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Effect of Dietary Vitamin B_{12} on *Trypanosoma brucei brucei*-Induced Cardiomyopathy of Male Wistar Albino Rats

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Abstract

Objective: The study was undertaken to determine the effect of vitamin B_{12} on *T. b. brucei*-induced cardiomyopathy of male Wistar albino rats. **Methods**: The rats were divided into six groups of three rats which were replicated three times. The rats were marked and kept in stainless wire cages labeled A - F. Groups A, B and C were normal, negative and standard controls respectively. Groups D, E and F were infected with 1.0 x 10^6 trypanosomes and treated with 0.2 mg/kg (low-dose), 0.3 mg/kg (enriched-dose) and 0.4 mg/kg (high-dose) body weight of vitamin B_{12} respectively. The experiment lasted for twenty-one days from the day *T. b. brucei* infection was established. A sample of heart tissue homogenate was collected weekly across the groups and subjected to biochemical determination of low-density lipoprotein (LDL), superoxide dismutase (SOD), nitric oxide (NO), and hydrogen peroxide (H_2O_2) concentrations. On the last day of the experiment, a sample of the heart tissues of each group was harvested and subjected to histological studies. **Results**: There were significant differences in the effects of vitamin B_{12} on the levels of the biomarkers of cardiomyopathy. Significant reductions were seen in the levels of LDL, SOD, and H_2O_2 of cardiac tissue homogenate of rats infected with *T. b. brucei* following treatment with the different doses of vitamin B_{12} when compared to the negative control. The result, however, showed the pathogenesis of *T. b. brucei* significantly decreased NO concentration and the administration with vitamin B_{12} raised the trypanosome-induced reduction of the nitric oxide level. **Conclusion**: There were significant reductions in the levels of LDL, SOD and H_2O_2 and an increase in the NO concentration of the experimental rats following treatment with vitamin B_{12}.

KEYWORDS: *Trypanosoma brucei brucei, Cyanocobalamin, Cardiomyopathy, LDL, SOD, Hydrogen peroxide, Nitric oxide.*

1. Introduction

Human African Trypanosomiasis (HAT) is one of the diverse range of neglected diseases that are widespread in Africa. It is a serious disease with severe consequences[1]. There are two distinct forms of sleeping sickness; the chronic form which is caused by *T. b. gambiense* and the acute zoonotic form caused by *T. b. rhodesiense*[2]. The clinical signs and symptoms are the same in both *gambiense* HAT and *rhodesiense* HAT, but they vary in terms of frequency, severity, and appearance between individuals and foci[3]. The main signs and symptoms of the first stage of HAT are intermittent fever, headache, pruritus, lymphadenopathies, weakness, anemia, asthenia, cardiac and endocrine disorders,

musculoskeletal pains and hepatosplenomegaly[4]. Cardiomyopathy is a disease that presents with symptoms of heart failure mainly due to the left ventricular systolic dysfunction and infections[5]. CD^{8+} lymphocytes are the dominant cells penetrating the tissues of the heart during infection with the intracellular protozoan parasite *T. cruzi*, the causative agent of Chaga's cardiomyopathy[6]. Variations in the levels of certain cardiac tissue biomarkers such as LDL, SOD, NO, catalase, reduced glutathione, malondialdehyde and hydrogen peroxide have been implicated in the Chaga's cardiomyopathy. These inflammatory processes may cause heart disorder, which can clinically evident as congestive heart failure, arrhythmias, sudden cardiac death, and thromboembolic event[7]. Vitamin B_{12} is a group of molecules that has a corrin ring structure and central cobalt atom. It is stored bound to the glycoprotein haptocorrin, a blood protein that is available only to storage heart cells. Vitamin B_{12} is a cofactor for methionine synthase conversion of homocysteine to methionine[8]. Vitamin B_{12} status is evaluated by serum vitamin B_{12}. Values below 170 pg/ml for adults indicate a vitamin B_{12} deficiency. Cognitive decline, neuropathy, myelopathy and sensory neuropathy has been implicated with vitamin B_{12} deficiency[9]. Symptoms of vitamin B_{12} deficiency can also include difficulty maintaining balance, depression, confusion, dementia, poor memory, and soreness of the mouth[10]. Vitamin B_{12} deficiency has also been linked to immuno-incompetence[11]. From the aforementioned reviews, the aim of this study was to elucidate the effect of dietary supplementation of Vitamin B_{12} on *Trypanosoma brucei brucei*-induced cardiomyopathy in male Wistar albino rats.

