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Effect of Vitamin E on glutathione reductase and reduced glutathione levels of male wistar albino rats infected with *Trypanosoma brucei brucei*

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Abstract

The research work was carried out to assess the antioxidant effect of Vitamin E on glutathione reductase and reduced glutathione levels of male wistar albino rats infected with *Trypanosoma brucei brucei*. 24 albino rats were divided into six groups of four test animal per group namely: control, Trypanosome infected, Trypanosome + diaminazine treated Trypanosome + 0.1mg Vitamin E, Trypanosome + 0.5mg Vitamin E and Trypanosome + 1.0mg Vitamin E respectively. Glutathione reductase and reduced glutathione were determined in all albino rats using oxidation -Reduction process. The data was subjected to statistical analysis using SPSS version 20. The result of this study shows that there was no significant difference ($p>0.05$) in reduced glutathione (µ/mg) concentrations of 26.73±8.89,33.48±0.31, 29.30±0.50, 30.35±0.44, 29.90±0.26 and 28.45±2.25 for control, Trypanosome infected, Trypanosome + diaminazine treated ,Trypanosome + 0.1mg Vitamin E, Trypanosome + 0.5mg Vitamin E and Trypanosome + 1.0mg Vitamin E respectively. The results also showed that there was no significant difference ($P>0.05$) in glutathione reductase(µ/mg) concentrations of 18.42±1.03, 18.70±0.17, 17.22±1.06, 17.65±0.29, 17.65±0.90 and 17.42±1.01Nfor control, Trypanosome infected, Trypanosome + diaminazine treated ,Trypanosome + 0.1mg Vitamin E, Trypanosome + 0.5mg Vitamin E and Trypanosome + 1.0mg Vitamin E respectively. The study showed significant reduction ($P<0.05$) in glutathione reductase and reduced glutathione levels of Vitamin E treated albino rats compared with the *Trypanosoma brucei brucei* infected albino rats. The result of this study suggested that oral administration of Vitamin E reduced the glutathione reductase and reduced glutathione levels induced by *Trypanosoma brucei brucei* infection.

Keywords: Vitamin E, Gluthathione reductase Reduced gluthathione, Trypanosoma.

1. Introduction

Vitamin E is found naturally in some foods, added to others, and available as a dietary supplement. “Vitamin E” is the collective name for a group of fat-soluble compounds with distinctive antioxidant activities [1]. Naturally occurring vitamin E exists in eight chemical forms (alpha-, beta-, gamma-, and delta-tocopherol and alpha-, beta-, gamma-, and delta-tocotrienol) that have varying levels of biological activity [1]. Alpha- (or α-) tocopherol is the only form that is recognized to meet human requirements. Serum concentrations of vitamin E (alpha-tocopherol) depend on the liver, which takes up the nutrient after the various forms are absorbed from the small intestine. The liver preferentially resecretes only alpha-tocopherol via the hepatic alpha-tocopherol transfer protein [1]; the liver metabolizes and excretes the other vitamin E forms. As a result, blood and cellular concentrations of other forms of vitamin E are lower than those of alpha-tocopherol and have been the subjects of less research [2, 3]. Antioxidants protect cells from the damaging effects of free radicals, which are molecules that contain an unshared electron. Free radical’s damage cells and might contribute to the development of cardiovascular disease and cancer [4]. Unshared electrons are highly energetic and react

rapidly with oxygen to form reactive oxygen species (ROS). The body forms reactive oxygen species endogenously when it converts food to energy, and antioxidants might protect cells from the damaging effects of reactive oxygen species. The body is also exposed to free radicals from environmental exposures, such as cigarette smoke, air pollution, and ultraviolet radiation from the sun. Reactive oxygen species are part of signaling mechanisms among cells. Vitamin E is a fat-soluble antioxidant that stops the production of reactive oxygen species formed when fat undergoes oxidation.

