



## **Effects of Vitamin C and Choline Chloride Supplementation on Temperature and Some Haematological Parameters in *Trypanosoma congolense* Infected Rats**

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### **Authors' contributions**

This reaserch work was carried out in collaboration among all the authors. Author CSU designed the work and wrote the first draft of the manuscript. Author MUE performed the statistical anylysis and literature search, while authors CSU and KJO carried out the laboratory work. All authors read and approved the final Manuscript.

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### **ABSTRACT**

**Aims:** Effects of Vitamin C and Choline chloride supplementation on temperature and some haematological parameters were investigated in *Trypanosoma congolense* infected rats.

**Methodology:** A total of twenty five (25) healthy albino rats weighing between 113.5-154.3 g were used for the study. They were randomly divided into groups (A-E) of five rats per group. Group A was uninfected untreated which served as the control, group B was infected untreated. Groups C, D and E were infected treated with Choline chloride, Vitamin C and combination of Choline chloride and Vitamin C respectively.

**Results:** The mean body temperature was significantly higher ( $p < 0.05$ ) in the infected untreated group on days 21 and 28 post infection, when compared to the infected supplemented treated groups. The mean packed cell volume (PCV) decreased significantly ( $p < 0.05$ ) in the infected untreated group on days 21 and 28 post infection, when compared to the other infected treated groups. The mean haemoglobin concentration (HB) decreased significantly ( $p < 0.05$ ) in the infected

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untreated group on days 7, 21 and 28 post infection, when compared to the other infected and treated groups. The mean red blood cell counts (RBC) decreased significantly ( $p < 0.05$ ) in the infected untreated group on day 28 post infection compared to the other infected and treated groups. The mean total white blood cell counts (TWBC) decreased significantly ( $p < 0.05$ ) in the infected untreated group on days 14 and 21 post infection compared to the infected treated groups. On day 28 post infection TWBC counts in the group treated with combination of vitamin C and Choline chloride increased ( $p < 0.05$ ) when compared with the other treated groups. There was no significant difference ( $p > 0.05$ ) in all the infected treated groups when compared with the control

**Conclusion:** It was concluded from the study that combined supplementation of Vitamin C and Choline chloride will be more effective in ameliorating the haematological impacts of trypanosomosis.

**Keywords:** Haematology; *Trypanosoma congolense*; vitamin C; choline chloride; rats.

## 1. INTRODUCTION

Trypanosomosis is a haemoprotozoan disease of domestic and wild animals [1]. African Animal Trypanosomosis affects both humans and animals; it is widely distributed in several parts of sub-Saharan Africa [2,3]. The disease is mainly transmitted cyclically by tsetse fly of the genus *Glossina* specie; but can also be transmitted by several biting flies such as tabanids, hipponboscides, stomoxys [4]. The species of trypanosomes that affects animals include *Trypanosoma vivax*, *T. congolense*, *T. brucei*, *T. simiae*, *T. evansi* and *T. equiperdium*. Susceptible hosts include cattle, sheep, goats, pigs, horses, camels, dogs and cats. Many wild animals are carriers of trypanosome infection, thus serving as reservoirs of infection to susceptible animals [5].

Anaemia and haematological derangements are the major clinical and laboratory finding in clinical trypanosomosis [1], characterized by pronounced decrease in packed cell volume, haemoglobin concentration, red blood cell counts and white blood cells [6,7]. Other symptoms may include pyrexia, anorexia, loss of condition and death [8]. The cause of anaemia in Trypanosomosis is multifactorial; haemodilution, erythrophagocytosis, haematopoietic response, haemolytic factor and bone marrow dyserythropoiesis [9,10] Oxidative stress and tissue damage have also been implicated in the anaemia observed in trypanosome infections [11,12]. It is hypothesized that the large amounts of peroxides and free radicals generated by trypanosome and activated mononuclear phagocytes predisposes erythrocytes to early ageing and fragmentation [12].

