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EFFECT OF VITAMIN E ON LIPID PROFILE OF ALBINO RATS INFECTED WITH *TRYPANOSOMA BRUCEI*

Adegoke O. Adebayo[1*], George –Opuda I.M.[1] and Bamigbowu E.O.[2]

[1]Department of Medical Laboratory Science, Madonna University, Elele, Nigeria.
[2]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

***Corresponding Author: Adegoke O. Adebayo**

Department of Medical Laboratory Science, Madonna University, Elele, Nigeria.

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ABSTRACT

This study was carried out to determine the effect of vitamin E on the lipid profile of male Wistar albino rats infected with *Trypanosomabruceibrucei.* 24 male Wistar albino rats were divided into 6 groups namely; Control (not infected not treated), Trypanosome infected, Diamenazene treated, 40mcg vitaminB_{12}, 60mcg vitaminB_{12}, 80mcg vitaminB_{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 ofTriglycerides (mg/dl), Cholesterol (mg/dl), HDL (mg/dl) and LDL (mg/dl) in trypanosome infected group (77.55±2.42, 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) (52.93±2.76, 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 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. The result of the study showed that Vitamin E reversed the changes in lipid concentration cause by *T brucei*.

KEYWORD: Vitamin E, Trypanosomiasis, Lipids.

INTRODUCTION

Vitamin E has been used for the treatment of health condition, often based on its antioxidantproperties and it also serves as a dietary supplement recommended for better health. Herbas *et al*. (2009)reported that the inhibition of alpha tocopherol transfer protein (α -TTP) a determinant of thevitamin E concentration in host circulation confers resistance to *Trypanosoma congolense* infection, evidently owing to oxidative damage to parasite DNA.

Human African trypanosomiasis (HAT or sleeping sickness) has been claimed to be more deadly than any other vector-borne disease such as malaria, because death is inevitable if a patient is not treated (Kennedy, 2004). In spite of the existence of a huge body of research findings on African trypanosomosis (trypanosomiasis), the disease has continued to wreak havoc on human and animal lives with consequent effects on the fragile economy of countries of Tropical Africa (Kristjanson *et al*., 1999; Bourn *et al*., 2005). Variable disorders occur sequel to trypanosome infection in animals. An in-depth knowledge of mechanisms of their development is pivotal to search for and identification of molecular targets, which could be exploited in evolution of therapeutic approaches, especially, in this cutting edge period of research in molecular medicine and biotechnology. Drug regimen are cumbersome in addition to being expensive (Kioy and Mattock, 2005; Moore, 2005). The search for new drugs and formulations that are safe and effective against both the early and late stages of the diseases is recommended (Jannin and Cattand, 2004; Chibale, 2005; Pink *et al*., 2005).

The aim of this study is to determine the effect of Vitamin E on lipid profile in rats infected with *Trypanosoma brucei*

MATERIALS AND METHOD

Study Animals

Twenty four male (24) 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.

Reagents

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

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.

Diamenazene Aceturate

Diamenazeneaceturate was purchased from Enugu

Procurement of Trypanosome Parasite

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

Animal model and experimental design

The 24 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.

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 and Lumbsden 1976). All rats except the control were inoculated, marked and kept in cages labeled A-R.

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% Giemsa, air dried and examined under the microscope using x40 and x100 objective. Identification of parasite was done using morphological description.

Animal experiment

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 0.1 mg ,0.5 mg and 1.0 mg of vitamin E respectively; 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 separated and stored in a clean sample bottle for biochemical parameter determinations.

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 et al 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 37^0C. 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 (Buccolo and 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 37^0C. 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 ofAllain et al (1974).

LDL-cholesterol was calculated using the formula of Friedwald et al (1972) as shown below.

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

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).

RESULT

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 (77.55±2.42, 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) (52.93±2.76, 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 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 1: Effect of graded doses of Vitamin E on Lipid Profile in Trypanosome infected rats.

Group	Triglycerides (Mg/ml)	Cholesterol (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
Diaminiazeneaceturate	77.55 ±2.42	56.18 ± 0.89	35.26 ± 1.00	38.38 ± 0.86
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
F	5.950	5.461	4.751	5.146
P	0.000	0.000	0.000	0.000

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 E concentrations are 75.19 ± 5.27, 68.45 ± 3.67, 39.91 ± 1.47 and 45.42 ± 3.74 respectively.

Table 2: Effect of vitamin E on lipids of male wistar albino rats infected with *Trypanosoma brucei brucei.*

Parameters	Triglycerides (Mg/ml)	Cholesterol (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
Diamenazine	77.55 ±2.42	56.18 ± 0.89	35.26 ± 1.00	38.38 ± 0.86
Vitamin E	75.19 ± 5.27	68.45 ± 3.67	39.91 ± 1.47	45.42 ± 3.74
F	4.363	6.172	2.029	10.571
P	0.002	0.000	0.000	0.000

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 *et al* (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 and Vander weed 1989).Thus the lowering if lipids in this study could be as result of utilization of the molecules.

Treatment of the rats infected with *T. brucei* 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.

CONCLUSION

The result of the study showed that Vitamin E reversed the changes in lipid concentration cause by *T brucei*.

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