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EFFECTS OF VITAMIN B12 AND E ON LIPID PROFILE OF MALE WISTAR ALBINO RATS INFECTED WITH *TRYPANOSOMABRUCEI* INFECTION

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ABSTRACT

This study was carried out to determine the effect of vitamin B_{12} and E on the lipid profile of male Wistar albino rats infected with *Trypanosomabruceibrucei*. 72 male Wistar albino rats were divided into Control, Trypanosome infected, Diamenazene treated, vitamin E, vitamin B_{12} and various combinations of vitamin B_{12} and vitamin E. The lipid profile indicators like Triglycerides, Total cholesterol, High Density Lipoprotein and Low Density Lipoprotein were determined using enzymatic methods while Low Density Lipoprotein was determined using Friedewald formula. The data was subjected to statistical analysis using Statistical Package for Social Science (SPSS) version 20. There was a significant decrease ($p<0.05$) in the 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) and diamenazene treated group (77.55±2.42, 56.18±0.89, 35.26±1.00 and 38.38±0.86) respectively when compared to the respective control concentrations (99.03±6.66, 64.83±2.41, 39.87±0.27 and 38.03±2.81).Vitamin B12 (68.08±5.25, 59.44±2.40, 36.61±0.30 and 38.51±2.89), Vitamin E (68.45 ± 3.67, 75.19 ± 5.27, 39.91 ± 1.47 and 45.42 ± 3.74) and Vitamin B12 and E combination (73.17 ± 1.94, 79.14± 2.56, 40.57 ± 0.97 and 58.96 ± 2.25) showed a significant increase ($p<0.05$) in the mean concentrations of Triglycerides (mg/dl), Cholesterol (mg/dl), HDL (mg/dl) and a significant increase in LDL (mg/dl) respectively when compared to the trypanosome infected group (52.93±2.76, 51.88±2.20, 37.31±0.81 and 28.85±1.78).The result suggested that oral administration of vitamin B12 and E reversed the changes induced by *Trypanosoma brucei brucei* infection in Triglycerides, Cholesterol, High Density Lipoprotein and Low Density Lipoprotein.

Contribution/Originality: This study was carried out to determine the effect of vitamin B_{12} and E on the lipid profile of male Wistar albino rats infected with *Trypanosomabruceibrucei*. 72 male Wistar albino rats were divided into Control, Trypanosome infected, Diamenazene treated, vitamin E, vitamin B_{12} and various combinations of vitamin B_{12} and vitamin E.

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* (Stevens & Brisse, 2004). 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. *Trypanosomabrucei*belongs to the salivaria group in general and subgenus *Trypanozoon*in particular. *Trypanosomabrucei*infection like other trypanosome infections precipitate increased red blood cell destruction which results in anaemia as well as tissue damage (Akanji, Adeyemi, Oguntoye, & Sulyman, 2009; Ekanem & Yusuf, 2008). These changes together with the need by the host to destroy the parasite are presumably responsible for the symptoms of African sleeping sickness. The application of anti trypanosomal drugs has been the most widely practised means of controlling trypanosomiasis in domestic livestock since the early 1950s, either as curative or prophylactic drugs.

Vitamin B12 is an essential water-soluble vitamin that is commonly found in a variety of foods such as fish, shellfish, meat, and dairyproducts. Vitamin B12 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 B12 is bound to the protein in food. Hydrochloric acid in the stomach releases B12 from protein during digestion. Once released, B12 combines with a substance called intrinsic factor (IF) before it is absorbed into the bloodstream. The human body stores several years' worth of vitamin B12, 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 B12.

Vitamin E consists of two families of compounds, the tocopherols and tocotrienols, characterised by a 6-chromanol ring and an isoprenoid side chain. The members of each family are designated alpha((a)-, beta(b)-, gamma(g)-, or delta(d)- according to the position of methyl groups attached to the chroman nucleus. Therefore, 8 stereoisomers of the large vitamin E family are possible but only the RRR-form occurs naturally. Tocopherols and tocotrienols are differentiated by their phenyl "tails" as these are saturated in the tocopherols but unsaturated in the tocotrienols (Combs, 1992). The vitamin is a peroxyl radical scavenger and especially protects polyunsaturated fatty acids (PUFAs) within membrane phospholipids and in plasma lipoproteins. The efficiency of vitamin E absorption is low in humans (Institute of Medicine, 2000).

The aim of this study is to ascertain the effect of vitamin E(tocopherol) and vitamin B12(cyanlcobalaminmale) on lipid profile of trypanosome-infected male wistar albino rats using Cholesterol, Triglycerides, High density Lipoprotein (HDL) and Low density Lipoprotein (LDL) as indicators.

