Vitamins C and E can ameliorate the negative effect on the body temperature and serum chemistry of Wistar rats infected with T. *brucei brucei* (Federe strain)

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ABSTRACT: Effect of oral administration of vitamins C and E on the body temperature and serum chemistry of Wistar rats infected with T. brucei brucei (Federe strain) was investigated. Twenty five Albino Wistar rats were randomly divided into five groups of five animals each. Group I was administered with 0.5 mL normal saline only, group II was inoculated with 0.1 × 106 of T. brucei brucei only. Groups III, IV, and V were administered the same dose of parasites as in group II, and in addition, they were administered with 150 mg/kg b.w. of Vitamin C; 150 mg/kg b.w. of Vitamin E, and the combination of both Vitamins in the last group, respectively. Body temperature increased consistently in all groups except group I. However, there was a significant ($P< 0.05$) difference in all treated groups compared to group II on day 28 post-infection. ALT was significantly ($P< 0.05$) lower only in the group treated with combined vitamins compared to group II. AST and Creatinine decreased significantly ($P< 0.05$), whereas the decrease recorded for Urea was highly significant ($P< 0.01$) in all the treated groups compared to group II, values recorded for ALP followed the same pattern but in a converse direction. In conclusion, the administration of vitamins C and E, particularly in its combined form ameliorated the negative effect on the body temperature and serum biochemistry profiles of the infected rats.

Key words: Albino Wistar rats, body temperature, serum chemistry, Trypanosoma brucei brucei (Federe strain), vitamins C and E.

INTRODUCTION

African animal trypanosomiasis (also called *Nagana)* is a major threat to livestock development and an economically wasting disease in sub-Saharan Africa [1]. This disease is transmitted via blood sucking tsetse flies of the genus *Glossina* spp. [2], and caused by *Trypanosoma congolense*, *T. vivax* and *T. brucei brucei;* or simultaneous infection with one or more of these trypanosomes.

T. brucei brucei infection causes weight loss, as a result of the excessive production of free radicals such as: superoxides, reactive oxygen and nitrogen species, and low level glutathione (GSH) in the cells and tissues of the host organism. The free radicals thus generated permits cleavage of the sialic acids on the surface cell membrane of the host erythrocytes, leading to anaemia and cellular damage of all the affected organs [3], [4], [5]. It has also been documented that excessive generation of free radicals is the precursor of lipid peroxidation and the attendant

decrease in systemic anti-oxidants, such as the plasma level of vitamin C or ascorbic acid. [6], [9], [10], [11].

However, it has been reported that a good nutrition for infected and uninfected control animals will cause both to grow at the same rate. [7], [8]. Reports have equally been documented that the destruction of homeostasis as a result of oxidative stress may be corrected if the body system is supplemented with natural antioxidants, such as vitamin C. [12], [13]. Since oxidative stress is prevented in the aqueous compartment and lipid bi-layer of cell membranes by anti-oxidants such as vitamins A and C, which are lypo- and hydro-soluble, respectively [14]. The ameliorative effect of vitamin C, vitamin E, and/or its combination on organ damage [6] and severity of anaemia [15] in *Trypanosoma brucei brucei* infected animals have all been reported, but from literature consulted there was paucity of information relating to the effects of this strain of trypanosome on serum chemistry.

Therefore, the objective of this study is to investigate whether or not vitamins C and E possess ameliorative effect on indicators such as the body temperature and serum chemistry of albino Wistar rats infected with *T. brucei brucei* (Federe strain).

MATERIALS AND METHOD

Experimental area

The study was carried out at the Nigerian Institute for Trypanosomiasis (and Onchocerciasis) Research (NITR) Kaduna, Nigeria, which is situated within latitude 10° 30' 00" N and longitude 7° 25' 50" E with an altitude of 614 metre above sea level.

