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Effect of Vitamin E on Serum Albumin, Total Protein, Total and Conjugated Bilirubin of Male Wistar Albino Rats infected with

Trypanosoma brucei brucei.

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Abstract

The research was undertaken to determine the effect of vitamin E serum albumin, total protein, total and conjugated bilirubin of male Wistar albino rats infected with *T. b. brucei*.. Fifty-four (54) male Wistar albino rats were randomly divided into six (6) groups of three (3) rats each replicated three (3) times. The rats were marked and kept in stainless wire cages labeled A-F. Groups A, B, and C were normal, negative and standard controls respectively. Groups D, E and F were infected with 1.0 x 10^6 trypanosomes and treated with 0.5mg/kg (low-dose), 2.5mg/kg (medium-dose), and 5.0mg/kg (high-dose) of vitamin E per body weight per day respectively. The experiment lasted for twenty-one days from the day *T. b. brucei* infection was established. The sample of serum was collected every seven days across the groups and subjected to biochemical determination of protein and bilirubin. There were significant differences ($p<0.05$) in the effect of vitamin E on the levels of the albumin, total protein, total and conjugated bilirubin. Hypoalbuminemia and hypoproteinemia were found in the negative control group which was dependent on the duration of study. Albumin and total protein levels were highest in the high-dose vitamin E group when compared to the low-dose vitamin E rats. Hyperbilirubinaemia was found in the negative control group which was week-dependent. There were significant reductions in the concentrations of the total and conjugated bilirubin between the negative control group and the vitamin E treated groups. In conclusion, the administration of vitamin E caused significant increase in the serum albumin and total protein levels and reductions in the activities of the total and conjugated bilirubin of male Wistar albino rats.

Keywords: *Trypanosoma brucei brucei, Vitamin E, Albumin, Total Protein, Total bilirubin, Conjugated bilirubin, Hypoalbuminemia, Hypoproteinaemia, and Hyperbilirubinaemia*

1.1 Background

The parasite of HAT is a protozoan hemoflagellates of the genus *Trypanosoma.* There are many species of trypanosomes but only two subspecies of the *T. brucei* are the cause of sleeping sickness. The two subspecies are *T. b. gambiense* and *T. b. rhodesiense.* A third subspecies, *T. b. brucei* is not pathogenic to humans, but infect both domestic and wild animals. The vector of human African trypanosomiasis is an infected tsetse fly (*Glossina* spp.). Tsetse fly has been classified into three sub-genera and about thirty species and subspecies, which are distributed to different habitats [1]. The most common mechanism developed by trypanosomes is the antigenic variation which they successively exhibit variant surface glycoprotein (VSG). This is the first phase of immunosuppression; however it proceeds from immunological exhaustion since trypanosomes force their host to elicit successive antibodies able to withstand the VSG variants, while a new variant is planned to develop before humoral response is effective [2]. Sleeping sickness affects the health, economy, and cultural development of Africa people. This dreadful disease hampers agricultural production, livestock-based rural livelihoods, and the health of people [3]. Both sleeping sickness and nagana has severely affected socio-economic, agricultural, veterinary, and forestry development of African people [4]. The fatality rate of human African trypanosomiasis is close to 100% [5]. The direct effects of the disease involve the annual expenditure of large sum of money on control and enormous economic losses through the death of cattle [6]. In endemic communities, HAT infections are feared because of their long-lasting and fatality [7]. It is also a stigmatizing disease, mainly because of the neuropsychological consequences on the infected individuals [7]. Trypanosomiasis in the blood produces significant changes in the biochemical indices [8]. Decreased albumin level has been shown in chronic liver diseases, such as cirrhosis and nephrotic syndrome. Albumin is a negative acute-phase protein in trypanosomes [9]. Total protein has been reported to increase in the serum of *T. b. brucei*-rats [10]. However, Edoga *et al.* [11] reported decreased total protein in the serum of rats infected with *T. b. brucei.* Adeyemi *et al.* [12] reported a significant increase in serum and tissue bilirubin levels of rats infected with *T. b. brucei.* Prevention and treatment of trypanosomiasis largely depend on methods directed to the vectors, the host, and parasites. The treatment options for trypanosomiasis are few and restricted to a small number of chemotherapeutic medications with mild to severe side effects and in some cases fatal [5]. Vitamin E is a fat-soluble compound that has distinct antioxidant functions [13]. Vitamin E is naturally present in some fat-containing foods, fortified to others, and available as a supplement and is stored within the fatty tissues of animals and humans