2. MATERIALS AND METHODS

2.1. Study Animals

Seventy two male (72) wistar albino rats aged 90 days, weighing between 180-320g were purchased from the Faculty of Veterinary Medicine, University of Nigeria Nsukka, and Enugu State.

2.2. Reagents

Commercially prepared Cholesterol, Triglycerides reagents as well as HDL cholesterol and LDL cholesterol precipitants were obtained from Randox Diagnostics UK.

2.3. Vitamin$_{b12}$

Vitamin B_{12} (cyanocobalamine) was procured at Science Line, New Parts, Onitsha, Anambra State, Nigeria with a molecular weight and volume of 1355.39g/mol and 96ml respectively. The working concentration was determined at the Faculty of Pharmacognosy, Madonna University, Nigeria, Elele campus as thus: The working volume of vitamin B_{12} was administered via intubation (orally) using distilled water as vehicle.

2.4. Vitamin E

Vitamin E (tocopherol) was procured at Science Line, New Parts, Onitsha, Anambra State, Nigeria in a powdered form with a molecular weight of 430.71g/mol The working concentration was determined at the Faculty

of Pharmacognosy of Madonna University, Nigeria, Elele campus. The working volume of vitamin E was administered via intubation (orally) using 2% ethanol as vehicle.

2.5. Diamenazene Aceturate

Diamenazeneaceturate was purchased from Enugu.

2.6. Procurement of Trypanosome Parasite

Trypanosomabruceibrucei infected male wistar albino rats were procured from Veterinary department, Faculty of Veterinary Medicine, University of Nsuka, Enugu state.

2.7. Animal Model and Experimental Design

The 72 male wistar albino rats purchased from the faculty of Veterinary Medicine, University of Nigeria Nsukka from were housed at the Animal House of the Department of Physiology, Faculty of Basic Medical Sciences, Madonna University, Elele, Rivers state and allowed to acclimatize for two weeks with access to feed and water before inoculation and treatment.

The rats were kept in stainless wire cage and fed with rat pellet and were provided with clean water. Also, the cages were cleaned daily to prevent infection of the animals and animal care and treatment were conducted in conformity with the Institutional guidelines, which are in compliance with the guide for the care of laboratory animals. They were kept under normal room temperature, humidity of 45% and 12 hours light and dark cycles. The weight and temperature of the rat was taken every day using weighing balance.

2.8. Inoculation of Rats with Trypanosomes

Trypanosomabruceibrucei was obtained from an experimental infected rat previously inoculated with the parasite from Veterinary Parasitology of the University of Nigeria Nsukka. This was used to inoculate one rat and after 7 days of inoculation, the blood of that rat was used to inoculate others in each group. Each experimental rat was administered 0.1ml of infected blood in 0.3ml normal saline containing $1x10^6$ trypanosomes using rapid matching method to determine the level of parasitaemia (Herbert & Lumsden, 1976). All rats except the control were inoculated, marked and kept in cages labeled A-R.

2.9. Determination of Parasitaemia

About one microlitre of blood smear was placed on a clean grease-free glass slides, thin and thick smears were made with the aid of another microscope slide. The slide was air dried and fixed in methanol for three minutes. It was then stained in 10% Giesmsa, air dried and examined under themicroscope using x40 and x100 objective. Identification of parasite was done using morphological description.

2.10. Animal Experiment

The LD50 was done by Arithmetic method of Karber (Dede, Kagbo, & Igbigbi, 1997).At the end of the acclimatization, animals were randomly selected into eighteen groups of four rats each. The groups include Group A (Control), Group B (Trypanosome) was infected with 1x 10^6 trypanosomes, Group C (diaminazemeaceturate) was infected with trypanosome($1x10^6$) and was treated with a known trypanosoma drug. Groups D, E and F were infected with $1x10^6$ of trypanosome and treated with 40mcg,60mcg and 80mcg of vitamin B_{12}respectively. Groups G,H and I were infected with $1x10^6$ of trypanosome and treated with 0.1 mg ,0.5 mg and 1.0 mg of vitamin E respectively; Groups J ,K, L,M,N,O,P,Q and R were infected with $1x10^6$ of trypanosome and treated with 40mcg of vitamin B_{12} and 0.1mg vitamin E, 40mcg of vitamin B_{12} and 0.5mg vitamin E , 40mcg of vitamin B_{12} and 1.0mg vitamin E, 60mcg of vitamin B_{12} and 0.1mg vitamin E , 60mcg of vitamin B_{12} and 0.5mg vitamin E, 60mcg of

