

Original Research Article

BIOCHEMISTRY ASSESSMENT OF DOGS EXPERIMENTALLY INFECTED WITH SINGLE *T. BRUCEI*, *A. CANINUM* AND CONJUNCT *T. BRUCEI* AND *A. CANINUM* INFECTIONS AND TREATED WITH DIMINAZENE ACETURATE AND MEBENDAZOLE

Abstract

Trypanosomosis is a wasting disease characterized by progressive loss of body condition, to the point of weakness, anaemia and death. For a fact serum biochemistry profile is one of the most informative laboratory tests in Veterinary Medicine used in diagnosis. Its usefulness necessitated the study of serum biochemistry of dogs experimentally infected with single and conjunct *T. brucei*, and *A. caninum*. The dogs were randomly assigned into 4 groups with 4 animals in each group as follows: GP I: Uninfected (control), GROUP II: *Ancylostoma caninum* infected, GROUP III: *Trypanosoma brucei* infected, GROUP IV: Conjunct *Trypanosoma brucei* and *A. caninum* infection. Post acclimatization *Ancylostoma caninum* infection was done on GPII and GPIV alone. Two weeks later, *Trypanosoma brucei* infection was done on GPIII and GPIV. Three weeks post trypanosome infection; GPIII and GPIV were treated with diminazene aceturate. Mebendazole was used only on GPII and GPIV and a repeat treatment given 2 weeks later. The serum biochemistry parameters showed a significant ($p < 0.05$) increase in Total protein in GPIII and GPIV. A significant ($p < 0.05$) decrease in Total protein was recorded in GPII. Conversely, there were significant ($p < 0.05$) decrease in albumin in all the infected groups (GPII, GPIII and GPIV). Significant ($p < 0.05$) increases in the levels of cholesterol and bilirubin were observed in GPII, GPIII and GPIV. However there was no significant ($p > 0.05$) alteration in calcium level in all the infected groups compared with control.

Keywords: Biochemical changes, *Trypanosoma brucei*, *A. caninum*, diminazene aceturate, mebendazole.

27 Introduction

28 Trypanosomosis and ancylostomosis are parasitic diseases which may occur in mixed infection in
 29 dogs especially in the southeastern region of Nigeria (Ezeokonkwo *et al.*, 2010; Nwoha,
 30 2011).The effect of these parasites are best known by proper assessment of biochemistry profiles
 31 to reveal the clinical status of animal, the organ function, nutritional state and determine
 32 therapeutic prescription (Mundim *et al.*, 2007; Bukowski, 2012). Biochemistry assessment of
 33 infected animals would also helps to determine the prognosis of the diseases and the specific
 34 therapeutic protocols and their accompanying effects on the body (Payne and Payne, 1987). It
 35 may be used in determining the effect of prolonged use of therapy on vital organs of the body
 36 including liver or kidney (Bukowski, 2012). In clinical biochemistry, the use of wide range of
 37 properly chosen tests are preferred to a specific test in order to reveal the effect of a disease
 38 condition in different systems of the body through the process of pattern recognition. Hence in
 39 trypanosomosis, serum biochemistry analysis though variable and highly inconsistent reveals
 40 effects on vital organs of the body and results correlate to the degree of damage thus serves as
 41 substantial prognostic and diagnostic tool in clinical practice. Regrettably, previous studies on the
 42 effects of trypanosomes on biochemical parameters had focused on single parasite species
 43 infections with very little information available on ancylostomosis or conjunct infection of
 44 ancylostomosis and trypanosomosis in dogs. It is therefore imperative to assess the serum
 45 biochemistry changes in dogs with single *Trypanosoma brucei* and in combination with *A.*
 46 *caninum*.

