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Effect of Vitamin B_{12} on Liver function of albino rats infected with *Trypanosoma brucei*

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Abstract

This study was carried out to determine the effect of vitamin B_{12} on the liver function of male wistar albino rats infected with *Trypanosnma brucei brucei*, 24 male Wistar albino rats were divided into 6 groups namely; Control, Trypanosome infected, Diamenazene treated, 40mcg vitamin B_{12}, 60mcg vitaminB_{12}, 80mcg vitamin B_{12}, the liver indicators such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin and direct bilirubin were determined in the rats using kinetic method for ALP, AST and ALT and diazo method for total and direct bilirubin. The data was subjected to statistical analysis using SPSS vision 20. there was a significant increase ($p<0.05$) in the serum activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and concentration of total bilirubin and direct bilirubin with a mean value of (trypanosome infected 137.45±0.19U/L, 25.09±0.40U/L, 36.85±0.49U/L, 27.76±0.99mg/dl and 9.73±0.52 for alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin and direct bilirubin respectively), (Diamenazone 102.15± 0.12U/L, 14.61±0.04U/L, 24.07±0.05U/L, 20.64±0.18mg/dl and 3.73±0.14mg/dl respectively), (40mcg vitamin B_{12} 112.60± 0.33U/L, 15.76±0.12U/L, 24.15±0.10U/L, 22.21±0.43mg/dl and 6.48±0.80mg/dl respectively), (60mcg vitamin B_{12} 115.88±0.22U/L, 18.79±0.05U/L, 24.59±0.14U/L, 18.79±0.44mg/dl and 7.10±0.37mg/dl respectively), (80mcg vitamin B_{12} 116.22±0.14U/L, 17.55±0.71U/L, 24.02±0.03U/L, 20.42±0.50mg/dl and 6.13±0.80mg/dl) respectively. The mean value of the parameters in all the groups was significant at the 0.05 level ($p< 0.05$). in conclusion the result of this study suggested that oral administration of vitamin B_{12} reduces the changes in liver dysfunction associated with *Trypanosnma brucei brucei* infection in wistar albino rats

Keywords: *Trypanosnma brucei brucei,* vitamin B_{12}, Liver.

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1. Introduction

Infections with *T. brucei brucei* and *T. congolense* usually produce severe disease conditions in dogs and rodents [1]. Other species of canine trypanosomes have differing virulence and produce disease of varying severity [2]. *Trypanosoma brucei brucei* and *T. congolense* are naturally transmitted through tse-tse bites producing severe disease conditions in dogs [1]. *Trypanosoma brucei* infection often produces acute disease of about 2 to 4 weeks duration with high rate of mortality in the absence of treatment [3]. *Trypanosoma congolense* infection in rodents is dependent on the infecting strain of the parasite, and some strains produce sub-acute to acute disease conditions in rodents [4]. There is usually massive destruction of the erythrocytes by both species in the rodents resulting in anaemia which is the cardinal sign of the disease in animals [5].

Anaemia from trypanosomiasis is correlated to decreased productivity as most anaemic animals become progressively weak and eventually unfit for work, hence the name "Nagana"- a Zulu word for useless/ powerless [6]. In addition, trypanosomes affect other body components such as the serum biochemical constituents [7] and the immune system [8]. Alterations of these body components produce diverse clinical conditions such as pyrexia, anaemia, immunosuppression, listlessness, emaciation, hair loss and oedema of lower jaw, fore and hind limbs [9]. The profound immunosuppression that occur following trypanosome infections is considered a very significant complicating factor in the disease process [10]. Immune dysfunctions in trypanosomosis are contributed largely by the phenomenon of antigenic variation in the parasite which has also militated against the development of effective vaccine for the disease.

Vitamin B_{12} is an essential water-soluble vitamin that is commonly found in a variety of foods such as fish, shellfish, meat, and dairy products. Vitamin B_{12} is frequently used in combination with other B vitamins in a vitamin B complex formulation. It helps maintain healthy nerve cells and red blood cells and is also needed to make DNA, the genetic material in all cells. Vitamin B_{12} is bound to the protein in food. Hydrochloric acid in the stomach releases B^{12} from protein during digestion. Once released, B_{12} combines with a substance called intrinsic factor (IF) before it is absorbed into the bloodstream.