2. Materials and methods

2.1. Procurement and inoculation of trypanosomes

Trypanosoma brucei brucei was obtained from an experimentally infected rat previously inoculated with the parasite from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Each experimental rat was administered 0.1 ml of infected blood in 0.3 ml normal saline containing 1 x 10^6 trypanosomes using the rapid matching method[12] to determine the level of parasitemia. Inoculation was done intraperitoneally.

2.2. Determination of parasitaemia

Wet blood preparations were covered with a coverslip on a slide and examined under x40 and oil immersion using a microscope at x100 magnification. The identification of parasites was done using morphological description[13].

2.3. Formulation and administration of Vitamin B_{12}

Vitamin B_{12} (Cyanocobalamin) was procured from Basic Nutrition Company Limited, No. 425, Yishan Road, Shanghai. The sub-lethal dosages used in this study were determined at the Departmental of Biochemistry, Madonna University, Elele from the result of acute oral toxicity (LD_{50} Oral (mouse) > 5,000 mg/kg) test of Vitamin B_{12} as thus: Mild dose = 0.2 mg/kg b.w, Enriched dose = 0.3 mg/kg b.w and High dose = 0.4 mg/kg b.w. The working concentrations were administered via intubation using 2 ml of distilled water as a vehicle.

2.4. Standard drug

Diminazene aceturate was procured from the Faculty of Veterinary Medicine Clinic, University of Nigeria, Nsukka, Nigeria in a 2.36 g granules. The working dosage was 0.2 mg/kg. The administration was intravenous.

2.5. Animal model and experimental protocol

Fifty-four (54) male albino Wistar rats (*Rattus norvegicus*) aged 3 months, weighing between 180 – 220 g were procured, housed and allowed to acclimatize for two weeks at the Pharmacy Animal House, Madonna University, Elele, Rivers State, Nigeria. The rats were randomly assigned to six (6) treatment groups labeled A – F comprising three replicates and three per replicate. The animals were kept under normal room temperature with *ad libitum* access to feed and water. The cages were cleaned daily to prevent infection of the animals and to minimize extraneous variables. The treatment groups (A – F) were: Group A (Normal Control) where rats were neither infected with trypanosomes nor treated with vitamins, Group B (Negative Control) where rats were infected with 1.0 x 10^6

trypanosomes but not treated, Group C (Standard Control) where rats were infected with 1.0 x 10^6 trypanosomes and treated with 0.2 mg/kg Diminazene aceturate body weight), Group D (Low-dose of vitamin B_{12}) where rats were infected with 1.0 x 10^6 trypanosomes and treated with 0.2 mg/kg body weight of Vitamin $B_{12,}$ Group E (Enriched dose of Vitamin B_{12}) where rats were infected with 1.0 x 10^6 trypanosomes and treated with 0.3 mg/kg body weight of Vitamin B_{12} and Group F (High dose of Vitamin B_{12}) where rats were infected with 1.0 x 10^6 trypanosomes and treated with 0.4 mg/kg body weight of vitamin B_{12}. The experiment lasted for twenty-one days after *T. b. brucei* infection was established. A sample of cardiac tissue homogenate was collected weekly from the three (3) rats across the groups and taken to Divine Chemicals and Analytical Laboratory, Nsukka for biochemical determination of LDL, SOD, NO and H_2O_2 concentrations. On the last day of the experiment, one rat heart from each replicate was harvested and taken to the Anatomical Pathology Unit, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka for histopathological studies.

2.6. Preparation of cardiac tissues homogenate

The heart was weighed and homogenized with a Potter-Elvenhjem tissue homogenizer in a potassium phosphate buffer 10 Mm pH 7.4. The crude tissue homogenate was centrifuged at 10,000 revolutions per minute, for 15 minutes in a cold centrifuge, and the resultant supernatant was used for the different estimations of proteins.