47 Materials/Methodology

48 Experimental Animals

Sixteen indigenous breed of dogs of both sexes weighing between 4.0 and 8.0kg were used in this experiment. The dogs were acclimatized for 4 weeks before commencement of the experiment during which they were screened for blood parasites and confirmed negative by Giemsa-stain, thin blood smears and haematocrit buffy coat method (Woo, 1970). They were dewormed with tablets of mebendazole (Vermin[®], Janssen-Cilag Ltd 50 - 100 Holmers Farm Way, High Wycombe, Bucks, HP12 4EG UK) at the dose of 100mg/kg twice daily for 3 days and also treated with sulfadimidine at the dose of 48mg/kg intramuscularly against systemic opportunistic bacterial infections. The experiment commenced a week later. The animals were kept in clean cages in a fly proof house and fed twice daily. Water was given *ad libitum*.

Care of Experiment Animals

The care of the animals was in conformity with the guideline for animals' experimentation of Council for International Organization of Medical Sciences (CIOMS) for biomedical research involving animals. The dogs were humanely cared for and treated throughout the study. They were comfortably housed in properly ventilated pens in good hygienic condition and provided good and adequate feeding with clean portable drinking water.

Parasites and infections

***Trypanosoma brucei* isolate**

Trypanosoma brucei isolate used in the study was a local isolate obtained from a clinically infected dog from Nsukka area of Enugu State. The isolate was typed and confirmed in the department of Veterinary Parasitology and Entomology, University of Nigeria Nsukka. The parasites were maintained in rats and subsequently passage in a donor dog from where the experimental dogs were inoculated.

Estimated 2.5×10^6 of *T. brucei* suspended in 1ml of normal saline was used to infect each experimental dog in the group, and 1 ml of whole blood containing an estimated 2.5×10^6 parasites. The quantity of parasites inoculated was estimated using the rapid matching method of Herbert and Lumsden (1976).

***Ancylostoma caninum* Infection**

The concentration of larval suspension was estimated using an automatic pipette (Bioht Peoline®), according to the method of MAFF (1977). Small doses of 20 µl larval suspensions were placed as drops on a microscope slide and counted under $\times 4$ objective of a light microscope (Olympu®). Dogs were starved prior to infection so as to establish infection. A dose of 200 infective L₃ suspended in 1ml of distilled water were delivered *per os* to each of the experimental dogs, using a 2 ml syringe without needle.

Reconstitution of Diminazene aceturate

A 2.36g Veribin® a brand of trypanocide containing 1.05g of diaminazene aceturate was reconstituted with 15 ml distilled water according to manufacturer's recommendation. The volume of diminazene acetate administered to individual dog in GPIII and GPIV, were calculated from their weight at the dose of 7 mg/kg via the intramuscular route.

Administration of Mebendazole

Tablets of mebendazole (Vermin®, Janssen-Cilag Ltd 50 - 100 Holmers Farm Way, High Wycombe, Bucks, HP12 4EG UK) was given at the dose of 100mg/kg *per os* twice daily for 3 consecutive days. Treatment was repeated 2 weeks later.

Experimental Design

Dogs were randomly divided into 3 groups of 4 members in each group. GROUP I was uninfected dogs (control), GROUP II was infected with *Ancylostoma caninum*, GROUP III was *Trypanosoma brucei* infection, and GROUP IV was mixed infections of *Trypanosoma brucei* and *A. caninum*.

Post acclimatization, *Ancylostoma caninum* infection was done on GPIV alone. Two weeks later *Trypanosoma brucei* infections was done on GPIII and superimposed on GPIV. Three weeks post trypanosome infection; GPIII and GPIV were treated with diminazene aceturate. Mebendazole was used only on GPII and GPIV and a repeat treatment given 2 weeks later.

Serum Biochemistry

Blood Collection and Preparation of Serum Samples

Five milliliters of blood was collected through the cephalic vein of each of the experimental dogs and dispensed into dried appropriately labeled sterile test tubes with screw caps and kept slanted and allowed to clot. The blood samples were immediately transported to the Department of Veterinary Medicine Laboratory. The samples were left at room temperature for about 2 hours to yield sera and then centrifuged at 11000 revolutions per minute for 5 minutes and sera obtained were separated into clean labeled tubes and stored at -20°C until analyzed for biochemical constituents.

