International Journal of Biomedical and Advance Research
ISSN: 2229-3809 (Online); 2455-0558 (Print)
Journal DOI: https://doi.org/10.7439/ijbar
CODEN: IJBABN

Original Research Article

Effect of vitamin E on hydrogen peroxide and nitrogen levels in male *wistar albino* rats infected with *trypanosoma Brucei Brucei*

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Abstract

This research work was conducted to assess the antioxidant effect of vitamin E on hydrogen peroxide and nitrogen oxide levels. 24 albino rats were divided randomly into six groups of four test animal per group. 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. Hydrogen peroxide and nitric oxide were assayed by scavenging Assay methods. The result of this study shows that hydrogen peroxide levels showed a significant difference ($p<0.05$) when compared with value of 0.31±0.03 of the control except with groups treated with 0.5mg and 0.1mg of vitamin E which showed no significant difference ($p>0.05$) with values of 0.36±0.03 and 36±0.03 respectively. However, nitric oxide levels showed significant difference ($p>0.05$) when compared with value of 21.44±0.91 of control except group treated with 0.5mg of vitamin E which showed significant difference ($p<0.05$) with value of 17.09 ±0.00. The findings from this study therefore show that Vitamin E caused changes in Hydrogen peroxide and Nitric oxide levels based on the concentration of the vitamin E.

Keywords: Vitamin E, Trypanosomiasis, Nitric Oxide, Hydrogen Peroxide.

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***Article History:**
Received: 19/10/2018
Revised: 21/11/2018
Accepted: 01/12/2018
DOI: https://doi.org/10.7439/ijbar.v10i01.4934

QR Code

How to cite: Adebayo O., Ibitoroko GOM, Miracle EE. Effect of vitamin E on hydrogen peroxide and nitrogen levels in male wistar albino rats infected with *trypanosoma Brucei Brucei*. *International Journal of Biomedical and Advance Research* 2019; 10(01): e4934. Doi: 10.7439/ijbar.v10i01.4934 Available from: https://ssjournals.com/index.php/ijbar/article/view/4934

1. Introduction

African Trypanosomiasis is one of the mostly neglected tropical diseases, consisting of a number of important human and animal pathologies caused by parasitic protista of the order Kinetoplastida. Human African Trypanosomiasis (HAT), or sleeping sickness, and Animal African Trypanosomiasis (AAT), or nagana, are vector-borne diseases, which are primarily cyclically transmitted by tsetse fly. The animal trypanosomiasis challenge, caused by several species of trypanosome, e.g. *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma brucei brucei* cause about 3 million deaths annually in cattle and has a marked impact on African agriculture [1,2].

African trypanosomosis caused by the *Trypanosoma brucei* sub-group is associated with hepatocellular degeneration, glomerulonephritis and anaemia [3,4]. There is considerable variation in the pathogenicity of different strains and the susceptibility of different host species. *T. brucei brucei*, like other pathogenic trypanosomes is covered by a dense protein layer consisting of a single protein called the variable surface glycoprotein (VSG), which acts as a major immunogen and elicits the formation of specific antibodies. The parasites are able to evade the consequences of these immune reactions by switching the VSG, a phenomenon known as antigenic variation [5].

The hematological and biochemical abnormalities induced by trypanosomes arose from their direct effect via their products on host cells such as red blood cell (RBC), white blood cell (WBC), platelets and tissues such as liver, kidney, bone marrow and lymphoid organs, resulting in cell

destruction and organ malfunction as well as extractions from and additions to host chemistry associated with parasite metabolism [6-8]. The oxidative stress which occurs in trypanosomiasis host is as a result of systematic vitamin E depletion due to decreased vitamin E consumption in infected animals; this oxidative stress leads to peroxidative tissue damage, which elevates erythrocyte free radicals, oxidative haemolysis and depletion of erythrocyte and liver glutathione by free radicals generated by the trypanosome. As a result, membrane Phospholipids and Proteins are attacked leading to alteration in membrane structure, which also affects the membrane fluidity.

Vitamin E is a fat-soluble compound comprising tocopherols and tocotrienols [9]. As a fat-soluble antioxidant, it stops the production of reactive oxygen species (e.g. Oxygen ion and peroxides) formed when fat undergoes oxidation [10,11]. This work was carried out to investigate the Vitamin E Oral administration on cardiomyopathies in male albino Wistar Rats infected with *Trypanosoma brucei brucei* (Federe strain).

The role of oxidative stress in the pathogenesis of the disease is becoming increasingly relevant. Blood stream forms of *T. brucei. brucei* were reported to produce enormous amounts of H_2O_2 [12] and the systemic oxidative stress thus imposed was corroborated by decreases in hepatic reduced glutathione levels in *Trypanosoma brucei brucei*-infected rats [13], decreases in liver retinol and ß-carotenes in *T. brucei* infected rats [14] as well as increased susceptibility of erythrocytes to oxidative haemolysis in *T. brucei*-infected rats [13].