Trypanosomiasis is a parasitic disease of people and animals, caused by protozoa of the Trypanosoma genus and transmitted by the tsetse fly [5]. It ranks high in importance amongst protozoan infections of animals and man. It occurs in tropical and subtropical regions of the world and affects different species of animals like horse, camel, dog, cattle, sheep and goats [6]. The aim of this study is to determine the effect of vitamin E (tocopherol) on glutathione reductase and reduced glutathione activities of male wistar albino rats infected with *Trypanosoma brucei brucei.*

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.

Reagents: Hydrogen Peroxide, Phosphate Buffer, Serum Sample, Physiological Saline, Blank Solution, Griess Reagent, Sodium Nitroprusside, Phosphate Buffer Saline, Sulfanilic Acid Reagent, 20%Glacial Acetic Acid, Naphthylenediamine Dichloric, Saline Water, Distilled Water.

2.2 Procurement and administration of Vitamin E

Vitamin E was procured at science line of new parts, Onitsha, Anambra state, Nigeria. The working volume of vitamin E will be administered via intubation (orally) using 2% ethanol as vehicle.

2.3 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.

2.4 Acute toxicity study of vitamin E

The method of Karber [7] was used in the LD_{50} determination twenty adult male albino wistar rats. The first group of four rats was administered with 2% ethanol which served as a vehicle for vitamin E. however, the second, third and fourth four rats each and received 400mcg, 900mcg and 1400mcg of vitamin E respectively. The interval mean of a number of mortality in each group and dose difference across the group were key parameters in this method

2.5 Oral toxicity findings

The administration of vitamin E by intubation to albino wistar rat up to 1400mcg recorded mortality as shown in table 1. Thus, the LD_{50} was considered to be not more than 1100mcg /kg body weight.

2.6 Inoculation of rats with trypanosome

2ml of blood sample was acquired from rats already infected with *Trypanosoma 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.

2.7 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 [8] to rate the degree of infection.

2.8 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 (diaminazene acetuate). 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.

2.9 Glutathione Reductase (GR) Assay

The ubiquitous tripeptide glutathione (GSH), which is the most abundant low molecular weight thiol in almost all cells, is involved in a wide range of enzymatic reactions. A major function of GSH is to serve as a reductant in oxidation-reduction processes; a function resulting in the formation of glutathione disulfide (GSSG).

Procedure: The method illustrated by Kakkar *et al* [9] is as follows: Hearts (about 400 g) are obtained from killed rats (200–250 g). The hearts are cut into small pieces and homogenized in 9 mL of 0.25 M ice-cold sucrose per g of

rat heart in a blender. The homogenate is centrifuged for 45 min at 14,000 rpm. The pellets are suspended in a small volume of 0.25 M sucrose and centrifuged. The supernatants are combined with the previous centrifugate. The pooled material is adjusted to pH 5.5 with cold 0.2 M acetic acid and centrifuged again for 45 min at 14,000 rpm. The rate of oxidation of NADPH by GSSG at 30 °C is used as a standard measure of enzymatic activity.

The reaction system of 1 mL contained: 1.0 mM GSSG, 0.1 mM NADPH, 0.5 mM EDTA, 0.10 M sodium phosphate buffer (pH 7.6), and a suitable amount of the glutathione reductase sample to give a change in absorbance of 0.05–0.03/min. The oxidation of 1 µM of NADPH/min under these conditions is used as a unit of glutathione reductase activity. The specific activity is expressed as units per mg of protein.

2.10 Reduced Glutathione (GSH) estimation

GSH is an intra-cellular reductant and plays major role in catalysis, metabolism and transport. It protects cells against free radicals, peroxides and other toxic compound [10]. Deficiency of GSH in the lens leads to cataract formation. Glutathione also plays an important role in the kidney and takes part in a transport system involved in the reabsorption of amino acids.

The method illustrated by Ellman [11] can be used for determination of antioxidant activity. The tissue homogenate (in 0.1M phosphate buffer pH 7.4) is taken and added with equal volume of 20% trichloroacetic acid (TCA) containing 1 mm EDTA to precipitate the tissue proteins. The mixture is allowed to stand for 5minutes prior centrifugation for 10minutes at 2000rpm.The supernatant (200ul) is then transferred to a new set of test tubes and added with 1.8ml of Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid (0.1mM) prepared in 0.3M phosphate buffer with 1% of sodium citrate solution). Then all the test tubes are made up to the volume of 2ml. After completion of total reaction, solutions are measured at 412nm against blank. Absorbance values were compared with a standard curve generated from know GSH.

