

## EFFECTS OF DIETARY VITAMINS C AND E ON BODY TEMPERATURE, BODY WEIGHT AND HAEMATOLOGICAL PARAMETRES IN WISTAR RATS INFECTED WITH *T. brucei brucei* (Federe strain) DURING THE HOT RAINY SEASON

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### ABSTRACT

Effects of the administration of vitamins C and E on body temperature, body weight and haematological parametres in Wistar Rats infected with *T. brucei brucei* (Federe strain) were evaluated. Twenty five adult rats were randomly divided into five groups of five rats each. Group A was neither treated nor infected, group B was infected with  $1 \times 10^6$  in 0.2 mL blood/PBS of innoculum, groups C, D and E were given the same dose of innoculum and in addition, they were treated orally with vitamins C, E, and the combination of both at a dosage of 150 mg/kg body weight, respectively. Body temperature showed a significant ( $p < 0.05$ ) decrease in all infected and vitamin treated groups either singly or in their combined form compared to group B. Observed result of the initial and final live body weights in groups A and E were not significantly ( $p > 0.05$ ) different. Groups C showed a significant ( $p < 0.05$ ) decrease, while the decrease in groups B and D were highly significant ( $p < 0.01$ ), respectively. Haematological parametres were significantly ( $p < 0.05$ ) higher in all infected and treated groups compared to group B. In conclusion, combined anti-oxidants capacity of both vitamins C and E mitigated the negative effects observed in the measured parametres in the Wistar rats better than single administration.

**Keywords:** Body temperature, haematological profiles, live body weight, *Trypanosoma brucei brucei* (Federe strain), vitamins C and E, Wistar rats.

### INTRODUCTION

African trypanosomiasis is a disease of humans and animals caused by several species of trypanosomes and spread by tsetse flies in 38 countries within the sub-Saharan region (Welburn *et al.*, 2001). The disease is mainly transmitted cyclically by the genus *Glossina* spp., but can also be transmitted by several biting flies like tabanids, hippoboscids, stomoxys, etc. (Kneeland *et al.*, 2012; Stijlemans *et al.*, 2017), and of recent Votypka *et al.* (2012) have indicted the *Culex* mosquito in the transmission of an avian trypanosome *Trypanosoma culicavium* spp. nov. Two tsetse-transmitted parasites, *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodensienne*, cause human African trypanosomiasis (HAT) which is commonly known as sleeping sickness, while animal African trypanosomiasis (AAT) which is the focus of this study (in this case cattle) on the other hand is mainly caused

by *Trypanosoma congolense*, *T. vivax* and *T. brucei brucei*.

An estimated 45-50 million cattle are at risk of infection in sub-Saharan, with an estimated economic loss of up to US\$1.3 billion in cattle production (Kristjanson *et al.*, 1999). To date, it has been documented that the most feasible control of the AAT relies on the application of trypanocides on infected host, even though their efficacy continues to wane by the day (Anene *et al.*, 2006). Established drugs such as diminazene di-acetate, isometamidium chloride and homidium chloride are used in animal infection, their therapeutic and prophylactic values are now overwhelmed by numerous limitations such as increased toxicity, prevalence of fake ones and the development of resistance by the parasites (Legros *et al.*, 2002).

With exacerbated drug resistance initiated through a complex mechanism called antigenic variation, the