vitamin B_{12} and 1.0mg vitamin E , 80mcg of vitamin B_{12} and 0.1mg vitamin E, 80mcg of vitamin B_{12} and 0.5mg vitamin E and 80mcg of vitamin B_{12} and 1.0mg vitamin E. The albino rats were given the treatment for 14 days. Blood samples were collected through the retro-bulbar plexus of the medial canthus of the eye of the rats. A microcapillary tube was inserted into the canthus of the eye to puncture the retro-bulbar plexus and thus enable the out flow of about 2ml of blood into a clean test tube. The blood sample was kept at room temperature for 30minutes to clot. Afterwards, the test tube containing the clotted blood sample was centrifuged at 3,000 revolutions per minute for 10 minutes using a table centrifuge to enable a complete separation of the serum from the clotted blood. The clear serum supernatant was carefully collected with syringe and needle and were stored in a clean sample bottle for biochemical parameter determinations.

2.11. 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 (Allain, Poon, Chan, Richmond, & Fu, 1974).

Ten microlitre (10 μl) of sample, control, standard and distilled water was pipette into respective test tube then 1000 μl of cholesterol working reagent was added. It was mixed and incubated for 5 minutes at 37^{0}C. The absorbance of the sample was measured against the reagent blank at 520nm. The concentration of sample 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 (Bucolo & David, 1973).

Ten microlitre (10) μl of sample, control, standard and distilled water was pipetted into respective test tube then 1000 μl of triglyceride reagent was added. It was mixed and incubated for 5 minutes at 37^{0}C. The absorbance of the sample was measured against the reagent blank at 520nm. The concentration of 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, 1000 μl 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 10 μl 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. (1974).

LDL-cholesterol was calculated using the formula of Friedwald, Fredrickson, and Levy (1972) as shown below:

LDL-cholesterol (Mmol/L)=Total cholesterol (Mmol/L)-(HDLC (Mmol/L)+TG/2.22)(Mmol/L).

2.12. Statistical Analysis

The data generated were subjected to statistical analysis including the mean (x), standard deviation (SD) and analysis of variance (ANOVA) using statistical package for social sciences (SPSS) version 21.

3. RESULT

Table 1 shows the result of effect of vitamin B_{12} and E 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.

Table-1. Effect of different doses of vitamin B_{12} and vitamin E on Lipid Profile of male wistar albino rats infected with *Trypanosomabruceibrucei*.

Group	Cholesterol (Mg/ml)	Triglycerides (Mg/ml)	HDL Cholesterol (Mg/ml)	LDL Cholesterol (Mg/ml)
Control	99.03±6.66	64.83 ±2.41	39.87 ± 0.27	38.03 ± 2.81
Trypanosome	52.93±2.76	51.88 ± 2.2	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 Vitamin B12	53.90± 1.07	59.73± 4.6	35.88 ± 0.19	41.77± 4.53
60mcg of Vitamin B12	77.95± 4.54	52.15 ± 0.82	36.26 ± 0.31	29.31 ± 1.49
80mcg of Vitamin B12	72.40 ±13.25	66.45 ± 2.72	37.69 ± 0.52	44.44 ± 5.07
0.1mg of Vitamin E	63.13 ±7.59	57.95 ± 8.03	35.08± 1.25	38.28 ± 9.43
0.5mg of Vitamin E	96.05± .80	70.85 ± 4.54	42.04 ±2.26	43.23 ± 4.17
1.0mg of Vitamin E	66.40 ± 5.26	76.55± 1.49	42.6± 2.34	54.75 ± 0.5
40mcg Vitamin B12+0.1mg vitamin E	72.60 ± 9.71	83.00 ± 7.42	51.12 ±2.86	58.44± 7.44
40mcg Vitamin B12+0.5mg vitaminE	83.88± 12.53	71.18 ± 2.05	39.18 ± 1.03	56.14 ± 7.28
40mcg Vitamin B12+1.0mg vitamin E	52.23 ± 1.16	82.33 ± 1.56	36.63 ±2.53	59.93± 3.7
60mcg Vitamin B12+0.1mg vitamin E	75.13 ± 0.73	61.88 ± 11.03	39.68 ± 3.21	60.07± 4.75
60mcg Vitamin B12+0.5mg vitamin E	77.38 ±3.85	64.73± 1.98	38.12± 2.33	77.33± 9.70
60mcg Vitamin B12+1.0mg vitamin E	82.33 ± 2.18	82.15 ±0.82	39.86 ± 1.49	59.89± 9.38
80mcg Vitamin B12+0.1mg vitamin E	85.28 ± 1.87	66.45± 2.72	37.00 ± 1.41	55.35± 6.22
80mcg Vitamin B12+0.5mg vitamin E	87.7 ± 2.43	67.95 ± 1.5	39.12 ± 2.77	52.66 ± 1.06
80mcg Vitamin B12+1.0mg vitamin E	95.78 ± 1.99	78.85 ± 2.75	44.67± 0.27	50.80± 3.08
F	5.950	5.461	4.751	5.146
P	0.000	0.000	0.000	0.000