The aim of this study is to ascertain the effect of vitamin B_{12} (cyanocobalamin) on bilirubin, aspartate aminotransferase (AST), alkaline phosphate (ALP) and alanine aminotransferase (ALT) of trypanosome-infected male wistar albino rats.

2. Materials and method

2.1 Study animals

The animals used in this experiment were male albino wistar rats. A total of 24 male rats weighing between 100-180g were obtained from animal house of the Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State. They were housed and allowed to acclimatize for two weeks at the Pharmacy animal house of Madonna University, Elele, Rivers state. The animals were kept under normal room temperature and were fed with rat pellet and water *ad libitum*, the cages were cleaned daily to prevent infection of the animals.

2.2 Reagents

Commercially prepared Aspartate aminotransferase (AST) and Alanine amino transferase (ALT), were obtained from Randox Limited UK, Alkaline Phosphatase reagent from QCA Spain and Bilirubin reagent were obtained from Agappe Diagnostics Switzerland GmbH

2.2.1 Procurement and administration of vitamin B

Vitamin B12 (cyanocobalamine) was procured at Science Line, New Parts, Onitsha, Nigeria (molecular weight 1355.39g/mol and 96ml volume). The working concentration was determined at the Faculty of Pharmacognosy, Madonna University, Nigeria, Elele campus. The working volume of vitamin B12 was administered via intubation (orally) using distilled water as vehicle.

2.2.2 Procurement of trypanosome parasite

Trypanosoma brucei brucei infected male wistar albino rats were procured from Veterinary department, Faculty of Veterinary Medicine, University of Nsukka, Enugu state.

2.2.3 Inoculation of rats with trypanosome

2ml of blood sample was acquired from rats already infected with *trypanosome brucei brucei* via cardiac puncture and diluted with 2ml of saline water, after which those in groups (B, C, D, E and F) were inoculated with 0.1mililiters of infected blood containing 1million *trypanosome brucei brucei* retro-peritoneally.

2.2.4 Determination of parasitaemia

Wet blood preparations were covered with a cover slip on a slide and viewed under the microscope (×40). The microscopic field was compared to the standard using rapid matching method [11] to rate the degree of infection.

2.2.5 Animal model and experimental design

At the end of the acclimatization, animals were randomly selected into six groups of four rats each. Group A served as control and were given normal rat chow and water. Group B served as trypanosome treated and were infected with 1×10^6 trypanosome. Group C were infected with 1×10^6 trypanosome and treated with the standard drug (diaminazeneacetuate). Group D were infected with 1×10^6 trypanosome and treated with 0.1mg/kg body weight of vitamin E (low dose). Group E were infected with 1×10^6 trypanosome and treated with 0.5mg/kg body weight of vitamin E (moderate dose). Group F were infected with 1×10^6 trypanosome and treated with 1.0mg/kg body weight (high dose) for 14 days. The animals were sacrificed by medial decapitation along the stomach and blood was collected from the heart, transferred to plain test tubes, allowed to clot and subsequently centrifuged to obtain the serum component which was used for further biochemical analysis.

2.3 Biochemical analysis

Determination of ALT and AST was done by monitoring the concentrations of pyruvate hydrazone formed with 2, 4 dinitrophenyl hydrazine. Five hundred microlitre (0.5ml) of buffer solution was dispensed into test tubes labeled blank, sample, control blank and control respectively for AST and ALT respectively. One hundred

microlitre (0.1ml) of sample and control was dispensed into their respective test tubes. All the tubes were incubated at 37ºC for 30minutes. Five hundred microlitre (0.5ml) of 2, 4 Dinitrophenylhydrazine was dispensed into all test tubes. One hundred microlitre (0.1ml) of sample and control was dispensed into their respective blank test tube. The contents of each test tube was mixed and allowed to stand for 20minutes at 25ºC. 5ml of 0.4N sodium hydroxide was added to each tube, mixed and read at 550nm against the respective blank prepared. The activity of the unknown was extrapolated from the calibration curve already prepared [12].

Alkaline Phosphatase activity was done by Phenolphthalein Monophosphate method. The test tubes were respectively labeled sample, standard and control. One millilitre (1.0ml) of distilled water was pipetted into each tube followed by a drop of the substrate into each test tube. All the test tubes were incubated at 37ºC for 5minutes. Ten microlitre (0.1ml) of sample, standard and control were dispensed into their respective test tubes. The test tubes were incubated at 37ºC for 20minutes. Five milliliter (5ml) of colour developer was added to each test tube, mixed, and read at 550nm using water as blank. The activity of sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard [13].