parasites now have a field day in the infected animals thereby triggering a host of somatic anomalies. It has been reported that this process is initiated when the parasite penetrate and cleave the erythrocyte cells of the host which in turn cause the release of pyrogens in to the circulatory system (Robinson *et al.*, 1999; Morrison *et al.*, 2009). With increased circulating pyrogens, the host neuro- and immunological mechanism is set in motion to restore homeostasis which leads to unabated pyrexia and consequently oxidative stress if not arrested in time. Oxidative stress has been implicated in the anaemia observed in trypanosomiasis (Igbokwe *et al.*, 1994; Umar *et al.*, 2000). Oxidative haemolysis is assumed to occur due to high production of free radicals in the body of infected animals (Slater, 1984; Baltz *et al.*, 1985) and depletion of endogenous antioxidant reserves of the body (Zwart *et al.*, 1991). Anaemia is a major clinical and laboratory finding in trypanosomiasis and is characterized by a pronounced decrease in white blood cell, red blood cell, packed cell volume, and haemoglobin levels (Losos and Ikede, 1972). *Trypanosoma brucei* infection like other trypanosome infections precipitate increased red blood cell destruction which results in anaemia as well as tissue damage (Ekanem and Yusuf, 2008; Akanji *et al.*, 2009). It has been hypothesized that the large amounts of peroxides and free radicals generated by trypanosome and activated mononuclear phagocytes predispose erythrocytes to early ageing and fragmentation.

However, several reports claim that vitamin E ( $\alpha$ -tocopherol) is an indispensable lipid soluble non-enzymatic antioxidant that protects cell membranes from oxidation, thus stabilizing and maintaining their selective permeability (Herrera and Barbas, 2001; Traber and Atkinson, 2007).

Vitamins C on the other hand, is a water-soluble anti-oxidant, which protect against oxidative injuries in the aqueous compartment of cell membranes (Halliwell and Gutteridge, 1985). The ability of vitamin C to reduce organ damage (Umar *et al.*, 2000) and vitamins C and E to reduce the severity of anaemia (Umar *et al.*, 1999) in *Trypanosoma brucei* infected animals have all been reported.

Furthermore, a number of studies have also demonstrated that dietary supplementation of vitamins C and E elevates the activities of antioxidant enzymes such as Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) which in turn leads to reduced oxidative stress and intravascular damage of internal organs (Ammouche *et al.*, 2002, Kiron *et al.*, 2004).

However, from literature consulted, no information was found on the use of these antioxidants in combating the menace of this strain of trypanosome. This study was

carried out therefore, to determine the effects of dietary vitamins C and E oral administration on body temperature, body weight and haematological parameters in Wistar Rats infected with *T. brucei brucei* (Federe strain) during the hot rainy season.

## MATERIALS AND METHODS

### Experimental site

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

### Experimental animals

The experiment was approved by the Research Ethics Committee of NITR, and the study was conducted under protocols in the guidelines established by the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy of Sciences, Washington, D. C. 1996). Twenty five Albino Wistar rats purchased from the rat colony of NITR, Kaduna, were used as subjects for the experiment. They were randomly divided into five groups (A, B, C, D and E) of five rats each, in well ventilated plastic cages with a 15×22×10 m<sup>3</sup> dimension equipped with wire mesh lids. The rats were acclimatised for two weeks and duly dewormed with standard drugs (Praziquantel® Shijiazhuang Jiuding Animal Pharmaceutical, China) before commencement of the experiment. Group A was neither treated nor infected (positive control), group B was intraperitoneally infected with  $1 \times 10^6$  in 0.2 mL blood/PBS of inoculum containing *T. brucei brucei* (Federe strain) parasites only (negative control), while groups C, D and E were given the same dose of inoculum 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. 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*. The rats had average weight of between 200–240 g at the commencement of the experiment. Feed constituents and calculated bromatological analyses of the basal diet are as shown in Table 1. The basal diet contain 11.50 MJ/Kg of metabolisable energy (ME), 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 for laboratory animals (NRC, 1995).

**Table 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	17.0
Sodium chloride	5.0
Pre-mix Vitamins <sup>(a)</sup> and Minerals <sup>(b)</sup>	2.5
<b>Calculated analysis /Kg</b>	
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
Cl, g	0.50

Source: Dale and Batal (2006).