Biochemical Analysts

The serum Total protein, Bilirubin, albumin, cholesterol, and calcium were determined using Randox Text Kits according to the manufacturer's prescriptions.

Results

Total Protein

114 The result of total protein is shown in Table 1. There was significant ($p < 0.05$) decrease in total protein
 115 by week 3 in GPII compared to other groups (Table 1). The decreases in GPII progressed up to week 5.
 116 Conversely a significant ($p < 0.05$) increase was observed by week 4 in GPIII and by week 5 in GPIV.
 117 Increases in both groups progressed up to week 7 in GPIII and to week 9 in GPIV. Treatment was
 118 instituted on week 6 with 7 mg/kg diminazene aceturate and 200 mg/kg mebendazole in all the infected
 119 groups (GPII, GPIII and GPIV). Subsequently from week 6 there was no difference between GPII
 120 compared to GPI. Repeat treatment with diminazene aceturate was done on 8 and 9. From week 10 to
 121 12, there was no significant difference between both GPIII and GPIV compared to GPI.

122 Total Albumin

123 The result of the serum albumin level is shown in Table 2. From the result a significantly ($p < 0.05$)
 124 decrease in Albumin conc. was only observed in GPII on week 3 compared to GPI through out the
 125 experimental period. However from week 3 there was a significant decrease in GPIII and GPIV
 126 compared to GPI. The decreases in both groups progressed up to week 7 in GPIII and week 8 in GPIV.
 127 Treatment with 7 mg/kg diminazene and 200mg/kg mebendazole was done in all the groups (GPII,
 128 GPIII and GPIV) on weeks 6, 8, and 9. From week 9 onwards no significant difference was observed
 129 between GPIII and GPIV compared to GPI. However by week 12 there was a sudden decrease in both
 130 GPIII and GPIV compared to GPI.

131 Total Bilirubin

132 The result of total bilirubin is shown in Table 3. A Significant ($p < 0.05$) increase in bilirubin conc. was
 133 observed on week 6 In GPII compare to GPI through out the experiment. Nevertheless by week 3 there
 134 were significant ($p < 0.05$) increases in both GPIII and GPIV. The increases progressed up to week 6 in
 135 the groups. Treatment with 7 mg/kg diminazene and 200mg/kg mebendazole was done in all the groups

(GPII, GPIII and GPIV) on weeks 6, 8, and 9. Subsequently there was no significant difference between GPIII and GPIV compared to GPI.

Cholesterol

The result of serum cholesterol level is shown in Table 3. Significant ($p < 0.05$) increase in cholesterol level occurred in GPII on week 3 to 6. There was a sudden decrease were observed in both GPIII and GPIV on weeks 4, 5 and 6. Treatment with 7 mg/kg diminazene and 200mg/kg mebendazole was done in all the groups (GPII, GPIII and GPIV) on weeks 6, 8, and 9. Subsequently there was no significant difference between the groups compared to GPI.

Calcium ion

The result of serum calcium ion level is shown in Table 2. There was no significant alteration in calcium ion concentration in all the experimental groups (GPII, GPIII and GPIV) except on week 3 which shows a significant ($p < 0.05$). Treatment with 7 mg/kg diminazene and 200mg/kg mebendazole was done in all the groups (GPII, GPIII and GPIV) on weeks 6, 8, and 9. Afterwards there was no observable difference between the groups (GPII, GPIII and GPIV) compared to GPI.

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Table 1. Mean $\pm$ SE Total protein (mg/dl) of dogs with experimental single *T. brucei*, *T. brucei* and conjunct *T. brucei* / *A. caninum* infections and treated with diminazene aceturate and mebendazole.