Total Bilirubin was estimated using the method of Jendrassik, and Grof[14], as modified by agappe diagnostic. The Principle showed that Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. Total bilirubin reacts with diazotized sulfanilic acid in the presence of TAB to form azobilirubin. Two test tubes were labeled test and blank. 1000microlitres (µl) of the working reagent was added to each test tubes, 50 microlitres (µl) of serum was added to the test tube labeled test, 20 microlitres (µl) of the activator total was added to each test tube. It was well mixed and incubated for exactly 5minutes, the absorbance was read at 546nm. Total bilirubin was calculated by subtracting absorbance of test from absorbance of sample blank and multiplies by factor 20.9

Direct Bilirubin was estimated using the method of Jendrassik, and Grof[14], as modified by agappe diagnostic. The principle showed that Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. Total bilirubin reacts with diazotized sulfanilic acid in the presence of TAB to form azobilirubin. Two test tubes were labeled test and blank. 1000microlitres (µl) of the working reagent was added to each test tubes, 50 microlitres (µl) of serum was added to each test tube, 20 microlitres (µl) of the activator total was added to the test tube labeled test. It was well mixed and incubated for exactly 5minutes, the absorbance was read at 546nm. Direct bilirubin was calculated by subtracting absorbance of test from absorbance of sample blank and multiply by factor 16

2.4 Data Analysis

The data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 16. The values were reported as Mean ± SEM. Students t-test was used to test for differences between treatments groups. A value of $P<0.05$ was accepted as significant.

3. Result

The alkaline phosphatase (U/L) activity was 90.2875±7.55, 137.45±1.89, 102.15±.13, 112.60±3.29, 115.88±.24 and 116.22±.14, in Control, Trypanosome, Diamenazine, 40 Vitamin B12, 60 Vitamin B12 and 80 Vitamin B12, respectively. The AST (U/L) activity was 14.6125 ±.04366, 25.0950±.04193, 14.092±.04423, 15.7675 ±.11693, 18.79±0.046, 17.55±0.70, in Control, Trypanosome, Diamenazine, 40 Vit B, 60 Vit B and 80VitB, respectively. The ALT (U/L) activity was 24.00±0.046, 36.85±0.50, 24.07±0.053, 24.15±0.10, 24.59±0.13, 24.02±0.03, in Control, Trypanosome, Diamenazine, 40 Vitamin B12, 60 Vitamin B12 and 80 Vitamin B_{12}, respectively. The Total bilirubin (Umol/L) concentration was 18.24±0.42, 27.76±0.99, 20.64±0.17, 22.21±0.43, 18.79±0.44, 20.42±0.50, in Control, Trypanosome, Diamenazine, 40 Vitamin B12, 60 Vitamin B12 and 80 Vitamin B12, respectively. The Direct bilirubin (Umol/L) concentration was 2.07±0.16, 9.37±0.52, 3.73±0.14, 6.48±0.80, 7.10±0.37, 6.13±0.81, in Control, Trypanosome, Diamenazine, 40 Vitamin B12, 60 Vitamin B12 and 80 Vitamin B12, respectively.

Table 1: Effect of graded dose of Vitamin B_{12} on Liver function of albino rats infected with *Trypanosoma brucei*

Group	Alkaline Phosphatase (U/L)	AST (U/L)	ALT (U/L)	Total Bilirubin (Umol/l)	Direct Bilirubin (Umol/l)
Control	90.29±7.55	14.09±0.04	24.00±0.047	18.24±0.42	2.07±0.16
Trypanosome	137.45±1.89	25.09±0.04	36.85±0.50	27.76±0.99	9.37±0.52
Diamenazine	102.15±0.13	14.61 ±0.04	24.07±0.05	20.64±0.17	3.73±0.14
40 Vitamin B12	112.60±3.29	15.77 ±0.11	24.15±0.10	22.21±0.43	6.48±0.80
60 Vitamin B12	115.88±0.24	18.79±0.04	24.59±0.13	18.79±0.44	7.10±0.37
80 Vitamin B12	116.22±0.14	17.55±0.70	24.02±0.03	20.42±0.50	6.13±0.81
F	14.972	15.159	86.292	17.031	18.985
P	0.000	0.000	0.000	0.000	0.000