Experimental animals

Twenty five Wistar rats weighing between 200 – 240 g at the commencement of the experiment were purchased from the rat colony of Nigerian Institute for Trypanosomiasis (and Onchocerciasis) Research, Kaduna. After a two-week period of acclimatization, the rats were left to acclimatize for another two weeks, and duly dewormed with standard drugs before commencement of the experiment. They were randomly divided into five groups of five rats each, and kept in a well ventilated standard plastic cages as follows: Group I was neither treated nor infected (positive control), group II was intraperitoneally infected with 1×10^6 innoculum containing *T. brucei brucei* (Federe strain) parasites only (negative control), while groups III, IV and V were given the same dose of innoculum and in addition they were treated orally with 150 mg/kg body weight of vitamin C; 150 mg/kg body weight of vitamin E and the combination of 150 mg/kg body weight each of vitamins C and E, respectively. The vitamins were administered once daily and throughout the experimental period. Vitamins C and E were products of a commercial company (VMD, n.v./S.A, Arendonk, Belgium) and were obtained from a veterinary commercial outlet in Kaduna, Nigeria. The animals were fed with a basal diet obtained from a commercial feed outlet (Vital Feeds Plc., Kaduna, Nigeria) and water was given *ad libitum.* Feed constituents and calculated bromatological analysis of the basal diet are shown in Table (1). The basal diet contained 11.50 MJ/kg metabolizable energy (ME) and 16.50 g of crude protein (CP), 5.50 g of calcium and 1.45 g of available phosphorus, calculated to be slightly above the nutrient requirement recommended by National Research Council [16].

Figure 1: Composition and calculated bromatological analysis of basal diet.

Nutrients/constituents	Quantity in g/kg diet
Maize	480.0
Soya cake	175.0
Wheat offal	160.0
Fishmeal	100.0
Brewer's dried grain	20.0
Vegetables oil	25.0
Limestone	5.0
Monocalcium phosphate	10.5
Dry molasses	15.0
Sodium chloride	5.0
Pre-mix Vitamins[(a)] and Minerals[(b)]	2.5
Calculated analysis /Kg	
ME, MJ /kg	11.50
CP, g	16.50
Lysine	1.65
Methionine +Cystine, g	0.92
Tryptophan, g	0.20
Threonine, g	0.61
Ca, g	5.50
P (a), g	1.45
Na, g	0.50
CI, g	0.50

Source: [17]. [(a)] **Vitamin supplement per (kg) diet**: Vitamin A, 6000 IU, vitamin $D_{3,}$ 5000 IU, vitamin E; 23.0 mg; vitamin k_3, 4.0 mg; thymine, 11.0 mg; riboflavin, 4.0 mg; vitamin $B_{12,}$ 0.005 mg; pyridoxine, 1.8 mg; pantothenic acid, 20,0 mg; nicotinic acid, 35 mg; folic acid, 2.5 mg; choline chloride, 615
[(b)] **Mineral supplement (mg/kg diet):** Cobalt, 0.40 mg; iron, 130 mg; copper, 5 mg; zinc, 18 mg; iodine, 1.55 mg.

Inoculation of rats with parasite

The parasites *T. brucei brucei* (Federe strain) was obtained from the cryopreserved stabilates kept in Vector and Parasitology Department of NITR, Kaduna, Nigeria. The parasite was inoculated into a clean rat which served as donor rat. Infected blood from the donor rat at peak parasitaemia, that is, 4 days post infection (DPI) was collected by means of tail picking and diluted with cold physiological saline. The number of parasite in the diluted blood was determined through the method described by [18] and a volume containing approximately 1×10^6 parasites was injected intraperitoneally into each rat in groups II-V.

Measurement of body temperature

The body temperature of all the animals in all groups was measured daily between the hours of 12:00 and 15:00 pm. Briefly, each animal was gently caught and a digital thermometer with a maximum gauge of 42 °C (accuracy ± 0.1°C MODE: ECT-1, MAXICOM), was inserted 3 cm into the wall of the colorectum of each rat and at the sound of a beep, the thermometer was immediately withdrawn and values obtained recorded accordingly. Live body weight of all the animals in the groups was taken twice per week and throughout the experimental period with the aid of a standard electronic weighing balance (Salter, pocket balance, England) with a maximum calibration of 5 kg and a precision of 0.1 g.

Blood sample collection and measurement of serum profiles

Tail blood was collected daily for monitoring parasitaemia as described by [18] and PCV by the micro-haematocrit method. On 28 DPI, the rats were sacrificed by humane decapitation prior anesthesia with sterile cotton impregnated with chloroform, approximately 50% of blood was collected in plain vacutainers, serum was harvested and used for the estimation of alanine amino-transferase (ALT), aspartate amino-transferase (AST) and alkaline phosphatase (ALP) activities employing the method described by [19] with the aid of commercial reagent kit (Gasellch aft fur Biochemica und Diagnostica, Wiesbgden, Germany).The serum samples were also used for the estimation of Urea and Creatinine by the Diacetylmonoxime and Jaffe's reactions, respectively as described by [20].