Experimental Period(Weeks)	GPI (control)	GPII (Ac)	GPIII (Tb)	GPIV (Tb/Ac)
0	6.4 $\pm$ 0.90 ^a	5.0 $\pm$ 1.00 ^a	5.2 $\pm$ 1.00 ^a	6.0 $\pm$ 0.50 ^a
1 	6.7 $\pm$ 0.30 ^a	6.2 $\pm$ 0.30 ^a	6.0 $\pm$ 0.40 ^a	5.8 $\pm$ 0.20 ^a
2	6.8 $\pm$ 0.20 ^a	6.0 $\pm$ 0.70 ^a	6.1 $\pm$ 0.30 ^a	5.9 $\pm$ 0.10 ^a
3 	6.9 $\pm$ 0.50 ^a	5.0 $\pm$ 0.30 ^b	6.1 $\pm$ 0.60 ^a	6.0 $\pm$ 0.30 ^a
4	6.1 $\pm$ 0.20 ^a	4.9 $\pm$ 0.20 ^b	7.0 $\pm$ 0.50 ^c	6.2 $\pm$ 0.40 ^a
5	6.0 $\pm$ 0.30 ^a	4.0 $\pm$ 0.30 ^b	7.7 $\pm$ 0.70 ^b	6.6 $\pm$ 0.20 ^b

6 * +	6.7±0.80 ^a	5.9±0.60 ^a	7.6±0.60 ^b	7.8± 0.50 ^b
7	6.9±0.10 ^{ab}	6.0±0.20 ^a	7.0±0.40 ^{ab}	8.0±0.40 ^b
8 * +	6.5±0.20 ^a	5.2±0.40 ^a	6.1±0.80 ^a	8.0±0.60 ^b
9 *	6.8±0.60 ^a	5.9±0.70 ^a	6.3±0.20 ^a	8.4±0.40 ^b
10	6.5±0.50 ^a	6.0±0.30 ^a	6.5±0.50 ^a	6.5±0.20 ^a
11	6.6±0.20 ^a	6.3±0.20 ^a	6.6±0.30 ^a	6.5±0.30 ^a
12	6.2±0.20 ^a	6.0±0.20 ^a	7.0±0.30 ^a	6.0±0.30 ^a

Superscripts a b c represents the homogeneity between the experimental groups at probability P≤ 0.05.

- ↑ Infection with *A. caninum*
- ⚡ Infection with trypanosomes
- + Treatment with mebendazole
- * Treatment with diminazene aceturate
- Ac *Ancylostoma caninum*
- Tb *Trypanosoma brucei*

Experimental Period(Weeks)	GPI (control)	GPII (Ac)	GPIII (Tb)	GPIV (Tb/Ac)
0	1.9±0.50 ^a	1.5±0.30 ^a	1.9±0.20 ^a	2.2±0.10 ^a
1 ↑	2.6±0.20 ^a	2.4±0.10 ^a	2.1±0.40 ^a	2.1±0.20 ^a
2	2.7±0.20 ^a	2.3±0.30 ^a	2.3 ±0.20 ^a	2.1±0.40 ^a
3 ⚡	2.8±0.50 ^a	2.2±0.20 ^b	2.4±0.10 ^a	2.2±0.30 ^b
4	2.3±0.20 ^a	2.5±0.20 ^a	2.0±0.10 ^{ab}	1.6±0.20 ^b
5	2.6±0.20 ^a	2.4±0.30 ^a	1.5±0.10 ^c	1.3±0.00 ^c
6 * +	2.0±0.50 ^a	2.0±0.20 ^a	1.4±0.20 ^b	1.3±0.30 ^b
7	2.4±0.20 ^a	2.3±0.20 ^a	1.7±0.10 ^{cb}	1.4±0.20 ^{cd}
8 * +	2.2±0.20 ^a	2.6±0.30 ^a	2.0±0.10 ^a	2.0±0.10 ^b
9 *	2.5±0.10 ^a	2.5±0.40 ^a	2.2±0.10 ^a	2.3±0.20 ^a
10	2.5±0.20 ^a	2.3±0.10 ^a	2.3±0.20 ^a	2.3±0.10 ^a

