigerian Journal of Parasitology 30(1): 43- 47.
Mbaya AW, Aliyu MM, Nwosu CO (2008b). Effects of Cymelarsan® and Berenil® on clinico-pathological changes in Red Fronted, Gazelles (Gazella rufifrons) experimentally infected with Trypanosoma. brucei. Nigerian Veterinary Journal 29(3):27-40.
Mbaya AW, Aliyu MM, Nwosu CO, Egbe-Nwiyi TNC (2009b). The relationship between parasitaemia and anaemia in a concurrent Trypanosoma brucei and Haemonchus contortus infection in Red Fronted Gazelles (Gazella rufifrons). Veterenarski Arhiv 79(5): 451-460.
Mbaya AW, Aliyu MM, Nwosu CO, Egbe-Nwiyi TNC (2009e). Effects of melarsamine hydrochloride (Cymelarsan) and diminazene aceturate (Berenil) on the pathology of experimental Trypanosoma brucei infection in Red Fronted Gazelle (Gazelarutifrans) Veterinary Parasitology 163:140-143.
Mbaya AW, Aliyu MM, Nwosu CO, Ibrahim UI (2008c). Captive wild animals as potential reservoirs of haemo and ecto-parasitic infections of man and domestic animals in the Arid Region of Northeastern Nigeria. Veterenarski Arhiv 78(5):429-440.
Mbaya AW, Aliyu MM, Nwosu CO, Ibrahim UI (2009a). Effect of DL-α-difluoromethylornithine on Biochemical Changes in Baboons (Papioanubis) Experimentally Infected with Trypanosoma brucei gambiense. Nigerian Veterinary Journal 30(1):35-44.
Mbaya AW, Kumshe HA, Nwosu CO (2012). The mechanisms of anaemia in trypanosomosis. In: Anaemia, (D. S. Silverberg. Ed.), In Tech.Publishers, Croatia, pp. 270-282.
Mbaya AW, Nwosu CO, Onyeyili PA (2007). Toxicity and anti-trypanosomal effects of ethanolic extract of Butyrospermum paradoxum (Sapotacea) stem bark in rats infected with Trypanosoma brucei and *T. congolence*. Journal of Ethnopharmacology 111:536-530.
Moon AP, Williams JS, Witherspoon C (1968). Serum biochemical changes in mice infected with *Trypanosoma rhodesiense* and *Trypanosoma duttoni*. Experimental Pathology 22:112-121.
Murray PK, Jennings FW (1982). African Trypanosomosis: Chemotherapy in Rodents Models of Sleeping Sickness. In: Experimental Bacterial and Parasite Infection (Keash, I. and Wadstream, I., Eds.) Elsevier Biochemical, New Yorke, pp. 343-344.
Murray PK, Trail JCM, Turner DA, Wissoeg Y (1983). Livestock Productivity and Trypano-tolerance Network Training Manual. International Livestock Centre for Africa, Addis Ababa (Ethiopia), pp.159–165.
Mwiinde AM, Simuunza M, Namangala B, Chama-Chiliba CM, Machila N, Anderson N, Shaw A, WelburnSC (2017). Estimating the economic and social consequences for patients diagnosed with human African trypanosomiasis in Muchinga, Lusaka and Eastern Provinces of Zambia (2004-2014). Infectious Diseases of Poverty 6:1–13.
Nwosu CO, Ikene MM (1992). Parasitaemia and clinical manifestations in Trypanosoma brucei infected dogs, Revenue, Elevage: et de Medicine Veteriniare des pays tropicaux 45: 273-277.
Ochei J, Kolhatkar A (2000). Medical Laboratory Science Theory and Practice Tata McGraw — Hill publishing Company Limited, New Deltti India 6(1):1213.
Onyeyili PA, Egwu O (1995). Chemotherapy of African animal trypanosomosis: A historical perspective. Prototozoological Abstract 19:5-10.
Parashar R, Singla LD (2019). Challenges in control of trypanosomosis in ruminants: Alarming bells on drug resistance. Ruminant Science 8(2):153-162.
Parashar R, Singla LD, Gupta M, Sharma SK (2018). Evaluation and correlation of oxidative stress and haemato-biochemical observations in horses with natural patent and latent trypanosomosis in Punjab state of India. Acta Parasitologica 63(4):733-743.
Simwango M, Ngonyoka A, Nnko HJ, Salekwa LP, Ole-Neselle M, Kimera SI, Gwakisa PS (2017). Molecular prevalence of trypanosome infections in cattle and tsetse flies in the Maasai Steppe, northern Tanzania. Parasites Vectors 10:1-11.
Sorden SD, Andreasen CB (2008). Trypanosomosis, Nagana tsetse disease, tsetse fly disease, trypanosomosis. In: Emerging and exotic disease of animals. 3rd Edition. Iowa State University Institute for Cooperation in Animal Biologics p. 238.
Soulsby EJL (1982). Helminthes, Arthropods and Protozoa Parasites of Domesticated Animals, Bailere Tindal, London, pp. 46-49.
Sumbria D, Singla LD (2017). Chemotherapeutic approaches in management of equine trypanosomosis and piroplasmosis: An update. In: Recent Developments in Drug Development, Bhagwat DP, Sapra S and Ahlawat P (Eds), Bharti Publications, New Delhi pp. 66-77.
Tadesse B, Terefe G, Kebede N, Shibeshi W (2015). In vivo anti-trypanosomal activity of dichloromethane and methanol crude leaf extracts of Dovyalis abyssinica (Salicaceae) against *Trypanosoma congolense*. BMC Complementary and Alternative Medicine 15:278.
Tekle Y (2014). An ethno-veterinary botanical survey of medicinal plants in Kochore district of Gedeo Zone, Southern Nations Nationalities and Peoples Regional State (SNNPRs), Ethiopian Journal of Scientific and Innovative Research 3(4):433.
Tesfaye A, Terefe G, Giday M, Shibeshi W (2015). Invivo Antitrypanosomal activity of the leaf extracts of *Albizia schimperiana* (Fabaceae) Against *Trypanosoma Congolense* Infection in Mice. Clinical and Experimental Pharmacology and Physiology 5:171.
World Health Organization (2015a). Investing to Overcome the Global Impact of Neglected Tropical Diseases Third WHO Report on Neglected Tropical Diseases.
Welled BT, Lotzsch R, Diehl G, Sadun E, Williams J, Warui G (1974). *Trypanosome congolense*. I. Clinical Parasitology 36:6-19.
Wurochekke AU, Nuhu N, Anyanwu GO (2014). Trypanocidal potential of *Carrisa edulis* in male wistar rats infected with *T. congolense*. American Journal of Research Communication 2:234-244.