Natural products and formulations such as micronutrients boost the host immune system and contribute extensively to the control and

management of diseases [13,14]. Vitamin C is a potent antioxidant which protects the living cells from oxidative stress and tissue damage by neutralizing the reactive oxygen species [15]. The immune system can be boosted with Vitamin C in a number of ways which include protection from free-radicals, mediated protein inactivation in macrophages, chemotaxis, stimulation in production of antibody and interferon, signaling of rapid proliferation of immune cells and complement activation [16]. Vitamin C plays a very vital role in cell division, development and maintenance of scar tissue, blood vessels and cartilage [17]. Choline chloride is a water-soluble micro nutrient that is usually grouped within the B-complex vitamins [18]. Choline is a dietary component that is important for the structural integrity of cell membrane, methyl metabolism, cholinergic neurotransmission, transmembrane signaling, and lipid and cholesterol transport and metabolism [19]. Choline accelerates the production of acetylcholine, a substance that is responsible for nerve impulse [20].

Drug resistance of trypanosomes is a major impediment towards control and eradication of trypanosomes in sub-Saharan Africa [1]. The available drugs such as diminazene di-aceturate, isometamidium chloride and homidium chloride used in animal therapeutic and prophylactic treatments are now overwhelmed by numerous limitations such as increased toxicity and the development of resistance by the parasites [21]. With the allusion that oxidative stress has a major role to play in the pathogenesis of this disease; some workers have reported the efficacy of antioxidant supplementation in ameliorating the scourge of trypanosomosis in livestock [12,22]. Therefore this research was designed to study the effects of micronutrients; vitamin C and Choline chloride on the temperature and haematological parameters of

albino rats infected with *Trypanosoma congolense*.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Animals

Twenty five (25) healthy albino rats of both sexes weighing between 113.5-154.3 g were used for the study. The rats were obtained from Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture; Umudike, Abia State. They were housed in clean rat cages with wood shavings used as beddings. The cages were kept in a well ventilated fly proof house and the animals were humanely cared for in compliance with the principles of laboratory animal care [23]. They were fed with commercial growers ration (vital feed) and water was given *ad libitum*.

### 2.2 Vitamin C and Choline Chloride Dietary Supplementation

Vitamin C (Hodo pharmaceuticals Wuxi china, food grade type) was supplemented 1000mg/Kg of feed and Choline Chloride (VMD, n.v./s.A Arendonk, Belgium) and was supplemented at 800 mg/kg of feed. The supplemented feeds were pelleted with 10ml syringe and were dried with hot air oven at 40°C. The rats were fed with the experimental diet ten days (10) prior to the inoculation of the rats with trypanosomes and to the end of the experiment.

### 2.3 Inoculation of Rats with Parasites

*Trypanosome congolense* used in this study was obtained from National Institute for Trypanosomosis Research (NITR) Vom, Plateau State Nigeria. The trypanosomes were passaged into donor rats before infection of experimental animals intra peritoneally at a dose of  $1 \times 10^6$  trypanosomes per milliliter of saline diluted infected blood. The numbers of infective trypanosomes were determined using the rapid matching method of Herbert and Lumsden [24].

### 2.4 Experimental Procedures

The albino rats were acclimatized for two weeks before the commencement of the study and were randomly divided into five groups of five rats. Group A, un-infected untreated which served as negative control, group B infected untreated, while groups C, D and E were infected and

treated with Choline chloride, Vitamin C and combination of Vitamin C and Choline chloride respectively. Temperature and haematological parameters including packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell counts (RBC) and total white blood cell counts (TWBC) were monitored weekly for a period of four weeks after infection.

### 2.5 Temperature

The rectal temperatures of the rats were determined using a digital clinical thermometer and values obtained were recorded in degree Celsius (°C).