Statistical analysis

All data were presented as Means ± SEM and analyzed by one way ANOVA. In addition differences between means were compared by means of [21] post-hoc test using the SPSS version 19 statistical package and values of ($p<0.05$) were considered significant.

RESULTS

Result of body temperature values obtained during the study period is as shown in Figure 2; There was a general significant ($p<0.05$) increase in all infected groups compared to the positive control group. This group I showed no increase in the measured parameter throughout the experimental period as opposed to the sinusoid or undulating pattern of increase on 7 DPI, followed by a decrease on day 14 and 21 post infection, and finally a return to the path of increase on 28 DPI in groups II, III, IV and V, respectively. However, there were significant ($p<0.05$) decrease in all infected and vitamin treated groups either singly or in their combined form compared to the values recorded for the positive control group.

Figure 3; shows the result of serum chemistry indicators recorded during the biochemical analysis in this experiment. The levels of the serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea and creatinine increased significantly ($P< 0.05$) in the infected group II compared to uninfected group 1

The levels of alanine aminotransferase, aspartate aminotransferase, and urea decrease in all the treated groups compared to group II but ALP increase significantly ($P< 0.05$) in the treated

groups .Likewise, there is a significant decrease in the creatine level obtained in the treated groups compared to group II

The combined vitamins(C+E) show a significant ($P< 0.05$) decrease in all the levels of serum chemistry analysed except alkaline phosphatase which shows a significant ($P< 0.05$) increase.

Figure 2: Body Temperature of Wistar rats infected with *T. brucei brucei* (Federe strain) and administered with oral vitamins C and E (Means ± SEM, n = 5).

GROUP	Uninfected untreated I	Infected Untreated II	Infected + Vit. C III	Infected + Vit. E IV	Infected + Vit. C and E V
Initial	37.54 ± 0.16	37.44± 0.13	37.60 ± 0.21	37.54 ± 0.19	37.64 ± 0.15
7DPI	37.50 ± 0.18^{c}	39.53 ± 0.17^{a}	38.31 ± 0.12^{b}	38.36 ± 0.15^{b}	38.23 ± 0.14^{b}
14DPI	37.58 ± 0.17^{b}	38.41 ± 0.12^{a}	37.93 ± 0.13^{b}	37.95 ± 0.12^{b}	37.85 ± 0.12^{b}
21DPI	37.42 ± 0.18^{c}	38.54 ± 0.32^{a}	37.93 ± 0.13^{b}	37.95 ± 0.12^{b}	37.85 ± 0.12^{b}
28DPI	37.51 ± 0.17^{c}	39.85 ± 0.17^{a}	38.48 ± 0.12^{b}	38.64 ± 0.13^{b}	38.33 ± 0.13^{b}

DPI = Day post-infection; Mean values with different superscripts along the same row are statistically ($P< 0.05$) different [21].

Figure 3: Serum chemistry of Wistar rats infected with *T. brucei brucei* (Federe strain) and administered with vitamins C and E (Means ± SEM, n = 5).

Parameters	Uninfected and untreated I	Infected and untreated II	Infected + Vit C III	Infected + Vit E IV	Infected + Vits C and E V
ALT	19.80 ± 0.68^{c}	32.20 ± 0.70^{a}	31.40 ± 0.31^{a}	30.30 ± 0.63^{ab}	28.50 ± 0.85^{b}
AST	31.70 ± 0.78^{c}	42.90 ± 0.94^{a}	36.90 ± 1.01^{b}	36.70 ± 1.13^{b}	35.40 ± 1.10^{b}
ALP	78.40 ± 0.67^{c}	211.20 ± 0.70^{b}	228.30 ± 1.67^{a}	230.10 ± 2.52^{a}	229.70 ± 1.19^{a}
Urea	174.50 ± 2.01^{b}	319.30 ± 2.79^{a}	149.80 ± 0.83^{c}	147.50 ± 0.92^{c}	145.20 ± 0.76^{c}
Creatinine	59.40 ± 0.56^{d}	106.00 ± 1.90^{a}	63.80 ± 0.63^{c}	67.60 ± 1.02^{b}	61.70 ± 0.94^{cd}

ALT= Alanine aminotransferase; AST= Aspartate aminotransferase; ALP= Alkaline phosphatase. Mean values with different superscripts along the same row are statistically different (P< 0.05).

