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Effect of Vitamin B_{12} on Lipid Profile 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 lipid profile of male Wistar albino rats infected with *Trypanosoma brucei brucei.* 24 male Wistar albino rats were divided into 6 groups namely; Control, Trypanosome infected, Diamenazene treated, 40mcg vitamin B_{12}, 60mcg vitamin B_{12}, 80mcg vitamin B_{12}, The lipid profile indicators such as Triglycerides, Total cholesterol, High Density Lipoprotein and Low Density Lipoprotein were determined in all the albino rats using enzymatic methods while Low Density Lipoprotein was determined using Friedewald formula. The data was subjected to statistical analysis using SPSS version 20. The result showed a significant decrease ($P<0.05$) in the mean value of Triglycerides (mg/dl), Cholesterol (mg/dl), HDL (mg/dl) and LDL (mg/dl) in trypanosome infected group (52.93±2.76, 51.88±2.20, 37.31±0.81 and 28.85±1.78) respectively when compared to the mean value of control group (99.03±6.66, 64.83±2.41, 39.87±0.27 and 38.03±2.81) respectively. The diamenazene treated group showed a significant decrease ($p<0.05$) in the mean value of Triglycerides (mg/dl), Cholesterol (mg/dl), HDL (mg/dl) and a significant increase in LDL (mg/dl) (77.55±2.42, 56.18±0.89, 35.26±1.00 and 38.38±0.86) respectively when compared to the mean value of control group (99.03±6.66, 64.83±2.41, 39.87±0.27 and 38.03±2.81) respectively.The Vitamin B_{12} treated group showed a significant decrease ($p<0.05$) in the mean value of Triglycerides (mg/dl) at dose of 40mcg (53.90±1.07), 60mcg (77.95±4.54), 80mcg (72.40±13.25), HDL (mg/dl) at dose of 40mcg (35.88±0.19),60mcg (36.26±0.31),80mcg (37.69±0.52) as compared to mean value of control group (99.03±6.66 and 39.87±0.27) respectively and a significant increase in mean value of cholesterol(mg/dl) at dose of 80mcg (66.45±2.72), LDL (mg/dl) at dose of 40mcg (41.77±4.53), 80mcg (44.44±5.07) when compared to mean value of control group (64.83±2.41 and 38.03±2.81) respectively. The result of this study suggested that oral administration of vitamin B_{12} and E increased the changes induced by *Trypanosoma brucei brucei* infection in serum levels of Triglycerides, Cholesterol, High Density Lipoprotein and Low Density Lipoprotein.

Keywords: Triglycerides, Cholesterol, High Density Lipoprotein and Low Density Lipoprotein.

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1. Introduction

Trypanosomiasis is a debilitating protozoan disease caused by parasites classified in the Phylum Sarcomastigophora, the Order Kinetoplastida, Family Trypanosomatidae and of the Genus *Trypanosoma* [1]. The pathogenic trypanosomes are further divided into two sections; salivaria and stercoraria according to their site of development in the vector and mode of transmission either through the saliva or by fecal contamination of the wound caused by bite of the vector. *Trypanosoma brucei* belongs to the salivaria group in general and subgenus *Trypanozoon* in

particular. While Rhodesian sleeping sickness, the acute form caused by *T.b. rhodesiense* is endemic in East Africa, Gambian sleeping sickness, a chronic form of the disease caused by *T.b. gambiense*, occurs in West and Central Africa. The region around Lake Victoria has been identified as the meeting point of the two forms of the disease [2]. *T brucei brucei* on the other hand has no regional epizootical limitations and causes virulent disease described as Nagana in animals side by side with Sleeping Sickness in several parts of Sub-Saharan Africa.

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 human body stores several years' worth of vitamin B_{12}, so nutritional deficiency of this vitamin is extremely rare. Elderly are the most at risk. However, deficiency can result from being unable to use vitamin B_{12}. Inability to absorb vitamin B_{12} from the intestinal tract can be caused by a disease known as pernicious anemia. Additionally, strict vegetarians or vegans who are not taking in proper amounts of B_{12} are also prone to a deficiency state.

This study was carried out to determine the effect of vitamin B_{12} on the lipid profile of male Wistar albino rats infected with *Trypanosoma brucei brucei* using Cholesterol, Triglycerides, HDL cholesterol and LDL Cholesterol as indicators.

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 Cholesterol, Triglycerides, HDL cholesterol were obtained from Randox Limited UK, Alkaline Phosphatase reagent from QCA Spain and Bilirubin reagent were obtained from Agappe Diagnostics Switzerland GmbH

2.3 Procurement and Administration of Vitamin B

Vitamin B_{12} (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 B_{12} was administered via intubation (orally) using distilled water as vehicle.