2.11 Statistical Analysis

The biochemical data were subjected to statistical analysis groups using Statistical Package for Social Sciences (SPSS) version 18. Values were reported as Mean ± SD while student's t-test and analysis of variance (ANOVA) was used to test for differences between treatment A value of $P<0.05$ was accepted as significant.

3. Result

The administration of vitamin E by intubation to albino wistar rat up to 1.0mcg recorded mortality as shown in table1. Thus, the LD50 was considered to be not more than 0.8mcg /kg body weight.

Table 1: Acute Oral Toxicity

Group	No of Rats	Dose (mcg/kg)	Mortality	Clinical sign
1	4	2% ethanol	None	0
2	4	0.1	None	0
3	4	0.5	Significant	1
4	4	1.0	Significant	3

The results showed that reduced glutathione were 26.73±8.89, 33.48±0.31, 29.30±0.50, 30.35±0.44, 29.90±0.26 and 28.45±2.25 for control, Trypanosome infected, diaminazine treated, 0.1mg Vitamin E, 0.5mg Vitamin E and 1.0mg Vitamin E respectively.

The results showed that glutathione reductase were 18.42±1.03, 18.70±0.17, 17.22±1.06, 17.65±0.29, 17.65±0.90 and 17.42±1.01 for control, Trypanosome infected, diaminazine treated, 0.1mg Vitamin E, 0.5mg Vitamin E and 1.0mg Vitamin E respectively.

Table 2: Activity of Vitamin E at different concentration on reduced glutathione and Glutathione reductase

Groups		(Reduced Glutathione) (µ/mg)	(Glutathione Reductase) (µ/mg)
Control		26.73±8.89	18.42±1.03
Trypanosome		33.48±0.31	18.70±0.17
Diaminazene aceturate		29.30±0.50	17.22±1.06
0.1mg vitamin E		30.35±0.44	17.65±0.29
0.5mg vitamin E		29.90±0.26	17.65±0.90
1.0mg vitamin E		28.45±2.25	17.42±1.01
F		1.434	1.987
P		0.260	0.129
POST HOC			
Control	Trypanosome	0.806	1.000
	Diaminazene aceturate	1.000	0.768
	0.1mg vitamin E	0.992	0.839
	0.5mg vitamin E	0.997	0.959
	1.0mg vitamin E	1.000	0.879
Trypanosome	Control	0.806	1.000
	Diaminazene aceturate	0.000	0.353
	0.1mg vitamin E	0.000	0.015
	0.5mg vitamin E	0.000	0.487
	1.0mg vitamin E	0.118	0.425
Diaminazene aceturate	Control	1.000	0.768
	Trypanosome	0.000	0.353
	0.1mg vitamin E	0.167	0.995
	0.5mg vitamin E	0.514	1.000
	1.0mg vitamin E	0.996	1.000
0.1mg Vitamin E	Control	0.992	0.839
	Trypanosome	0.000	0.015
	Diaminazene aceturate	0.167	0.995
	0.5mg vitamin E	0.693	1.000
	1.0mg vitamin E	0.746	1.000
0.5mg Vitamin E	Control	0.997	0.959
	Trypanosome	0.000	0.487
	Diaminazene aceturate	0.514	1.000
	0.1mg vitamin E	0.693	1.000
	1.0mg vitamin E	0.896	1.000
1.0mg Vitamin E	Control	1.000	0.879
	Trypanosome	0.118	0.425
	Diaminazene aceturate	0.996	1.000
	0.1mg vitamin E	0.746	1.000
	0.5mg vitamin E	0.896	1.000

The results showed that reduced glutathione were 26.73±8.89, 33.48±0.31, 29.30±0.50, and 29.57±1.47 for control, Trypanosome infected, diaminazine treated, and Vitamin E respectively.