2.6.1. Low-density lipoprotein (LDL) assay

The LDL oxidation was determined by measuring the generated amount of lipid peroxides and also by the thiobarbituric acid reactive substances (TBARS) assay at 532 nm, using malondialdehyde (MDA) for the standard curve as described by El-Saadani *et al.*[14].

2.6.2. Determination of superoxide dismutase (SOD)

The method described by McCord and Fridovich[15] was applied for the determination of superoxide dismutase.

2.6.3. Nitric oxide scavenging activity

The method illustrated by Marcocci *et al.*[16] was used for the determination of nitric oxide.

2.6.4. Hydrogen peroxide (H_2O_2) scavenging assay

Hydrogen peroxide was determined according to the method of Ruch *et al.*[17].

2.7. Histopathological examination

2.7.1. Tissue preparation

The surviving experimental animals were humanely sacrificed at the end of the study. Gross lesions were recorded as observed during the post mortem examination. Section of the heart was harvested, processed and examined for histopathological changes[18]. The samples after excision were fixed in 10 % phosphate-buffered formalin for 72 hours. The tissues were subsequently trimmed, dehydrated in 4 grades of alcohol (70, 80, 90 % and absolute alcohol), cleared in 3 grades of xylene and embedded in molten parafin wax. On solidifying, the blocks were cut into 5 µm thick tissue sections using a rotary microtome, floated in water bathe and incubated at 60˚C for 30 minutes. The 5 µm thick sectioned tissues were subsequently cleared in 3 grades of xylene and rehydrated in 3 grades of alcohol (90, 80 and 70 %). The sections were then stained with Hematoxylin for 15 minutes. Blueing was done with ammonium chloride. Differentiation was done with 1 % acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant (DPX).

2.7.2. Slide examination and photomicrography

The prepared slides were examined with a Motic BA410E Elite Research Compound Microscope (trioccular) at x40 to x400 magnifications. The photomicrographs were taken using a Motic 2.0 Megapixels Microscope Camera at x160 and x400 magnifications.

2.8. Statistical analysis

Data were subjected to a two-way analysis of variance (ANOVA) using SPSS for window (version 21). The difference between means was separated using Duncan's multiple range tests. The test for significance was considered at the 0.05 probability level. The results were expressed as mean ± SEM.

3. Results

3.1. Biochemical parameters

Vitamin B12 had a dose and duration dependent effect on the LDL activity of cardiac tissue homogenate of rats infected with trypanosomes (Table 1). In week 1, the uninfected and untreated rats had the lowest level of LDL (113.478 ± 0.028 ɥ/mg), followed by infected rats treated with standard drug (120.756 ± 0.320 ɥ/mg), while the infectd and untreated rats had the highest level of LDL (154.398 ± 1.387 ɥ/mg). The the third week the LDL concentrations followed the saame trend, with the Vitamin B12 treated groups having significantly higher ($p<0.05$) values the normal and standard controls.

Table 1: Effect of cyanocobalamin on the LDL (ɥ/mg) of cardiac tissue homogenate

Groups	Treatments	Week 1		Week 2		Week 3	
A	Normal control	113.478 ±	0.028^{a1}	111.981 ±	0.260^{a1}	113.123 ±	0.329^{a1}
B	Negative control	154.398 ±	1.387^{b1}	162.368 ±	3.182^{b2}	171.745 ±	0.506^{b3}
C	Standard control	120.756 ±	0.320^{c1}	117.955 ±	0.724^{c1}	120.618 ±	0.407^{c1}
D	Low dose	144.659 ±	1.628^{d1}	144.674 ±	1.057^{d1}	151.501 ±	0.657^{d2}
E	Enriched dose	144.593 ±	0.850^{d1}	144.988 ±	0.655^{d1}	150.983 ±	0.169^{d2}
F	High dose	141.801 ±	0.306^{d1}	143.365 ±	0.613^{d1}	149.137 ±	0.421^{d1}