11	2.4±0.10 ^a	2.4±0.10 ^a	2.3±0.10 ^a	2.2±0.20 ^a
12	2.6±0.10 ^a	1.9±0.40 ^{ab}	1.6±0.10 ^b	1.1±0.20 ^b

Table 2. Mean ± SE Total Albumin (mg/dl) of dogs with experimental single *T. brucei*, *T. brucei* and conjunct *T. brucei* / *A. caninum* infections and treated with diminazene aceturate and mebendazole.

Superscripts a b c represents the homogeneity between the experimental groups at probability $P \leq 0.05$.

- ↑ Infection with *A. caninum*
- ⚡ Infection with trypanosomes
- + Treatment with mebendazole
- * Treatment with diminazene aceturate
- Ac* *Ancylostoma caninum*
- Tb* *Trypanosoma brucei*

Table 3. Mean ± SE Total Bilirubin (mg/dl) of dogs with experimental single *T. brucei*, *T. brucei* and conjunct *T. brucei* / *A. caninum* infections and treated with diminazene aceturate and mebendazole.

Experimental Period(Weeks)	GPI (control)	GPII (<i>Ac</i>)	GPIII (<i>Tb</i>)	GPIV (<i>Tb/Ac</i>)
0	0.3±0.10 ^a	0.4±0.00 ^a	0.5±0.10 ^a	0.4±0.40 ^a
1	0.3±0.10 ^a	0.3±0.10 ^a	0.2±0.10 ^a	0.2±0.10 ^a
2	0.2±0.10 ^a	0.3±0.10 ^a	0.3±0.10 ^a	0.3±0.00 ^a
3 ⚡	0.3±0.10 ^a	0.3±0.00 ^a	0.7±0.10 ^b	0.7±0.10 ^b
4	0.2±0.10 ^a	0.2±0.00 ^a	0.7±0.20 ^b	0.4±0.10 ^a
5	0.2±0.10 ^a	0.3±0.10 ^a	0.3±0.10 ^a	0.2±0.00 ^a
6 * +	0.2±0.10 ^a	0.4±0.00 ^b	0.5±0.10 ^b	0.5±0.10 ^{ab}
7	0.4±0.10 ^a	0.5±0.10 ^a	0.3±0.10 ^a	0.4±0.10 ^a
8 * +	0.3±0.10 ^a	0.3±0.10 ^a	0.3±0.10 ^a	0.3±0.10 ^a
9 *	0.2±0.10 ^a	0.4±0.20 ^a	0.3±0.20 ^a	0.3±0.10 ^a
10	0.3±0.30 ^a	0.2±0.20 ^a	0.3±0.10 ^a	0.2±0.20 ^a
11	0.2±0.10 ^a	0.3±0.20 ^a	0.2±0.10 ^a	0.3±0.10 ^a
12	0.3±0.10 ^a	0.3±0.10 ^a	0.3±0.10 ^a	0.2±0.00 ^a

Superscripts a b c represents the homogeneity between the experimental groups at probability $P \leq 0.05$.

↑ Infection with *A. caninum*

⬮ Infection with trypanosomes

+ Treatment with mebendazole

* Treatment with diminazene aceturate

Ac *Ancylostoma caninum*

Tb *Trypanosoma brucei*

Table 4. Mean $\pm$ SE Cholesterol level (mg/dl) of dogs with experimental single *T. brucei*, *T. brucei* and conjunct *T. brucei* / *A. caninum* infections and treated with diminazene aceturate and mebendazole.