Supplementation of infected animals with antioxidant vitamins tended to reduce the oxidative stress and the associated degeneration of tissues and organs. The administration of vitamin E to *T. brucei brucei*-infected rats boosted the reserves of endogenous antioxidants and reduced the tissue damages caused by the disease. Thus antioxidant vitamin supplementations may reduce the severity of trypanosome infections by offering protection against possible oxidative injuries associated with the disease. Control of trypanosomes presently relies mainly on use of trypanocidal drugs, but has not been satisfactory largely due to the slow discovery of new drugs and high cost of existing ones as well as toxicity to the hosts and acquired resistance to the drugs by the parasite [15].

It is postulated that combining trypanocidal drugs with antioxidant therapy may improve the efficacy and efficiency of treatment of trypanosome infections. The aim of this study is to determine the antioxidant effect of vitamin E on *Rattus albus* infected with *trypanosome brucei brucei* using Hydrogen Peroxide and Nitric oxide 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

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

2.3 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.

2.4 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 Acute toxicity study of vitamin E

The method of Karber [16] was used in the LD50 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

2.6 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.

2.7 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.

2.8 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 [17] to rate the degree of infection.

2.9 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.

2.10 Biochemical analysis

2.10.1 Hydrogen peroxide scavenging (H_2O_2) assay

According to the method of Ruch *et al* [18], a solution of hydrogen peroxide (40mM) is prepared in phosphate buffer (50mM pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230nm using a spectrophotometer. One mililitre of serum sample in two mililitres of physiological saline is added to hydrogen peroxide and absorbance at 230nm is determined after 10min against blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows:

% scavenged (H_2O_2) =

$$[(A_i-A_t)/A_{ij}\times 100\% \text{ scavenged } (H_2O_2).$$

Where A_i is the absorbance of control and A_t is the absorbance of test.

2.10.2 Nitric oxide scavenging activity

Using Griess reagent [19], two (2) ml of 10Mm sodium nitroprusside dissolved in 0.5ml phosphate buffer saline (pH 7.4) is mixed with 0.5ml of sample at various concentrations (0.2-0.8mg/ml). The mixture is then incubated at 25°C. After 150min of incubation, 0.5ml of the incubated solution is withdrawn and mixed with 0.5ml of Griess reagent [(1.0ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5min with 1ml of naphthylethylenediamine dichloric (0.1% w/v)].

The mixture is then incubated at room temperature for 30min and its absorbance pouring into a cuvette is measured at 546nm. The amount of nitric oxide radical inhibition is calculated following this equation:

$$\%\text{inhibition of NO radical} = [A_0-A_1]/A_0 \times 100.$$

Sample (µM TE/g).

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 Lethal dose 50 (LD50) of vitamin E as shown is 1100mcg/kg

Table 1: Acute oral toxicity of vitamin E on albino wistar rat

Group	No of Rats	Dose (mcg/kg)	Clinical Sign	Mortality
1	5	2% ethanol	None	0
2	5	400	None	0
3	5	900	Significant	1
4	5	140	Significant	5

The results also showed that hydrogen peroxide were 0.31±0.03, 0.58±0.00, 0.49±0.02, 0.41±0.01, 0.36±0.03 and 0.36±0.03 for group A, group B, group C, group D, group E and group F respectively. The results showed that nitrogen peroxide were 21.44±0.91, 20.88±0.05, 18.85±0.89, 18.75±0.91, 17.09±0.00 and 19.35±0.77 for Group A, Group B, Group C, Group D, group E and group F respectively.

According to table 2, hydrogen peroxide levels shows there is significant difference ($p<0.05$) when compared with control except the groups treated with 0.5mg and 0.1mg of vitamin E which showed no significant difference ($p>0.05$). Nitric oxide levels also shows significant difference ($p<0.05$) when compared with control except trypanosome infected group without treatment and group treated with 0.5mg of vitamin E which showed no significant difference ($p>0.05$).