<sup>(a)</sup> **Vitamin supplement per (kg) diet:** Vitamin A, 6000 IU, vitamin D<sub>3</sub>, 5000 IU, vitamin E; 23.0 mg; vitamin k<sub>3</sub>, 4.0 mg; thymine, 11.0 mg; riboflavin, 4.0 mg; vitamin B<sub>12</sub>, 0.005 mg; pyridoxine, 1.8 mg; pantothenic acid, 20.0 mg; nicotinic acid, 35 mg; folic acid, 2.5 mg; choline chloride, 615

<sup>(b)</sup> **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 stabilates kept in Vector and Parasitology Department of NITR, Kaduna, Nigeria. The parasite was inoculated into a clean rat which serve as donor rat. Infected blood from a 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 Herbert and Lumsden (1976), and a volume containing approximately  $1 \times 10^6$  in 0.2 mL blood/PBS of parasites was injected intraperitoneally into each rats in the infected groups.

#### ***Measurement of body temperature and body weight***

The body temperature of all the animals in all groups were 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  $\pm 0.1$  °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 were 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 haematological profiles***

Tail blood was collected daily for the estimation of parasitaemia (Herbert and Lumsden, 1976) and blood samples were taken using heparinised tubes to determine the PCV using micro-haematocrit method. 28 DPI, the rats were sacrificed by humane decapitation and blood samples were collected for haematological studies. White blood cell (WBC), neutrophils, lymphocytes and eosinophils counts were determined using the Automated Haematology

Analyser, (Sysmex, KX-21, Japan) as described by Dacie and Lewis (1991).

### Statistical analyses

All data were presented as means  $\pm$  SEM and analysed by one way ANOVA. The data of live body weight were analysed using student paired *t*-test and expressed as means  $\pm$  SEM. In addition, differences between means were compared through the post-hoc test method described by Duncan (1955) with aid of 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 Table 2. There was a general significant ( $p < 0.05$ ) increase in all infected groups compared to the positive control group. This group A 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 B, C, D and E, 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 negative control group.

The result of the initial and final live body weights of the experimental animals are presented in Table 3. Even though, there were no significant ( $p > 0.05$ ) difference in the live weights values observed for groups A and E, there was a slight increase in the final values observed in group A when compared to the initial value, whereas, the reverse was the case in Group E where the observed final liveweight was lower than the initial value. However, in the case of the paired values of group C, a significant ( $p < 0.05$ ) decrease was observed in the final value compared to the initial. But more intense ( $p < 0.01$ ) were the decrease observed in the final values of groups B and D compared to their initial values.

Observed haematological values are recorded in Table 4. All haematological indicators decreased consistently in all infected groups when compared to uninfected group. PCV, Neutrophil and Eosinophil showed similar patterns of significant decrease at ( $p < 0.05$ ), with highest decrease observed in group B, followed by the groups D and C, while the least decrease in values were observed in the group treated with both vitamins when compared to the positive control group. The highest significant ( $p < 0.05$ ) decrease for WBC was observed in group B and closely followed by C and D with the same range of probability, while the least decrease was observed for group E when compared to group A. Interestingly, values recorded for lymphocyte in all infected groups were significantly ( $p < 0.05$ ) reduced within the same range when compared to group A.

**Table 2:** Body Temperature of Wistar rats infected with *Trypanosoma brucei brucei* (Federe strain) and administered with oral vitamins C and E (Means  $\pm$  SEM, n = 5).

GROUP	Uninfected untreated	Infected Untreated	Infected + Vit. C	Infected + Vit. E	Infected + Vit. C and E
Initial	37.54 $\pm$ 0.16	37.44 $\pm$ 0.13	37.60 $\pm$ 0.21	37.54 $\pm$ 0.19	37.64 $\pm$ 0.15
7DPI	37.50 $\pm$ 0.18 <sup>c</sup>	39.53 $\pm$ 0.17 <sup>a</sup>	38.31 $\pm$ 0.12 <sup>b</sup>	38.36 $\pm$ 0.15 <sup>b</sup>	38.23 $\pm$ 0.14 <sup>b</sup>
14DPI	37.58 $\pm$ 0.17 <sup>b</sup>	38.41 $\pm$ 0.12 <sup>a</sup>	37.93 $\pm$ 0.13 <sup>b</sup>	37.95 $\pm$ 0.12 <sup>b</sup>	37.85 $\pm$ 0.12 <sup>b</sup>
21DPI	37.42 $\pm$ 0.18 <sup>c</sup>	38.54 $\pm$ 0.32 <sup>a</sup>	37.93 $\pm$ 0.13 <sup>b</sup>	37.95 $\pm$ 0.12 <sup>b</sup>	37.85 $\pm$ 0.12 <sup>b</sup>
28DPI	37.51 $\pm$ 0.17 <sup>c</sup>	39.85 $\pm$ 0.17 <sup>a</sup>	38.48 $\pm$ 0.12 <sup>b</sup>	38.64 $\pm$ 0.13 <sup>b</sup>	38.33 $\pm$ 0.13 <sup>b</sup>