Experimental Period(Weeks)	GPI (control)	GPII (<i>Ac</i>)	GPIII (<i>Tb</i>)	GPIV (<i>Tb/Ac</i>)
0	78.3 $\pm$ 15.90 ^a	198.0 $\pm$ 55.00 ^a	182.0 $\pm$ 19.40 ^a	154.0 $\pm$ 39.30 ^a
1 ↑	138.1 $\pm$ 2.00 ^a	140.0 $\pm$ 25.00 ^a	131.1 $\pm$ 0.80 ^a	110.0 $\pm$ 0.10 ^a
2	128.0 $\pm$ 3.70 ^a	130.2 $\pm$ 8.90 ^a	123.2 $\pm$ 0.10 ^a	119.3 $\pm$ 3.00 ^a
3 ⬮	121.0 $\pm$ 10.80 ^a	138.0 $\pm$ 13.00 ^{ab}	124.0 $\pm$ 9.00 ^a	120.0 $\pm$ 5.70 ^a
4	121.0 $\pm$ 3.00 ^a	132.0 $\pm$ 3.00 ^a	184.0 $\pm$ 20.00 ^b	146.0 $\pm$ 18.70 ^{ab}
5	141.0 $\pm$ 26.80 ^a	222.0 $\pm$ 23.10 ^b	140.0 $\pm$ 3.70 ^a	190.0 $\pm$ 28.80 ^{ab}
6 * +	111.0 $\pm$ 6.30 ^a	177.0 $\pm$ 15.00 ^b	200.0 $\pm$ 38.40 ^b	161.0 $\pm$ 18.10 ^{ab}
7	130.0 $\pm$ 10.80 ^a	116.0 $\pm$ 19.00 ^a	160.3 $\pm$ 20.00 ^a	120.0 $\pm$ 10.00 ^a
8 * +	127.0 $\pm$ 6.90 ^a	88.0 $\pm$ 19.30 ^b	170.0 $\pm$ 16.50 ^a	130.0 $\pm$ 15.30 ^a
9 *	125.0 $\pm$ 3.00 ^a	121.0 $\pm$ 12.00 ^a	134.0 $\pm$ 3.00 ^a	125.0 $\pm$ 3.20 ^a
10	130.0 $\pm$ 2.90 ^a	125.0 $\pm$ 3.60 ^a	136.0 $\pm$ 2.00 ^a	126.0 $\pm$ 2.50 ^a
11	124.0 $\pm$ 2.00 ^a	136.0 $\pm$ 3.40 ^a	132.0 $\pm$ 2.00 ^a	130.0 $\pm$ 1.00 ^a
12	114.0 $\pm$ 23.30 ^a	134.0 $\pm$ 3.00 ^{ab}	119.0 $\pm$ 30.00 ^a	108.7 $\pm$ 29.00 ^a

Superscripts a b c represents the homogeneity between the experimental groups at probability $P \leq 0.05$.

Infection with *A. caninum*

⬮ Infection with trypanosomes

+ Treatment with mebendazole

190 * Treatment with diminazene aceturate
 191 *Ac* *Ancylostoma caninum*
 192 *Tb* *Trypanosoma brucei*

193

194 **Table 5. Mean $\pm$ SE Calcium ion conc.(mg/dl) of dogs with experimental single *T. brucei*, *T. brucei* and**
 195 **conjunct *T. brucei* / *A. caninum* infections and treated with diminazene aceturate and mebendazole.**