In a column, mean value with the same letter as superscript is not significantly different ($p>0.05$). In a row, mean value with the same number as superscript is not significantly different ($p>0.05$)

Vitamin B12 had a dose and duration dependent effect on the SOD activity of cardiac tissue homogenate of rats infected with trypanosomes (Table 2). In week 1, the infected and untreated rats had the lowest level of SOD (3.216 ± 0.136 ɥ/mg), followed by uninfected and untreated rats (3.444 ± 0.095 ɥ/mg), while the infected and treated rats with enhanced dose of Vitamin B12 had the highest level of SOD (3.992 ± 0.005 ɥ/mg), although there was no significant difference from other Vitamin B12 treatment values for SOD. By the third week the SOD concentrations followed the a different trend, with infected and untreated rats having the highest value for SOD (5.002 ± 0.096 ɥ/mg) and the Vitamin B12 treated groups having significantly higher ($p<0.05$) values the normal and standard controls. The level of SOD among the vitamin B12 treated groups did not differ significantly ($p>0.05$)

Table 2: Effect of vitamin B_{12} on superoxide dismutase (SOD) concentration (u/mg) of cardiac tissue homogenate

Groups	Treatments	Week 1		Week 2		Week 3	
A	Normal control	3.444 ±	0.095^{a1}	3.526 ±	0.162^{a1}	3.460 ±	0.167^{a1}
B	Negative control	3.216 ±	0.136^{b1}	4.972 ±	0.039^{b2}	5.002 ±	0.096^{b2}
C	Standard control	3.485 ±	0.057^{a1}	3.657 ±	0.071^{a1}	3.635 ±	0.090^{a1}
D	Low dose	3.992 ±	0.005^{c1}	4.044 ±	0.065^{c2}	4.111 ±	0.003^{c2}
E	Enriched dose	3.978 ±	0.007^{c1}	3.977 ±	0.035^{c2}	4.071 ±	0.032^{c2}
F	High dose	3.949 ±	0.029^{c1}	4.001 ±	0.066^{c1}	4.001 ±	0.002^{c1}

In a column, mean value with the same letter as superscript is not significantly different ($p>0.05$). In a row, mean value with the same number as superscript is not significantly different ($p>0.05$)

Vitamin B12 had a dose and duration dependent effect on the nitric oxide activity of cardiac tissue homogenate of rats infected with trypanosomes (Table 3). In week 1, the uninfected and untreated rats had the highest level of nitric oxide (20.827 ± 0.211 ɥ/mg), followed by infected rats treated with standard drug (20.627 ± 0.188 ɥ/mg), while the infected and untreated rats had the lowest

level of nitric oxide (18.065 ± 0.240 ɥ/mg). In the third week, the nitric oxide concentrations followed the same trend, with the uninfected and untreated rats had the highest level of nitric oxide (20.811 ± 0.187 ɥ/mg), followed by infected rats treated with standard drug (20.724 ± 0.193 ɥ/mg), while the infected and untreated rats had the lowest level of nitric oxide (15.879 ± 0.174 ɥ/mg). The Vitamin B12 treated groups having significantly lower ($p<0.05$) values the normal and standard controls.

Table 3: Effect of vitamin B_{12} on nitric oxide level (u/mg) of cardiac tissue homogenate

Groups	Treatments	Week 1	Week 2	Week 3
A	Normal control	20.827 ± 0.211^{a1}	21.024 ± 0.427^{a1}	20.811 ± 0.187^{a1}
B	Negative control	18.065 ± 0.240^{b1}	16.691 ± 0.217^{b2}	15.879 ± 0.174^{b3}
C	Standard control	20.627 ± 0.188^{a1}	20.927 ± 0.389^{a1}	20.724 ± 0.193^{a1}
D	Low dose	19.696 ± 0.084^{c1}	17.938 ± 0.400^{c2}	16.455 ± 0.447^{c2}
E	Enriched dose	19.848 ± 0.051^{c1}	18.099 ± 0.175^{c2}	18.389 ± 0.045^{d1}
F	High dose	19.892 ± 0.030^{c1}	18.289 ± 0.273^{c2}	18.437 ± 0.159^{d1}