The alkaline phosphatase (U/L) activity was 90.29±7.55, 137.45±1.89, 102.15±.13 and 114.90±1.11229, in Control, Trypanosome, Diamenazine, and Vitamin B12, Respectively. The AST (U/L) activity was 14.09±0.04, 25.09±0.04, 14.61±0.04 and 17.37±0.43 in Control, Trypanosome, Diamenazine and Vitamin B_{12}, Respectively. The ALT (U/L) activity was 24.00±0.05, 36.85±0.50, 24.07±0.05 and 24.25±0.09, in Control, Trypanosome, Diamenazine and Vitamin B_{12}, Respectively. The Total bilirubin (Umol/L) concentration was 18.24±0.42, 27.76±0.99, 20.64±0.17 and 20.47±0.48, in Control, Trypanosome, Diamenazine, Vitamin B_{12}, Respectively. The Direct bilirubin (Umol/L) concentration was 2.07±0.16, 9.37±0.52, 3.73±0.14 and 6.57±0.38 in Control, Trypanosome, Diamenazine and Vitamin B_{12} respectively.

Table 2: Effect of Vitamin B12 on Liver function of albino rats infected with *Trypanosoma brucei*

Group		Alkaline Phosphatase (U/L)	AST(U/L)	ALT (U/L)	Total Bilirubin (Umol/l)	Direct Bilirubin (Umol/l)
Control		90.29±7.55	14.09±0.04	24.00±0.05	18.24±0.42	2.07±0.16
Diamenazine		102.15±0.12	14.61±0.04	24.07±0.05	20.64±0.17	3.73±0.14
Trypanosome		137.45±1.89	25.09±0.04	36.85±0.50	27.76±0.99	9.37±0.52
Vitamin B_{12}		114.90±1.11	17.37±0.43	24.25±0.09	20.47±0.48	6.57±0.38
F		16.720	28.009	82.331	18.973	20.539
P		0.000	0.000	0.000	0.000	0.000
Post hoc						
Control	Diamenazine	0.043	0.000	0.001	0.007	0.003
	Trypanosome	0.002	0.000	0.001	0.031	0.007
	Vit B_{12}	0.001	0.000	0.001	0.015	0.041
Diamenazine	Trypanosome	0.784	0.002	0.980	0.045	0.003
	Vit B_{12}	0.254	0.000	0.266	0.060	0.000
Trypanosome	Control	0.002	0.000	0.001	0.031	0.007
	Vit B_{12}	0.000	0.001	0.717	1.000	0.000
Vit B	Control	0.001	0.000	0.001	0.015	0.041
	Diamenazine	0.254	0.000	0.266	0.060	0.000

4. Discussion

In an effort to further elucidate the possible effect of trypanosome infection on the liver and the therapeutic role of vitamin B_{12} in elevating the effects of trypanosome infection. 24 male albino rats were used, the rats were housed in clean cages and divided into 6 groups of 4 rats in a cage. The infected groups were intra-peritoneally inoculated with 10^6 ml of blood containing 1million *Trypanosoma brucei brucei* after 2 weeks of acclimatization.

The result of the study showed significant increase ($P<0.05$) in alkaline phosphatase(ALP), aspartate aminotransaminase (AST), alanine aminotransferase (ALT) activities as well as total bilirubin and direct bilirubin concentrations of albino rats infected with trypanosome when compared to their respective controls. This is similar to study by Ciccarell *et al* [15]. This is suggestive that *Trypanosoma brucei brucei* caused damage to the liver in the albino rats.

The result of the study showed there was significant difference ($P<0.05$) in alkaline phosphatase(ALP), aspartate aminotransaminase (AST), alanine aminotransferase (ALT) activities as well as total bilirubin and direct bilirubin concentrations of rats treated with Vitamin B12 when compared with the trypanosome treated and their respective controls. This is similar to previous study by Ciccarell *et al* [15] and Ciccarell *et al* [16] who reported that Vitamin B_{12} produced a marked decrease in epimastigote growth rate together with significant changes in motility of *T cruzi.*

The result further showed dose dependent changes in alkaline phosphatase (ALP), aspartate aminotransaminase (AST), alanine aminotransferase (ALT), activities as well as total bilirubin and direct bilirubin concentrations of rats treated with Vitamin B12 when compared with the trypanosome treated and their respective controls.