Experimental Period(Weeks)	GPI (Control)	GPII (<i>Ac</i>)	GPIII (<i>Tb</i>)	GPIV (<i>Tb/Ac</i>)
0	10.2 $\pm$ 3.70 ^a	9.6 $\pm$ 0.40 ^a	9.0 $\pm$ 1.20 ^a	10.4 $\pm$ 0.20 ^a
1 ↑	10.1 $\pm$ 2.00 ^a	9.5 $\pm$ 1.00 ^a	11.1 $\pm$ 1.00 ^a	9.3 $\pm$ 1.00 ^a
2	9.2 $\pm$ 1.00 ^a	10.3 $\pm$ 5.00 ^a	9.1 $\pm$ 1.00 ^a	9.0 $\pm$ 1.00 ^a
3 ⚡	10.4 $\pm$ 0.10 ^a	10.1 $\pm$ 0.20 ^a	9.0 $\pm$ 0.40 ^a	9.3 $\pm$ 0.50 ^a
4	9.2 $\pm$ 0.20 ^{ab}	10.2 $\pm$ 0.30 ^b	10.0 $\pm$ 0.60 ^b	9.0 $\pm$ 0.40 ^{ab}
5	8.0 $\pm$ 1.10 ^a	8.5 $\pm$ 1.00 ^a	7.6 $\pm$ 2.20 ^a	8.0 $\pm$ 1.00 ^a
6 * +	7.8 $\pm$ 1.00 ^a	4.6 $\pm$ 1.20 ^a	8.3 $\pm$ 1.80 ^a	3.6 $\pm$ 2.00 ^a
7	9.1 $\pm$ 1.30 ^a	9.0 $\pm$ 0.70 ^a	10.3 $\pm$ 2.30 ^a	9.9 $\pm$ 0.80 ^a
8 * +	7.8 $\pm$ 2.40 ^a	7.5 $\pm$ 10.00 ^a	7.7 $\pm$ 1.00 ^a	4.0 $\pm$ 2.40 ^a
9 *	8.3 $\pm$ 5.70 ^a	7.3 $\pm$ 0.50 ^a	9.0 $\pm$ 1.00 ^a	8.5 $\pm$ 3.00 ^a
10	9.0 $\pm$ 2.90 ^a	8.9 $\pm$ 2.00 ^a	9.8 $\pm$ 1.30 ^a	9.4 $\pm$ 2.90 ^a
11	10.2 $\pm$ 1.00 ^a	9.0 $\pm$ 2.00 ^a	9.8 $\pm$ 0.20 ^a	9.8 $\pm$ 2.00 ^a
12	10.3 $\pm$ 0.20 ^a	7.3 $\pm$ 0.40 ^a	7.7 $\pm$ 1.00 ^a	7.1 $\pm$ 2.40 ^a

196 Superscripts a b c represents the homogeneity between the experimental groups at probability P $\leq$ 0.05.

197

198 ↑ Infection with *A. caninum*
 199 ⚡ Infection with trypanosomes
 200 + Treatment with mebendazole
 201 * Treatment with diminazene aceturate
 202 *Ac* *Ancylostoma caninum*
 203 *Tb* *Trypanosoma brucei*

204

Discussion

The decrease ($p < 0.05$) in total protein (TP) observed in *A. caninum* infected group (GPII) agrees with Nwoha *et al.* (2013) who recorded hypoproteinaemia in ancylostomosis. This may be due to protein loss enteropathy often encountered in cases of ancylostomosis. The marked increase ($P \leq 0.05$) recorded by the 4th week in GPIII agrees with Ohaeri and Eluwa (2011) and Taiwo *et al.* (2003) who reported hyperproteinaemia in cases of *T. brucei brucei* infection in various species of animals. This increase may be due to increase inflammatory reactions associated with trypanosome infection (Taiwo *et al.*, 2003; Akanji *et al.*, 2009). In humans, inflammatory reactions cause release of variety of proteins with associated influx of proteins into the blood stream leading to hyperproteinaemia (Cardon, 1943). Anene *et al.* (2010) and Biryomumaisho *et al.* (2003) recorded no significant difference ($p > 0.05$) in the total protein of pigs and West Africa Dwarf Goat (WADG) infected with *T. brucei brucei*, respectively. The persistent increase in total protein in the conjunct group (GPIV) was due to relapse of infection with persistent inflammatory reactions. Hyperproteinaemia has been associated with series of untowards effects among which includes oxygen deficit. Negatively charged proteins attract positively charged rbcs forming a protein cover chain. This process though does not completely eliminate oxygen uptake but severely inhibits oxygen carrying capacity by rbcs (Cardon, 1943). This often led to cell aging and degeneration seen in trypanosomosis (Ekanem and Yusuf, 2008; Akanji *et al.*, 2009) and may account for the severe disease in the conjunct group. The significant ($p < 0.05$) decreases observed in total albumin in the trypanosome infected groups (GPIII and GPIV) may be attributed to the loss of albumin in urine which was documented in cases of trypanosomosis (Agu and Egbuji, 2002). The decrease recorded in the *Ancylostoma* group (GPII) agrees with the previous record of hypoalbuminaemia which was attributed to enteropathy and loss of essential nutrients for synthesis of albumin (Nwoha *et al.*, 2013).