In a column, mean value with the same letter as superscript is not significantly different ($p>0.05$). In a row, mean value with the same number as superscript is not significantly different ($p>0.05$)

Vitamin B12 had a dose and duration dependent effect on the hydrogen peroxide activity of cardiac tissue homogenate of rats infected with trypanosomes (Table 4). In week 1, the uninfected and untreated rats had the lowest level of hydrogen peroxide (0.308 ± 0.100 ɥ/mg), followed by infected rats treated with standard drug (0.320 ± 0.004 ɥ/mg), while the infected and untreated rats had the highest level of hydrogen peroxide (0.413 ± 0.007 ɥ/mg). In the third week, the hydrogen peroxide concentrations followed the same trend, with the uninfected and untreated rats had the lowest level of hydrogen peroxide (0.332 ± 0.010 ɥ/mg), followed by infected rats treated with standard drug (0.337 ± 0.012 ɥ/mg), while the infected and untreated rats had the lowest level of hydrogen peroxide (0.506 ± 0.010 ɥ/mg). The Vitamin B12 treated groups having significantly lower ($p<0.05$) values the normal and standard controls.

Table 4: Effect of vitamin B_{12} on hydrogen peroxide level (u/mg) of cardiac tissue homogenate

Groups	Treatments	Week 1	Week 2	Week 3
A	Normal control	0.308 ± 0.100^{a1}	0.306 ± 0.021^{a1}	0.332 ± 0.010^{a1}
B	Negative control	0.413 ± 0.007^{b1}	0.450 ± 0.012^{b2}	0.506 ± 0.010^{b3}
C	Standard control	0.320 ± 0.004^{a1}	0.318 ± 0.012^{a1}	0.337 ± 0.012^{a2}
D	Low dose	0.361 ± 0.002^{c1}	0.400 ± 0.004^{c2}	0.411 ± 0.003^{c2}
E	Enriched dose	0.367 ± 0.010^{c1}	0.402 ± 0.002^{c2}	0.417 ± 0.004^{c2}
F	High dose	0.370 ± 0.004^{c1}	0.395 ± 0.001^{c1}	0.389 ± 0.004^{c1}

In a column, mean value with the same letter as superscript is not significantly different ($p>0.05$). In a row, mean value with the same number as superscript is not significantly different ($p>0.05$).

3.2. Histopathology of heart tissues

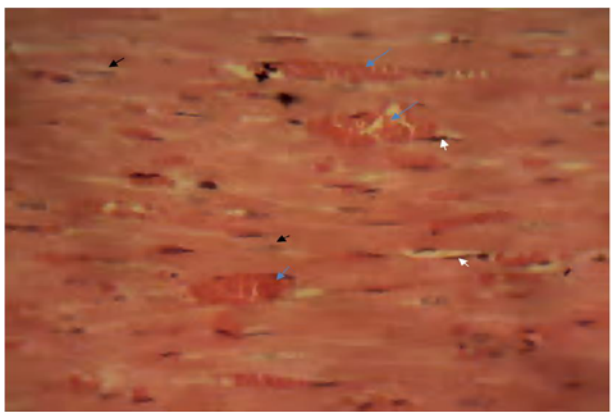

Figure 1: Transverse section of the heart of the rat group A. The nucleus of myocytes (black arrow); pericytes (white arrow); capillaries (blue arrow) (H & E, X400)

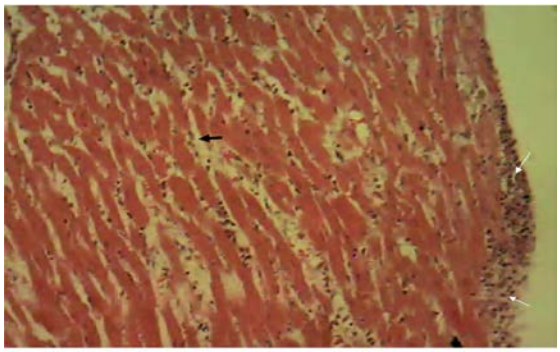

Figure 2: Transverse section of the heart of the rat in the group B. **{**Muscle fibres (black arrow) (H & E, X160, and X400)}