[14]. Vitamin E exists in eight chemical forms (alpha–, beta–, gamma–, and delta-tocopherol and alpha-beta–, gamma–, and delta-tocotrienol) that have distinctive biological functions [15]. Alpha- and gamma-tocopherol are the two major forms in which vitamin E occurs, with the relative proportions of these depending on the source. Alpha– (or α–) tocopherol is the only form that has met human requirements. Vitamin E functions in various physiological processes. *In vitro* studies have reported that vitamin E prevents oxidation of low-density lipoprotein (LDL) cholesterol and the consequent inflammation [16]. Vitamin E might also help stop the formation of blood clots that could result in a heart attack or venous thromboembolism [17]. Gamma-tocopherol functions to the better cardiovascular system by increasing the activity of nitric oxide synthase, which produces vessel-relaxing nitric oxide [18], thereby trapping the reactive nitrogen species (peroxynitrite) molecules and thus improving the endothelial function. Numerous studies have reported an inverse relationship between antioxidant intake and oxidative stress [19].

Vitamin E, mostly α-tocopherol can ameliorate the modifiable indexes by modulating free radical production [20]. Vitamin E has been established to cause a reduction in oxidative stress status in the small intestine of diabetic rats [21]. Vitamin E supplementation in humans ameliorates oxidative stress, lipid peroxidation and muscle soreness after exercise. Abbas *et al*. [22] reported that the administration of 800mg α-tocopherol for 48 days caused a significant reduction of exercise-induced oxidative injury. Antioxidant nutrients like vitamin E help defend cell constituents from the destructive effects of free radicals which might lead to cancer. Vitamin E possesses anti-cancer properties through the following mechanisms: the stimulation of wild-type p53 tumor suppressor gene, downregulation of mutant p53 protein, the activation of heat shock protein, and an antiangiogenic effect mediated by the blockage of transforming α-growth factor [23]. Vitamin E might also hinder the formation of nitric food carcinogenic nitrosamines in the stomach and protect against cancer development by enhancing immunity [24]. Alpha-, gamma-, and delta-tocopherols are the forms in which vitamin E molecules possess anti-cancer functions. Alpha-tocopherol was found to have inhibitory power on protein kinase C and collagenase; proteins that are specialized to promote the growth of cancer cells [25]. Gamma-tocopherol was reported as a more potent and efficient than alpha-tocopherol in its inhibitory activity on the growth of human prostate cancer cells, while delta-tocopherol demonstrated its peculiar growth-inhibitory power against mouse mammary cancer cells [26]. Gamma-tocopherol functions to inhibit the growth of cancer cells in cultures through one or a combination of the following mechanisms. It traps free radicals (the reactive nitrogen species molecules) that cause mutations in the

deoxyribonucleic acid strands and malignant transformations in the cells [27]. It also downregulates the control molecules (cyclins) that stop the cancerous cell cycle in the middle, thus preventing their proliferation [28].

Gamma-tocopherol has also been found to be more effective than alpha-tocopherol in inducing apoptosis [29], stimulating peroxisome proliferator-activated receptor-gamma activity in colon cancer cells [30], and in decreasing the formation of new blood vessels in tumors, thereby depriving them access to nutrients. In a similar way, tocotrienols were also found to possess antiproliferative and apoptotic functions on normal and cancerous cells in humans. The mechanisms include the induction of apoptosis by a mitochondria-mediated pathway or the suppression of cyclin D which necessitate the arrest of the cell cycle [31]. Several observations have been documented on the antitrypanosomal effects of vitamin E. The work of Yakubu *et al.* [32] reported that vitamin E caused a significant reduction in parasitemia levels of *T. b. brucei*-infected rats. Vitamin E deficiency cause changes in leucocytes levels and exacerbates the myocarditis and sympathetic denervation of ventricular hearts of *T. cruzi*-infected rats [33]. Dietary supplementation of vitamin E can enhance the resistance of trypanosomiasis, total white blood cells, mononucleated cells, polymorphonucleated cells and packed cell volume of *T. congolense*-infected rats [34, 35].

1.1.1 Purpose of the study

The purpose of the study was to determine the effect of vitamin E on serum albumin, total protein, total and conjugated bilirubin concentrations of rats infected with *T. b. brucei.*

2.0 Methodologies

2.1 Animal Model and Experimental Protocol

Fifty-four (54) male albino Wistar rats (*Rattus norvegicus*) aged 3 months, weighing between 180-220g were procured, housed and allowed to acclimatize for two weeks at the Pharmacy Animal House, Madonna University Elele, Rivers state. The rats were grouped into six (6) cages labeled A-F comprising three (3) rats that were replicated three (3) times from each group. The animals were kept under normal room temperature with *ad libitum* access to feed and water. The cages were cleaned daily to prevent infection of the animals and to minimize extraneous variables. The groups (A-F) were as thus: Group A (Normal Control) were neither infected with trypanosomes nor treated with vitamins; Group B (Negative Control) were infected with $1.0x10^6$ trypanosomes but not treated; Group C (Standard Control) were infected with $1.0x10^6$ trypanosomes and treated with 0.2 mg/kg diminazene aceturate body weight); Group D (Low-dose of vitamin E) were infected with $1.0x10^6$ trypanosomes and treated with 0.5 mg/kg body weight of vitamin E per day; Group E