DPI = Day post-infection; Mean values with different superscripts along the same row are statistically ( $p < 0.05$ ) different (Duncan, 1955).

**Table 3:** Live body weight of Wistar rats infected with *Trypanosoma brucei brucei* (Federe strain) and administered vitamins C and E at initial and final stage of the study (n=5)

Group	Initial	Final	t	p-value
A	221.50 ± 6.15	222.00 ± 4.95	-0.055	0.957
B	221.50 ± 6.26	185.10 ± 1.11	5.299	< 0.001**
C	221.60 ± 4.31	209.30 ± 2.12	3.119	0.012*
D	221.70 ± 4.87	196.40 ± 1.59	5.042	0.001**
E	221.50 ± 3.91	212.30 ± 2.63	1.919	0.087

Level of significance along rows: \* = p < 0.05; \*\* = p < 0.01

**Table 4:** Haematological parameters of Wistar rats infected with *Trypanosoma brucei brucei* (Federe strain) and administered vitamins C and E (Means ± SEM, n = 5).

Group	Uninfected untreated	Infected treated	not Infected	Infected and treated with Vit C	Infected and treated with Vit E	Infected and treated with Vit C + E
PCV	49.20 ± 0.25 <sup>a</sup>	29.20 ± 0.27 <sup>e</sup>	40.50 ± 0.21 <sup>c</sup>	39.70 ± 0.20 <sup>d</sup>	44.10 ± 0.26 <sup>b</sup>	
WBC	14.25 ± 0.12 <sup>a</sup>	6.64 ± 0.21 <sup>d</sup>	7.52 ± 0.19 <sup>c</sup>	7.14 ± 0.14 <sup>c</sup>	8.13 ± 0.10 <sup>b</sup>	
Neutrophil	31.45 ± 0.38 <sup>a</sup>	9.78 ± 0.11 <sup>e</sup>	13.53 ± 0.20 <sup>c</sup>	12.42 ± 0.23 <sup>d</sup>	14.19 ± 0.09 <sup>b</sup>	
Eosinophil	17.74 ± 0.15 <sup>a</sup>	3.04 ± 0.12 <sup>e</sup>	5.27 ± 0.07 <sup>c</sup>	4.80 ± 0.12 <sup>d</sup>	5.62 ± 0.10 <sup>b</sup>	
Lymphocyte	91.87 ± 0.31 <sup>a</sup>	54.33 ± 4.18 <sup>b</sup>	58.61 ± 1.49 <sup>b</sup>	59.76 ± 0.38 <sup>b</sup>	59.24 ± 0.63 <sup>b</sup>	

Mean values with different superscripts along the same row are statistically (p<0.05) different (Duncan, 1955).

## DISCUSSION

Increased body temperature observed in this study is a classical indication of physiological perturbation of the infected animals, more so, is the undulating pattern that coincides with the finding of Zwart *et al.* (1991) which reported this trend as a clinical manifestation normally seen in trypanosomiasis infested animals. Furthermore, it has been documented that changes in the body temperature regulating centre (hypothalamus) due to pyrogenic stimuli released during infection leads to pyrexia and oxidative stress observed in trypanosomiasis, because pyrexia is a direct response to successive waves of parasitaemia (Stephen, 1986; Baracos *et al.*, 1987). However, the significant decrease observed in all infected and treated groups compared to group B suggest that the non-enzymatic anti-oxidants possess anti-pyretic capacity, since this activity is achieved through their

ability to boost immune response to disease. (Passmore and Eastwood, 1986).