DISCUSSION

In this study, the body temperature of the infected rats increased as soon as the infection was established. The increased temperature (hyperthermia) that was observed in the infected rats could be due to endogenous activities of the pyrogens released by the parasites. This finding agrees with [22], who reported that the release of pyrogens affect a change in the body temperature set-point in the hypothalamus. The treated groups maintained a regular mean value of body temperature in the second and third week which was significantly (P< 0.05) lower than the infected control, this could suggest that the vitamin supplementation gradually suppressed the activities of the pyrogens released by the parasites. In the fourth week, the body temperature of both infected control and treated groups increased significantly (P< 0.05) compared to the previous week. This erratic body temperature corroborates with the findings of [23] that an unsteady body temperature is a clinical feature of trypanosomiasis resulting from a response to successive waves of parasitaemia.

It has been reported that analysis of haematological and serum/plasma chemistry parameters gives complementary information on the health status of the animal [24], [25], [26]. In this experiment, there were increase in levels of Alanine amino-transaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatise (ALP), urea and Creatinine due to infection. This agrees with the findings of [27], [28], [29], [30], [31], who reported increase in serum liver enzymes level in experimental trypanosomiasis. Increases in the level of these enzymes are indications of damage to liver, brain, and cardiac muscles as documented by [32],[33], [34], [35], [36]. Similarly, several workers have reported hepatocellular damage and generalized degenerative changes in other tissues and organs in trypanosomiasis [37], [38], [39], [40].

These enzymes functions increased due to the production of toxin as reported by [41], [42], [43]. Alkaline phosphatase is a 'Marker' enzyme for plasma membrane and endoplasmic reticulum, as it has been shown that this enzyme is involved in the mediation of membrane transport [44] and transphorylation [45]. Elevated enzyme levels may also result from effect of trypanosome lyses resulting from the host's defence mechanisms [46]. The increase in ALP values obtained in the study is not in agreement with the findings of [47], which reported decrease in the activity of the enzyme in rat testis following the repeated administration of mancozeb, this could be attributed to either non-leakage of the enzyme into the extracellular fluid as a result of the intactness of the lipid bilayer of the membrane or non-inhibition of the enzyme activity by this parasite, an effect that may be attributed to the administered anti-oxidant vitamins. Similar depletion in alkaline phosphatase was also observed in the testis of rats treated with mancozeb [48], and endosulphan [49] which reported that the reduction in the activity of this enzyme is due to the decreased metabolic activities.

The vitamin treatment significantly lowered the disease-induced increases in ALT and AST. This agrees with the findings of [15] which concluded in their own study that production of free radicals and peroxides in rats infected and treated with vitamins C and E was minimized, thereby inducing a reduction in oxidative challenge of cellular membranes of hepatocytes.

Similarly, damage to renal structures was obvious by the increases in serum urea and creatinine and these were significantly ($P< 0.05$) prevented due to the immunity conferred on them by the vitamins against the oxidative stress, caused by trypanosome-generated free radicals.

The combined vitamins was more effective compared to the singular vitamin administration in lowering the increases observed in the serum levels of ALT, AST, urea and creatinine; this

could be attributed to the function of vitamin E being able to substantially interact with (n-3) fatty acid thereby boosting the immune system as reported by [50], [51].

CONCLUSION

In conclusion, administration of vitamins C and E singly, but more particularly in its combined form ameliorated the negative effect caused by *T. brucei brucei* on the experimental animals. However, *T. brucei brucei* infection resulted in increase in the serum chemistry concentrations of ALT, AST, ALP, Urea and Creatinine in albino Wistar rats. The importance of the increase in serum levels of the observed biochemical molecules in all infected groups on the pathogenesis of African trypanosomiasis could give an insight into the deleterious effects of this etiology on the liver, spleen and kidney which could result into their malfunctions. Therefore, increase in all the observed serum levels may be one of the pathophysiological mechanisms in the development of some of the reported organ damage observed in trypanosome infected animals. Furtherance to our conclusion, we suggest the inclusion of both anti-oxidant vitamins in the traditional drug therapy of African trypanosomiasis as an interim measure against increased drug resistance by the parasites.

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