2.4 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.

2.5 Innoculation 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.

2.6 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 to rate the degree of infection.[3]

2.7 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.

2.8 Biochemical Studies

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxides [4].

Ten microlitre (10µl) of sample, control, standard and distilled water was pipette into respective test tube then 1000µl of cholesterol working reagent was added. It was mixed and incubated for 5 minutes at 37^0C. The absorbance of the sample was measured against the reagent blank at 520nm. The concentration of cholesterol (mg/dl) was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard.

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase [5].

Ten microlitre (10µl) of sample, control, standard and distilled water was pipetted into respective test tube then 1000µl of triglyceride reagent was added. It was mixed and incubated for 5 minutes at 37^0C. The absorbance of the sample was measured against the reagent blank at 520nm. The concentration of triglycerides (mg/dl) sample was calculated using the absorbance of sample against absorbance of standard multiplied by concentration of standard.

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, was determined.

Five hundred (500 µl) of sample, control standard and distilled water was added into respective test tubes, 1000µl of precipitant was added into all the tubes. It was mixed and allowed to stand for 10 minutes at room temperature. It was centrifuged for 2 minutes at 12,000 rpm. Then 10µl of supernatant from control, standard and distilled water was added into their respective test tubes and cholesterol concentration of supernatant was determined as shown above by method of Allain *et al* [4].

LDL-cholesterol was calculated using the formula of Friedwald et al [6] as shown below

LDL-cholesterol (mg/dl)=Total cholesterol (mg/dl)-(HDLC (mg/dl)+*TG*/2.22)(mg/dl).

2.9 Statistical Analysis:

The data generated were subjected to statistical analysis including the mean (x), standard deviation (SD), student's t – test, and Analysis of Variance (ANOVA).

3. Results

Table 1 shows the result of effect of vitamin B_{12} at different concentrations on serum lipids of male wistar albino rats infected with *Trypanosoma brucei brucei*. There was a significant decrease ($p<0.05$) in the mean value of Triglycerides (mg/dl), Cholesterol (mg/dl), HDL (mg/dl) and LDL (mg/dl) in trypanosome infected group (52.93±2.76, 51.88±2.20, 37.31±0.81 and 28.85±1.78) respectively when compared to the mean value of control group (99.03±6.66, 64.83±2.41, 39.87±0.27 and 38.03±2.81) respectively.

The diamenazene treated group showed a significant decrease ($p<0.05$) in the mean value of Triglycerides (mg/dl), Cholesterol (mg/dl), HDL (mg/dl) and a significant increase in LDL (mg/dl) (77.55±2.42, 56.18±0.89, 35.26±1.00 and 38.38±0.86) respectively when compared to the mean value of control group (99.03±6.66, 64.83±2.41, 39.87±0.27 and 38.03±2.81) respectively.

The Vitamin B_{12} treated group showed a significant decrease ($p<0.05$) in the mean value of Triglycerides (mg/dl) at dose of 40mcg (53.90±1.07), 60mcg (77.95±4.54), 80mcg (72.40±13.25), HDL (mg/dl) at dose of 40mcg (35.88±0.19), 60mcg (36.26±0.31), 80mcg (37.69±0.52) as compared to mean value of control group (99.03±6.66 and 39.87±0.27) respectively and a significant increase in mean value of cholesterol(mg/dl) at dose of 80mcg (66.45±2.72), LDL (mg/dl) at dose of 40mcg (41.77±4.53), 80mcg (44.44±5.07) when compared to mean value of control group (64.83±2.41 and 38.03±2.81) respectively.

Table 1: Effect of Vitamin B_{12} on Trypanosome infected rats

Treatment	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)
Control	99.03±6.66	64.83±2.41	39.87±0.27	38.03±2.81
Trypanosome	52.93±2.76	51.88±2.20	37.31±0.81	28.85±1.78
Diaminiazene Aceturate	77.55±2.42	56.18±0.89	35.26±1.00	38.38±0.86
40mcg of VIT B_{12}	53.90±1.07	59.73±4.60	35.88±0.19	41.77±4.53
60mcg of VIT B_{12}	77.95±4.54	52.15±0.82	36.26±0.31	29.31±1.49
80mcg of VIT B_{12}	72.40±13.25	66.45±2.72	37.69±0.52	44.44±5.07
F	5.950	5.461	4.751	5.146
P Value	0.000	0.000	0.000	0.000