5. Conclusion

The result of the study showed that there were increases in AST, ALT, ALP activities as well as Bilirubin in albino rats infected with *T cruzi* while supplementation with Vitamin B12 reduced the enzymes and the bilirubin.

References

[1]. Ezeokonkwo R. C., Ezeh I. O, Onukwu J. I., Obi, P. O. Onyenwe I. W., Agu W. E. Comparative haematological study of single and mixed infections of mongrel dogs with *Trypanosoma Congolense* and *Trypanosoma brucei brucei*. *Veterinary Parasitology*, 2010; 173: 48-54.

[2]. William S.R, Deborah A.W. Canine trypanosomosis. *Southwest. Vet*. 1997; 30(2).

[3]. Akpa P. O., Ezeokonkwo R. C., Eze C. A. and Anene B. M. Comparative efficiency assessment of pentamidine Isethionate and diminazene aceturate in the chemotherapy of *Trypanosoma brucei brucei* infection in dogs. *Veterinary Parasitology*. 2008; 151:139-149.

[4]. Bengaly Z, Sidibe I, Ganaba R, Desquesnes M, Boly H, Sawadogo L. Comparative pathogenicity of three genetically distinct types of Trypanosoma congolense in cattle: clinical observations and haematological changes. *Vet. Parasitiol*. 2002; 108(1):1-19.

[5]. Anosa V.O, Kaneko J. J. Pathogenesis of *T brucei* infection in deer mice (*P. maniculatus*). Ultra structural pathology of the spleen, liver, heart and kidney. *Veterinare Pathology*, 1984; 21:229-237

[6]. Steverding, D. The history of African trypanosomiasis. *Parasite Vectors*. 2008; 1: 3. doi: 10.1186/1756-3305-1-3.

[7]. Igbokwe, I.O. and A. Mohammed. Some plasma biochemical changes in experimental Trypanosoma brucei infection of Sokoto red goats. *Rev. Elev. Med. Vet. Pays Trop.*1992; 45: 287-290.

[8]. Gobert AP, Daulouede S, Lepoivre M, Boucher JL, Bouteille B, Buguet A, Cespuglio R, Veyret B, Vincendeau P. L-Arginine availability modulates local nitric oxide production and parasite killing in experimental trypanosomiasis. *Infect. Immun*. 2000; 68: 4653.

[9]. Eloy, L. J. and Lucheis, S. B. Canine trypanosomiasis: etiology of infection and implications for public health. *Journal of Venom and Animal Toxins in Tropical Disease*, 2009; 43(1): 15-23.

[10]. Magez S, Stijlemans B, Baral Toya and De Baetselier P. DNA from protozoan parasites *Babesia bovis*, *Trypanosoma cruzi*, and *T. brucei* is mitogenic for B lymphocytes and stimulates macrophage expression of interleukin-12, tumor necrosis factor-alpha, and nitric oxide. *Microbes Infect* 2002; 4: 999-1006.

[11]. Herbert W. J, and Lumsden W. H. Trypanosoma brucei: a rapid "matching" method for estimating the hosts parasitemia. *Exp. Parasitology*,1967; 40 (3): 427-31

[12]. Reitman, S. and Frankel, S.A. Colorimetric method for determination of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). *American Journal of Clinical Pathology*, 1957; 28: 56.

[13]. Babson, L. A., Greeley, S. J., Coleman, C. M.,Philips, G. D. Phenolphthalein monophosphate as a substrate for serum alkaline phosphatase. *Clinical Chemistry*, 196; 12: 482 –490.

[14]. Jendrassik, L and Grof, P. Estimation of total serum bilirubin level by spectrophotometrically in serum and plasma. *Biochemical Zeitschrift*, 1938; 297: 81-89

[15]. Ciccarelli A.B, Frank MF, Puente V, Malchiodi, E.L., Batlle A and Lombardoa, M.E. Antiparasitic Effect of Vitamin B_{12} on *Trypanosoma cruzi*. *Antimicrobial Agents and Chemotherapy* 2012; 56(10): 5315–5320.

[16]. Ciccarelli A, Araujo L, Batlle A, Lombardo E. Effect of hemin on growth, protein content and the antioxidant defense system in Trypanosoma cruzi. *Parasitology* 2007; 134:959–965.