The very high significant decrease in live body weight observed in infected untreated group is an indication of the severity of the disease infection. This phenomenon may possibly be as a result of parasite-induced anorexia. Our observation in this study agrees with the finding of Itard, (1989) which reported that the apparent weight loss was due to increased parasitaemia. However, weight loss recorded in the group of animals treated with combination of vitamins C and E was not significant, this corroborates several studies reports that overall antioxidant potential is possibly more efficient and crucial than single antioxidant nutrients (German and Traber, 2001; Umar *et al.*, 2007; Umar *et al.*, 2010).

Haematological parameters such as RBC, PCV, WBC, have been shown to be important indices of livestock health and production status (Oyewale and Fajimi,

1988), this is further supported by the report of Ekanem *et al.* (2005; 2006) which documented that the measurement of anaemia gives a reliable indication of the disease status and productive performance of trypanosome infected animals. PCV is a measure of anaemia, and the reduction in PCV indicator observed in this study particularly in group B suggests that the anaemia in this infection is haemolytic and that it involves a significant increase in the rate of erythrocyte destruction. Our finding in this study is in agreement with the reports of Preston *et al.* (1979) and Igbokwe and Nwosu (1997) which concluded in their studies that trypanosomiasis may cause anaemia as a result of massive erythrophagocytosis by an expanded and active mononuclear phagocytic system (MPS) of the host. In addition, several authors have reported that severity of anaemia usually reflects the intensity and duration of parasitaemia in trypanosomal infection (Ogunsanmi and Taiwo, 2001; Umar *et al.*, 2007; Ekanem *et al.*, 2006; Saleh *et al.*, 2009), as it has been reported by Mbaya *et al.* (2009) in their study on red fronted gazelles experimentally infected with *Trypanosoma brucei* and/or *Haemonchus contortus*, that there is an inverse relationship between parasitaemia and anaemia. The reduced haematological values of neutrophils, eosinophils and lymphocytes obtained from this study agree with previous studies reported by Anosa (1988); Igbokwe *et al.* (1994); Ekanem and Yusuf (2008) and Sulaiman and Adeyemi (2010), which reported such trend to be indicative of acute phase infection, parasitic infection and immunological reaction due to persistent intensity of parasitaemia, respectively.

The anaemia observed in this experiment decreased progressively without any period of drop, another indication of an acute phase of the disease. Similar observation was previously reported with a different strain (Basa) of the parasite (Umar *et al.*, 1999). Several factors contribute to the development of anaemia among which is the oxidative damage of RBC membranes by free radicals and peroxides generated during the course of the infection (Igbokwe *et al.*, 1994) on one hand, but on the other hand, infection with trypanosomes resulted in increased susceptibility of red blood cell membrane to oxidative damage probably as a result of depletion of reduced glutathione on the surface of the red blood cell (Igbokwe, 1994; Taiwo *et al.*, 2003; Akanji *et al.*, 2009).

The administration of the vitamins as shown in the PCV values obtained in all infected and treated group compared to group B significantly ( $p < 0.05$ ) ameliorated the disease induced anaemia and may be attributable to the antioxidant activity of these vitamins; by scavenging the trypanosome-generated free radicals; thus reducing the free radical load

(Kaikabo and Salako, 2006; Eze and Ochike, 2007). However, the inability of the vitamins to completely prevent the disease-induced anaemia suggests the involvement of other aetiological factors in the development of anaemia in the infected rats.

#### CONCLUSION AND RECOMMENDATION

In conclusion, the result obtained in this study has demonstrated that the combination of vitamins C and E ameliorated the increase in body temperature, reduce loss in body liveweight, and suppress parasitaemia than vitamins C and E in their individual application. It is our recommendation therefore, that further work should be carried out to evaluate the simultaneous administration of standard trypanocide with the combined anti-oxidants in order to combat the menace of this disease.

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