The results showed that glutathione reductase were 18.42±1.03, 18.70±0.17, 17.22±1.06, and 17.57±0.73 for control, Trypanosome infected, diaminazine treated and Vitamin E respectively.

Table 3: Activity of reduced glutathione and glutathione reductase in albino rats infected with *Trypanosoma brucei brucei* and treated with vitamin E

Groups		Glutathione (µ/mg)	Reductase (µ/mg)
Control		26.73±8.89	18.42±1.03
Trypanosome		33.48±0.31	18.70±0.17
Diaminazene aceturate		29.30±0.50	17.22±1.06
Vitamin E		29.57±1.47	17.57±0.73
F		2.375	3.563
P		0.101	0.033
POST HOC			
Control	Trypanosome	0.629	0.990
	Diaminazene aceturate	0.984	0.542
	Vitamin E	0.976	0.610
Trypanosome	Control	0.629	0.990
	Diaminazene aceturate	0.000	0.232
	Vitamin E	0.000	0.001
Diaminazene aceturate	Control	0.984	0.542
	Trypanosome	0.000	0.232
	Vitamin E	0.993	0.981
Vitamin E	Control	0.976	0.610
	Trypanosome	0.000	0.001
	Diaminazene aceturate	0.993	0.981

4. Discussion

In this study, it was observed that infection with *Trypanosoma brucei brucei* caused a significant increase in the activities of gluthathione reductase and reduced glutathione. The findings in this study are in conformity with the report of Muhmoud [16]. Muhmoud [16] reported an increase in oxidative stress and in lipid peroxidation in erythrocytes of cattle infected with *Trypanosoma brucei brucei* and, also oxidative stress has been cited as a cause of depletion of some endogenous antioxidants like glutathione in trypanosomiasis [12]. However, this study reported an increase in activities of gluthathione reductase and reduced glutathione in rats infected with *Trypanosoma brucei brucei* compared with their controls. This is suggestive that *Trypanosoma brucei brucei* infection could cause an increase in oxidative stress which would increase the activities of this two enzymes gluthathione reductase and reduced glutathione. Yusuf *et al* [13] also reported that the result of the effect of the extract on endogenous antioxidants show that infected control had higher GSH than normal control. This may be because their antioxidant defense system, which includes GSH, was mobilized to fight the presence of the parasite. It appeared that the antioxidant defense system of these animals has not suppressed or exhausted at the very early stage of infection. Perhaps the extract was able to stimulate *de novo* synthesis of glutathione or spared endogenous GSH, to fight trypanosome-generated free radicals. After treatment of *Trypanosoma brucei brucei* with Vitamin E, the result showed a significant decrease($p<0.05$) in the activities of glutathione reductase and reduced glutathione at dose of 0.1mg,0.5mg,1.0mg compared with the *Trypanosoma brucei brucei* infected rats. The decrease seen in the present study agrees with the work of Umar *et al* [12] who reported that supplementation of infected animals with antioxidant vitamins tend to reduce the oxidative stress and the associated degeneration of tissues and organs. The administration of vitamin E to *Trypanosoma brucei brucei* infected rats or rabbits Umar *et al* [14] boosted the reserves of endogenous antioxidants and reduced the tissue damages caused by the disease. Yakubu *et al* [15] also reported that the rats which received vitamin E treatment showed that there was significant decrease in the level of parasetemia supported by prolonged survival in vitamin E supplemented rats indicates that this macronutrients influence the immune response of *T. brucei* infected rats compared with non supplemented rats. Vitamin E supplementation keeps the parasitemia lower than those of non supplemented group. This could be attributed to their enhanced anti oxidant action following infection with T. *brucei*.

5. Conclusion

The result obtained in this study showed that infection with *Trypanosoma brucei brucei* will cause an evident increase in the activities of glutathione reductase and reduced glutathione. However, supplementation with vitamin E will decrease the activities of glutathione reductase and reduced glutathione and as thus reduce oxidative stress.

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