(Medium dose of vitamin E) were infected with $1.0x10^6$ trypanosomes and treated with 2.5 mg/kg body weight of vitamin E per day; Group F (High dose of vitamin E) were infected with $1.0x10^6$ trypanosomes and treated with 5.0 mg/kg body weight of vitamin E. The experiment lasted for twenty-one days after *Trypanosoma brucei brucei* infection was established. A sample of serum was collected weekly from the three (3) rats across the groups and taken to Divine Chemicals and Analytical Laboratory, Nsukka for the biochemical determination of serum protein and bilirubin.

2.2 Procurement and Inoculation of Trypanosomes

Trypanosoma brucei brucei was obtained from an experimentally infected rat previously inoculated with the parasite from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Each experimental rat was administered 0.1ml of infected blood in 0.3ml normal saline containing $1x10^6$ trypanosomes using the rapid matching method [36] to determine the level of parasitemia. Inoculation was done intraperitoneally.

2.3 Determination of Parasitaemia

Wet blood preparations were covered with a coverslip on a slide and examined under X40 and oil immersion using a microscope at X100 magnification. The identification of parasites was done using morphological description [37].

2.4 Formulation and Administration of Vitamin E

Vitamin E (α-tocopherol) was procured at Science Line, New Parts, Onitsha, Anambra State, Nigeria in a powdered bottle. The working concentrations were weighed at the Departmental of Biochemistry, Madonna University, Elele from the result of acute oral toxicity (LD_{50}) test of vitamin B_{12} as thus:

Mild dose: 0.5 mg/kg b.w.d.
Enriched dose: 2.5 mg/kg b.w.d.
High dose: 5.0 mg/kg b.w.d.

The working concentrations were dissolved in 2% ethanol as a vehicle and administered via intubation.

2.5 Standard Drug

Diminazene aceturate was procured from the faculty of Veterinary Medicine Clinic, University of Nigeria, Nsukka, Nigeria in a 2.36g granules. The working dosage was 0.2m/kg. The administration was intravenous.

2.6 Clinical Determination of protein and bilirubin

Blood samples for clinical determination of liver and renal functions were collected from the retro-bulbar plexus of the medial canthus of the eye of the rates. The blood sample was kept at room temperature for 30 minutes to clot. Afterward, 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 complete separation of the serum from the clotted blood. The clear serum supernatant was carefully collected with syringe and needle and stored in a clean sample bottle for the biochemical determination of protein and bilirubin concentrations.

2.6.1 Albumin estimation

Bromocresol Green method for the *in-vitro* determination of albumin in serum using Quimica Clinica Aplicada (QCA) Albumin Test Kits (QCA, Spain) [38] was employed for the study.

2.6.2 Total Protein estimation

The estimation of serum total protein concentration was carried out by the Biuret method, using Randox Test Kits (Randox Laboratories Ltd, USA) [39].

2.6.3 Bilirubin estimation

The serum bilirubin concentration was determined using Randox Test Kits (Randox Laboratories Ltd, USA) [40].

2.7 Statistical Analysis

The values of the parameters were expressed as mean$\pm SEM$. Data were subjected to a 2-way analysis of variance (ANOVA) using SPSS software for window (version 21) and the difference between means was separated using Duncan's multiple range tests. The test for significance was considered at the 0.05 probability level.

3.0 Principal Results

Table 1: Effect of vitamin E on serum albumin level (g/dl)

Groups	Week 1	Week 2	Week 3
A	2.424 ± 0.064^{a1}	2.400 ± 0.052^{a1}	2.444 ± 0.078^{a1}
B	1.616 ± 0.057^{b1}	1.102 ± 0.121^{b2}	1.023 ± 0.030^{b3}
C	2.377 ± 0.078^{a1}	2.308 ± 0.035^{a1}	2.120 ± 0.092^{a1}
D	1.915 ± 0.007^{c1}	1.912 ± 0.007^{c1}	1.919 ± 0.006^{c2}
E	1.890 ± 0.283^{c1}	1.912 ± 0.060^{c1}	1.926 ± 0.058^{c2}
F	2.013 ± 0.056^{c1}	2.010 ± 0.056^{c1}	2.118 ± 0.056^{a2}

In a column, mean value with the same letter as superscript is not significantly different (p>0.05). In a row, mean value with the same number as superscript is not significantly different (p>0.05).