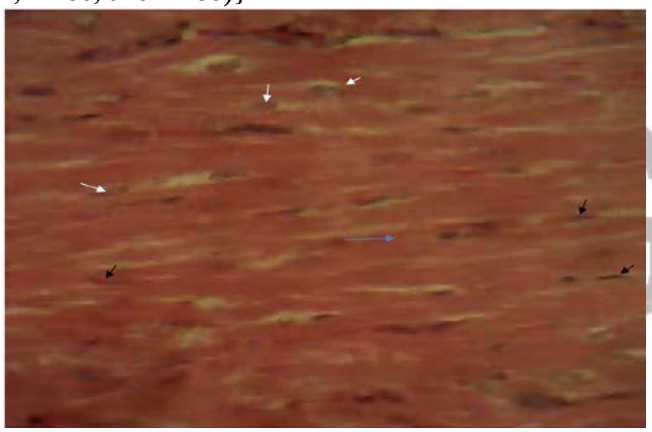

Figure 3: Transverse section of the heart of the rat in the group C. **{**The nucleus of myocytes (white arrow); pericytes (black arrow); blood capillaries (blue arrow) (H & E, X400)}

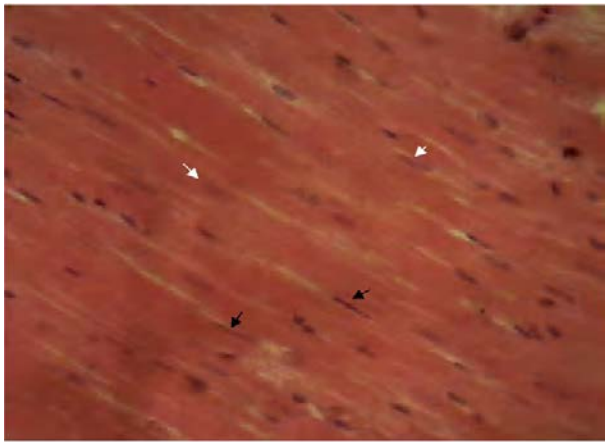

Figure 4: Transverse section of the heart of the rat in group D. **{**The nucleus of myocytes (white arrow); pericytes (black arrow) (H & E, X400)}

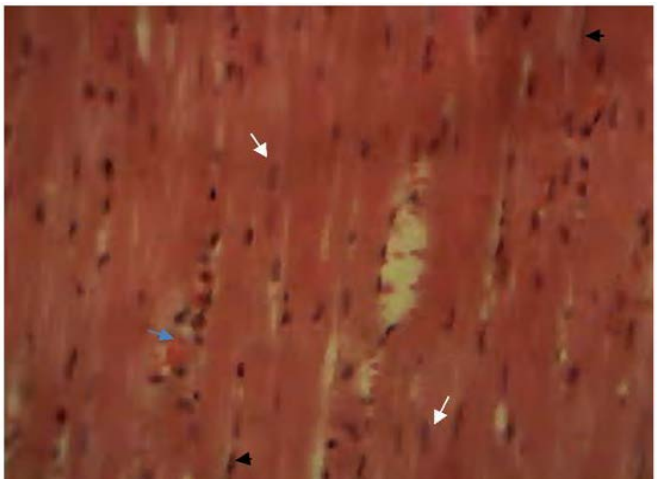

Figure 5: Transverse section of the heart of the rat in group E. The nucleus of myocytes (white arrow); pericytes (black arrow); blood capillaries (blue arrow) (H & E, X400)