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 Vitamin E treated group showed a significant decrease ($p<0.05$) in the mean value of Triglycerides (mg/dl) at dose of 0.1mg (63.13±7.59) ,0.5mg (96.05±0.80), 1.0mg (66.40±5.26) as compared to mean value of control group (99.03±6.66) and a significant increase in mean value of cholesterol (mg/dl) at dose of 0.5mg (70.85±4.54), 1.0mg (76.55±1.49), HDL (mg/dl) at dose of 0.5mg (42.04±2.26), 1.0mg (42.60±2.34) and LDL (mg/dl) at dose of 0.1mg (38.28±9.43), 0.5mg (43.23±4.17), 1.0mg (54.75±0.50) when compared to mean value of control group (64.83±2.41, 39.87±0.27 and 38.03±2.81) respectively.

Table 2 shows the result of effect of vitamin B_{12} and E on serum lipids of male wistar albino rats infected with *Trypanosomabruceibrucei*. 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 B12 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} and vitamin E on Lipid Profile of male wistar albino rats infected with *Trypanosomabruceibrucei*.

Parameters	Cholesterol (Mg/ml)	Triglycerides (Mg/ml)	HDL Cholesterol (Mg/ml)	LDL Cholesterol (Mg/ml)
Control	99.03 ± 6.66	64.83 ± 2.41	39.87 ± 0.27	38.03 ± 2.81
Trypanosome	77.55 ± 2.42	51.88 ± 2.20	37.31 ± 0.81	28.85 ± 1.78
Diamenazine	52.93± 2.76	56.18 ± 0.89	35.26 ± 1.00	38.38± 0.86
Vitamin B12	68.08 ± 5.25	59.44 ± 2.40	36.61± 0.30	38.51 ± 2.89
Vitamin E	75.19 ± 5.27	68.45 ± 3.67	39.91 ± 1.47	45.42 ± 3.74
VitaminB12+E	79.14± 2.56	73.17 ± 1.94	40.57 ± 0.97	58.96 ± 2.25
F	4.363	6.172	2.029	10.571
P	0.002	0.000	0.000	0.000

4. DISCUSSION

The result of the study observed that infection with *T brucei* caused a significant decrease in the triglycerides, cholesterol, HDL cholesterol and LDL cholesterol. This is similar to study by Biryomumaisho, Katunguka-Rwakishaya, and Rubaire – Akiiki (2003) and Adamu et al. (2008) in goats and sheep respectively. It has been reported that trypanosomes require lipoproteins for them to multiply under axenic culture (Black & Vandeweerd, 1989). Thus the lowering if lipids in this study could be as result of utilization of the molecules.

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 B12, HDL at dose of 40mcg,60mcg,80mcg of vitamin B12 and a significant increase in level of cholesterol at dose of 80mcg of vitamin B12, LDL at dose of 40mcg,80mcg of vitamin B12. The increase seen in this present study agrees with the works of Ciccarelli, Araujo, Batlle, and Lombardo (2007) who reported on the Anti-parasitic effect of vitamin B_{12} on *Trypanosomacruzi* where vitamin B12 showed a marked reduction in epimastigote growth rate, where its cytotoxic action is thought to occur through the generation of Reactive Oxidative Species. However, it could be inferred that since vitamin B12 reduces

the parasitic load of trypanosomes which causes a fall in serum levels of lipids, it induces an increased change in serum lipids.

Also treatment with Vitamin E showed a significant decrease ($P<0.05$) in the concentration of Triglycerides with significant increase in cholesterol, HDL cholesterol and LDL cholesterol. Mgbenka and Ufele (2004) reported that Vitamin E supplemented in nutritionally animal balanced diet leads to enhancement of Trypanosomiasis resistance and when combined enhanced resistance. Treatment with combination of Vitamin B12 and E showed a significant decrease ($P<0.05$) in the concentration of Triglycerides with significant increase in cholesterol, HDL cholesterol and LDL cholesterol when compared with the *Trypanosoma brucei* infected rats.

5. CONCLUSION

The study observed that infection with *T brucei* caused a significant decrease in the triglycerides, cholesterol, HDL cholesterol and LDL cholesterol while Treatment with combination of Vitamin B12 and E showed a significant decrease ($P<0.05$) in the concentration of Triglycerides with significant increase in cholesterol, HDL cholesterol and LDL cholesterol when compared with the Trypanosoma brucei infected rats.

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