Table 2: Effect of vitamin E on serum total protein level (g/dl)

Groups	Week 1	Week 2	Week 3
A	6.660 ± 0.168^{a1}	6.916 ± 0.048^{a1}	6.966 ± 0.045^{a1}
B	4.384 ± 0.191^{b1}	3.594 ± 0.094^{b2}	2.076 ± 0.100^{b3}
C	6.442 ± 0.130^{a1}	6.598 ± 0.117^{a1}	6.883 ± 0.052^{a1}
D	4.830 ± 0.048^{c1}	4.056 ± 0.123^{bc1}	3.313 ± 0.816^{c2}
E	4.448 ± 0.166^{b1}	4.282 ± 0.624^{c1}	3.997 ± 0.006^{c2}
F	4.873 ± 0.130^{c1}	5.333 ± 0.621^{c2}	4.573 ± 0.056^{d1}

In a column, mean value with the same letter as superscript is not significantly different (p>0.05). In a row, mean value with the same number as superscript is not significantly different (p>0.05).

Table 3: Effect of vitamin E on serum total bilirubin level (mg/dl)

Groups	Week 1	Week 3	Week 3
A	0.886 ± 0.018^{a1}	0.904 ± 0.024^{a1}	0.848 ± 0.017^{a1}
B	1.295 ± 0.036^{b1}	1.391 ± 0.040^{b2}	1.735 ± 0.027^{b3}
C	0.982 ± 0.008^{a1}	0.978 ± 0.010^{a1}	0.927 ± 0.006^{a1}
D	1.200 ± 0.008^{b1}	$1..142\pm0.048^{c1}$	1.541 ± 0.029^{c2}
E	1.160 ± 0.036^{c1}	1.164 ± 0.038^{c1}	1.548 ± 0.035^{c2}
F	1.072 ± 0.072^{c1}	1.156 ± 0.056^{c1}	1.490 ± 0.051^{c2}

In a column, mean value with the same letter as superscript is not significantly different (p>0.05). In a row, mean value with the same number as superscript is not significantly different (p>0.05).

Table 4: Effect of vitamin E on serum conjugated bilirubin level (mg/dl)

Groups	Week 1	Week 2	Week 3
A	0.274 ± 0.024^{a1}	0.278 ± 0.048^{a1}	0.284 ± 0.374^{a1}
B	0.686 ± 0.003^{b1}	0.733 ± 0.018^{b2}	0.860 ± 0.003^{b2}
C	0.329 ± 0.020^{a1}	0.347 ± 0.017^{a1}	0.304 ± 0.004^{a1}
D	0.494 ± 0.004^{c1}	0.471 ± 0.027^{c1}	0.495 ± 0.004^{c1}
E	0.473 ± 0.020^{c1}	0.470 ± 0.022^{c1}	0.481 ± 0.005^{c1}
F	0.476 ± 0.017^{c1}	0.463 ± 0.024^{c1}	0.491 ± 0.008^{c1}

In a column, mean value with the same letter as superscript is not significantly different ($p>0.05$). In a row, mean value with the same number as superscript is not significantly different ($p>0.05$).

4.0 Discussion and Conclusion

4.1 Discussion

The level of serum albumin significantly fall following increased parasitemia in the present study. This reduction aligns with the work of Korori *et al.* [9] who linked the reduced albumin level to chronic liver disease. The present study showed that the administration of vitamin E ameliorated the state of hypoalbuminemia which inferred that vitamin E has hepatoprotective function. This is in line with the work of Niki [41] which described vitamin E as the most effective scavenger of lipid peroxyl radicals. The result of the experiment also indicated the pathogenesis of *T. b. brucei* decreased the serum total protein and the administration of the rats with vitamin E hiked the trypanosome-induced serum total protein reduction. This is line with the work of Taiwo *et al.* [8] which observed a fall in the concentration of serum total protein during experimental *T. congolense* and *T. brucei* infections in sheep. The present study also aligns with the observations of Harald *et al.* [19] which reported an inverse relationship between vitamin E and cardiovascular risk factors. The significantly higher concentration in the total and conjugated bilirubin concentrations with growing infections observed in the study indicates that there could be acute hemolysis resulting from the activities of proliferating parasites. This agrees with the reports of Orhue *et al.* [42] which reported increased serum bilirubin in *T. brucei*-infected rabbits. The high levels of the total and conjugated bilirubin in the infected rats in this study support the earlier observation in the trypanosome-infected animals [43]. The administration of the infected rats with vitamin E to a significant extent reduced the trypanosome-induced serum bilirubin elevation. This agrees with the findings of Khan [44] which reported that antioxidant vitamin can protect bio-constituents from free radical damage.

4.2 Major Conclusion

From the study, it is advisable to include vitamin E in our daily foods and consumables because its effects on the biochemical parameters. The result indicated that vitamin E can be manipulated pharmacologically to supplement the existing trypanocidal drugs in a to improving on the treatment outcomes.

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