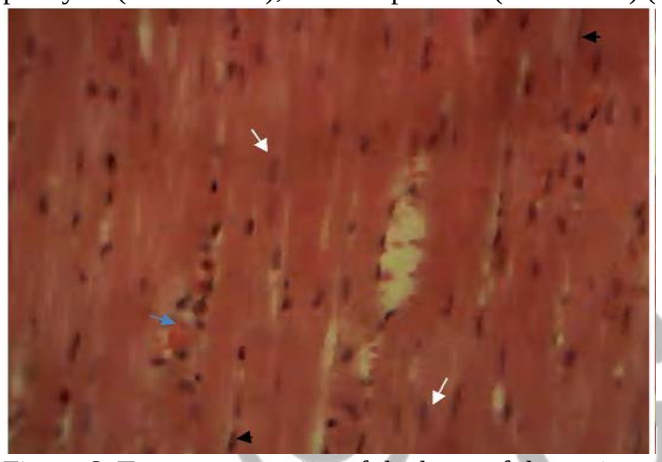

Figure 6: Transverse section of the heart of the rat in group F. The nucleus of myocytes (white arrow); pericytes (black arrow); blood capillaries (blue arrow) (H & E, X400)

4. Discussion

From the result of this study, it is reasonable to infer that *T. b. brucei* infection of Wistar albino rats caused a significant increase in the heart tissue low-density lipoprotein. The finding was in agreement with the reports made in *T. b. brucei*-infected rabbits[19]. The concentration of low-density lipoprotein of the heart tissues of albino rats infected with *T. b. brucei* and treated with the vitamin B_{12} significantly reduced the trypanosome-induce LDL elevation. This agreed with the work of Ullegaddi *et al.*[20] (2006) who reported that antioxidant vitamins and B-group vitamins cause a significant reduction in the biomarker of lipid peroxidation. The present experiment showed a higher level of superoxide dismutase in the heart of *T. b. brucei* infected rats from day 7 – 21 post-infection when compared to the normal control. This was in agreement with the report of Atalay and Laaksonen who observed that under the condition of oxidative stress, the activity of superoxide dismutase increases[21].

In the infected and treated groups, there was a statistically significant difference in the activity of SOD when compared to normal control. The result of the present experiment contradicted the observation of Eze *et al.* who reported that antioxidant vitamin supplementation enhances SOD activities of *T. b. brucei*-infected rats[22]. The present study has shown that the administration of vitamin B_{12} altered the pathogenesis in *T. b. brucei* infected rats. This is evidenced by the reduction in the concentration of hydrogen peroxide of cardiac tissue homogenate of rats infected with *T. b. brucei* following the treatments with different doses of vitamin B_{12}. This was consistent with the study of Birch *et al.* who reported that cobalamins inhibited intracellular hydrogen peroxide production, maintained intracellular glutathione levels and prevented apoptotic and necrotic cell death[23]. The

result indicated the pathogenesis of *T. b. brucei* decreased the nitric oxide concentration of the experimental rats and the administration of vitamin B_{12} raised the trypanosome-induced reduction of nitric oxide. The significant and progressive decrease in nitric oxide concentration observed in the infected groups as compared to the concentration obtained in the normal control is in agreement with the study of Buguet *et al.*[24]. They attributed the changes to an impaired iNOS activity that was evident in peritoneal macrophages collected from the same animals. Saha and Pahan had linked the reduced concentration of nitric oxide to iNOS activity in peripheral compartments [25]. The heart of negative control rats showed multifocal areas of degeneration and necrosis of the muscle fibers with infiltration of inflammatory mononuclear leukocytes. The affected areas also showed fragmentation of muscle fibers with loss of cross striation. This is consistent with the observation of Bonney *et al.* who observed degenerations of myocardial tissues in rats during the experimental *Trypanosoma cruzi* infection [26, 27, 28]. However, the groups infected and treated showed very mild inflammatory changes to normal myocardial histomorphology. This is consistent with the observations of Ullegaddi *et al.* [20] who reported the capacity of B-vitamins in reducing lipid peroxidation markers.

4.2 Conclusion

The results obtained in the present study indicated that the pathogenesis of *T. brucei brucei* caused a significant increase in the levels of LDL, SOD, H_2O_2 and a profound reduction in the concentration of nitric oxide. However, the administration of the rats with vitamin B_{12} caused dose-dependent reductions in the concentrations of LDL, SOD, H_2O_2 and increased nitric oxide of male Wistar albino rats infected with *T. b. brucei.*

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