229 Transitory hyperbilirubinaemia in the trypanosome infected groups could be attributed to
 230 trypanosome-induced liver damage. Sequestration of *Trypanosoma brucei* in the liver tissues
 231 could induce liver dysfunction affecting its excretory function. This agrees with Akpa *et al.*
 232 (2008) in *T. brucei* infection in dogs. Increase in cholesterol level in both trypanosomes (GPIII
 233 and GPIV) and *Ancylostoma* (GPII) groups were probably due to both liver and kidney
 234 impairment in the dogs. Liver tissues produce clearance enzymes that aids in excretion and
 235 elimination of triglycerides and these lipids tends to accumulate in cases of liver impairment
 236 (Kary, 2008). Trypanosomes induced liver dysfunction previously seen in hyperbilirubinaemia
 237 down played the excretory function of the liver resulting to increase in cholesterol. Similarly,
 238 hepatotracheal migration of *Ancylostoma caninum* in young dogs in GPII induced hepatic
 239 damage and resultant increase in serum cholesterol (Urquhart *et al.*, 1998). In addition for some
 240 unexplained reasons, cholesterol levels increases in cases of kidney damage. The triglycerides
 241 clearance enzymes produced in the liver could become less active and perhaps resistant in kidney
 242 damage and therefore could be the cause of increases in cholesterol levels in GPII, GPIII, and
 243 GPIV (Kary, 2008). Increase observed in trypanosome groups agree with the findings of
 244 Nakamura (1998) in *T. brucei* infection in rabbits. Also with Abenga and Anosa (2006); Takeet
 245 and Fagbemi (2008) in *T. b. gambiense* infection in Monkey and *T. congolense* infection in
 246 rabbits, respectively. It however contradicts the findings of decrease level of cholesterol in
 247 experimental *T. brucei* /*T. congolense* infections in goats (Biryomumaisho *et al.*, 2003) and in *T.*
 248 *brucei brucei* infection in pigs (Adamu *et al.*, 2009). The significant ($p < 0.05$) increases observed
 249 in GPVI and GPV post treatment was due to inapparent relapses. The transient increase in
 250 calcium ion concentration in all the infected group (GPII, GPIII and GPIV) could disregarded as
 251 an important factor in trypanosomosis and ancylostomosis infection in dogs. Previous works of

Osuna *et al.* (1990) observed a significant ($p<0.05$) increase in calcium ion of *T. cruzi* parasitized cells and attributed it to intracellular release of calcium deposit from the host mitochondria. Generally biochemistry assessment of the groups showed severe alterations in the conjunct group compared to the single infection. This was extrapolated from the extended period of alteration in serum biochemistry of the group compared to others. Treatment with 7mg/kg diminazene aceturate and 200mg/kg mebendazole twice improved most of the altered serum biochemistry as previously observed by (Rashid *et al.*, 2008). However the efficacy of diminazene aceturate was impaired by the resistant strains of *T. brucei*. Nevertheless repeated doses effectively improved the altered conditions.

Conclusion: Biochemical changes observed in single trypanosome brucei, *Ancylostoma caninum* and conjunct infection of *Trypanosoma brucei/A.caninum* were severe in the conjunct group compared to the single infections. Treatment of the infected group with diminazene aceturate and mebendazole improved the biochemistry conditions.

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