Table 2 shows the result of effect of vitamin B_{12} on serum lipids of male wistar albino rats infected with *Trypanosoma brucei brucei.* There was a significant decrease ($p<0.05$) in the mean value of Triglycerides (mg/dl), Cholesterol (mg/dl), HDL (mg/dl) and LDL (mg/dl) in Diamiazine treated group (77.55±2.42, 56.18±0.89, 35.26±1.00 and 38.38±0.86) respectively when compared to the mean value of control group (99.03±6.66, 64.83±2.41, 39.87±0.27 and 38.03±2.81) respectively. The trypanosome infected group showed a significant decrease ($p<0.05$) in the mean value of Triglycerides (mg/dl), Cholesterol (mg/dl), HDL (mg/dl) and a significant increase in LDL (mg/dl) (52.93±2.76, 51.88±2.20, 37.31±0.81 and 28.85±1.78) respectively when compared to the mean value of control group (99.03±6.66, 64.83±2.41, 39.87±0.27 and 38.03±2.81) respectively. The vitamin B_{12} treated group showed a significant decrease ($p<0.05$) in the mean value of Triglycerides (mg/dl), Cholesterol (mg/dl), HDL (mg/dl) and a significant increase in LDL (mg/dl) (68.08±5.25, 59.44±2.40, 36.61±0.30 and 38.51±2.89) respectively when compared to the mean value of control group (99.03±6.66, 64.83±2.41, 39.87±0.27 and 38.03±2.81) respectively.

Table 2: Effect of vitamin B_{12} on lipids of male wistar albino rats infected with *Trypanosoma brucei brucei.*

Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)
Control	99.03±6.66	64.83±2.41	39.87±0.27	38.03±2.81
Trypanosome	52.93±2.76	51.88±2.20	37.31±0.81	28.85±1.78
Diaminiazene Aceturate	77.55±2.42	56.18±0.89	35.26±1.00	38.38±0.86
Vitamin B_{12}	68.08±5.25	59.44±2.40	36.61±0.30	38.51±2.89
F	4.363	6.172	2.029	10.571
P value	0.002	0.000	0.000	0.000
Post Hoc				
Control vs diamenazine	0.024	0.198	0.105	1.000
Control vs trypanosome	0.259	0.070	0.432	0.284
Control vs vit B_{12}	0.079	0.801	0.122	1.000
Diamenazinevs Trypanosome	0.006	0.677	0.783	0.055
Diamenazinevs Vit B_{12}	0.243	0.942	0.899	1.000
Trypanosome vs vitB_{12}	0.776	0.372	0.994	0.152

4. Discussion

In this study, it was observed that infection with *Trypanosoma brucei brucei* caused a significant decrease in the concentration of serum Triglycerides, Cholesterol, HDL, and LDL. The findings in this study are in conformity with those in the reports of Biryomumaisho *et al* [7] and Adamu *et al* [8]. Biryomumaisho *et al* [7] and Adamu *et al* [8] reported a significant decrease in all the four serum lipids in *T. congolense* and *T. brucei* infection of goats, and in *T. congolense* infection of sheep respectively. The alterations observed in the serum concentrations of the triglyceride, high density lipoprotein, Low Density Lipoprotein and cholesterol could involve many pathophysiological mechanisms [8]. It has been reported that trypanosomes require lipoproteins for them to multiply under axenic culture [9]. Thus, the lowering of the serum the lipids and cholesterol as observed in the present and previous studies [7,8,10,11] could, partly, be the result of trypanosomal utilization of the molecules. However, it could be inferred that trypanosome infection causes a fall in serum levels of lipids. After treatment of the infected albino rats with Vitamin B_{12}, the result showed a significant decrease ($p<0.05$) in the level of Triglycerides at dose of 40mcg, 60mcg, 80mcg of vitamin B_{12}, HDL at dose of 40mcg, 60mcg, 80mcg of vitamin B_{12} and a significant increase in level of cholesterol at dose of 80mcg of vitamin B_{12}, LDL at dose of 40mcg, 80mcg of vitamin B_{12}. The increase seen in this present study agrees with the works of Ciccarelli *et al* [12] who reported on the Anti-parasitic effect of vitamin B_{12} on *Trypanosoma cruzi* where vitamin B_{12} showed a marked reduction in epimastigote growth rate, where its cytotoxic action is thought to occur through the generation of Reactive Oxidative Species. [13] However, it could be inferred that since vitamin B_{12} reduces the parasitic load of trypanosomes which causes a fall in serum levels of lipids, it induces an increased change in serum lipids.

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