Table 2: Activity of Vitamin E on Hydrogen peroxide and nitric oxide

Groups		(H_2O_2)(µ/mg)	(NO_2)(µ/mg)
Control		0.31±0.03	21.44±0.91
Trypanosome		0.58±0.00	20.88±0.05
Diaminazene aceturate		0.49±0.02	18.85±0.89
0.1mg vitamin E		0.41±0.01	18.75±0.91
0.5mg vitamin E		0.36±0.03	17.09±0.00
1.0 vitamin E		0.36±0.03	19.35±0.77
F		97.552	26.259
P		.000	.000
Post HOC			
Control	Trypanosome	0.002	0.917
	Diaminazene aceturate	0.001	0.063
	0.1mg vitamin E	0.028	0.053
	0.5mg vitamin E	0.161	0.015
	1.0mg vitamin E	0.345	0.119
Trypanosome	control	0.002	0.917
	Diaminazene aceturate	0.013	0.115
	0.1mg vitamin E	0.000	0.001
	0.5mg vitamin E	0.000	0.000
	1.0mg vitamin E	0.006	0.163
Diaminazene aceturate	Control	0.001	0.063
	Trypanosome	0.013	0.115
	0.1mg vitamin E	0.004	1.000
	0.5mg vitamin E	0.005	0.165
	1.0mg vitamin E	0.012	0.995
0.1mg Vitamin E	Control	0.028	0.053
	Trypanosome	0.000	0.001
	Diaminazene aceturate	0.004	1.000
	0.5mg vitamin E	0.028	0.003
	1.0mg vitamin E	0.387	0.814
0,5mg Vitamin E	Control	0.161	0.015
	Trypanosome	0.000	0.000
	Diaminazene aceturate	0.005	0.165
	0.1mg vitamin E	0.028	0.003
	1.0mg vitamin E	1.000	0.060
1.0mg Vitamin E	Control	0.345	0.119
	Trypanosome	0.006	0.163
	Diaminazene aceturate	0.012	0.995
	0.1mg vitamin E	0.345	0.814
	0.5mg vitamin E	1.000	0.060

According to table 3, hydrogen peroxide levels showed that there is significant difference ($p<0.05$) when compared with control. Nitrogen oxide levels also shows significant difference ($p<0.05$) when compared with control except group infected with trypanosome without treatment and group infected with trypanosome but treated with vitamin E

Table 3: Activity of Hydrogen Peroxide and nitric oxide in albino rats infected with *Trypanosoma brucei brucei* and treated with vitamin E

Groups		H_2O_2(µ/mg)	NO_2(µ/mg)
Control		0.31±0.03	21.44±0.91
Trypanosome		0.58±0.00	20.88±0.05
Diaminazeneaceturate		0.49±0.02	18.85±0.89
Vitamin E		0.38±0.03	18.40±1.08
F		105.008	14.537
P		0.000	0.000
Post HOC			
Control	Trypanosome	0.001	0.773
	Diaminazeneaceturate	0.001	0.032
	Vitamin E	0.038	0.007
Trypanosome	Control	0.001	0.773
	Diaminazeneaceturate	0.008	0.072
	Vitamin E	0.000	0.000
Diaminazene aceturate	Control	0.001	0.032
	Trypanosome	0.008	0.072
	Vitamin E	0.000	0.940
Vitamin E	Control	0.007	0.038
	Trypanosome	0.000	0.000
	Diaminazeneaceturate	0.940	0.000

4. Discussion

The result of the study showed a significant increase in Hydrogen peroxide concentration in albino rats infected with *T brucei* compared with the control while there was no difference in Nitric oxide of *T brucei* infected rats and control. Oxidative damage is a major source of many illnesses; as free radicals, and reactive oxygen species (ROS) attacks cell macromolecules. Antioxidants play key role in preventing cell being injured by ROS by counter acting these free radicals [20]. Nitric oxide (NO) acts as neurotransmitter through exerting their effect on different body operations, such as neurotransmission, synaptic plasticity, vasodilation, and CNS memory [21,22].

The result obtained indicated that administration of Vitamin E reduced the levels of hydrogen peroxide and nitrogen oxide compared to the trypanosome infected rats. This result indicated that there was significant decrease ($p<0.05$) in hydrogen peroxide and nitrogen levels of Vitamin E treated male albino wistar rats, when compared with albino rats treated with *T. brucei*. Antioxidants derived from plants provide protection to cell by scavenging free oxygen radical through offsetting ROS. This has been made possible due to the presence of certain bioactive substances, such as phenolic compounds, flavonoids, and essential oils, rendering plants with antioxidant activity[23]. The decrease in parasitaemia caused by the administration of antioxidant vitamins has been reported earlier [24] and was attributed to the vitamins' ability to boost immune response to disease.

There was an increased level of hydrogen peroxide and nitrogen oxide in infected group (trypanosome) when compared to the groups treated with 0.5mg, 0.1mg and 1.0mg of vitamin E. This shows that although vitamin E does not cure *Trypanosomia brucei brucei*, the result obtained in this study suggested it can reduce the free radical caused by *T brucei brucei* stress of trypanosomiasis.

5. Conclusion

From the study, it was concluded that infection with *Trypanosomia brucei brucei* cause increase level of hydrogen peroxide and nitrogen oxide while consumption of the vitamin E supplement decreased hydrogen peroxide and nitrogen oxide levels suggestive of reversal of oxidative stress.

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