### 2.6 Haematology

Packed cell volume was determined using microhaematocrit method [25], Haemoglobin Concentration was determined using cyanomethaemoglobin method [26]. Red blood cell and total White blood cell counts were determined using improved Neubauer counting technique [27].

### 2.7 Statistical Analysis

All data were presented as a means  $\pm$  SEM and analyzed by one way ANOVA. In addition, differences between means were compared through the post-hoc test method described by Duncan [28] (1955) with the aid of SPSS version 19 statistical package, and values of ( $p < 0.05$ ) were considered significant.

## 3. RESULTS

### 3.1 Temperature

The mean temperature was significantly higher ( $p < 0.05$ ) in the infected untreated group on days 21 and 28 post infection, when compared to the other infected and treated groups. However, there was no significant difference ( $p > 0.05$ ) in all the infected and treated groups when compared with the control (Table 1).

### 3.2 Packed Cell Volume

The mean packed cell volume decreased significantly ( $p < 0.05$ ) in the infected untreated group on day 21 and 28 post infection, when compared to the other infected and treated groups. However, there was no significant difference ( $p > 0.05$ ) in all the treated infected groups, when compared with the control (Table 2).

### 3.3 Haemoglobin Concentration

The mean haemoglobin concentration decreased significantly ( $p < 0.05$ ) in the infected untreated groups on days 7, 21 and 28 post infection, when compared with the other infected and treated groups. On day 21 post infection, the HB concentration was significantly higher ( $p < 0.05$ ) in the group treated with combination of vitamin C and choline chloride compared with the group treated singly with vitamin C or choline chloride and the control group (Table 3).

### 3.4 Red Blood Cell Counts

The mean red blood cells decreased significantly ( $p < 0.05$ ) in the infected untreated group on day 28 post infection compared to the other infected and treated groups. There was no significant ( $p > 0.05$ ) difference between all the infected treated groups and the control group (Table 4).

### 3.5 Total White Blood Cell Counts

The Mean white blood cell count decreased significantly ( $p < 0.05$ ) in the infected untreated group on day 14 and 21 post infection when compared to the infected and treated groups. On day 28 post infection, TWBC counts in the group treated with combination of vitamin C and choline chloride increased significantly ( $p < 0.05$ ) when compared with the other treated groups (Table 5).

## 4. DISCUSSION

The significantly ( $p < 0.05$ ) increased body temperature observed on days 21 and 28 post infection in the untreated infected group is an indication of physiological disorder of the hypothalamic temperature regulating centre; and

is a common feature of African animal trypanosomosis [29]. The fluctuating pattern of the body temperature observed in the study agrees with the findings [30], who reported this trend as a clinical manifestation normally seen in the trypanosome infected animals. This results further confirms pyrexia and oxidative stress as a common feature of trypanosomes [31], triggered by the appearance of parasites in the blood of infected rats [32]. The significantly ( $p < 0.05$ ) decreased body temperature, observed in the infected and treated groups is suggestive of anti-pyretic and anti-oxidant [15] nature of Choline chloride and vitamin C respectively; thus the ability of these micronutrients to boost the host's immune response to the trypanosomes. This result also corroborates with the findings of Umar et al. [33] who reported ameliorated anaemia and reduced oxidative damage after intraperitoneal administration of vitamin C and E in *Trypanosoma brucei* infected mice. The decreased PCV, HB concentration, RBC counts on days 21 and 28 in the infected untreated group agrees with previous studies which showed that infection with trypanosomes resulted in increased susceptibility of red blood cell membrane to oxidative damage probably as a result of depletion of vitamins on the surface of the red blood cell [34-36].

Anaemia in trypanosomosis is not directly dependent on the number of parasites; but controlled by host factors such as cytokines [37]. The significant decrease ( $p < 0.05$ ) in red blood cell parameters in the infected; untreated group when compared to other infected groups that were supplemented may be partly due to the protective effects of vitamin C from oxidative injuries in the aqueous compartments of cell membranes [38] and cellular structural integrity by Choline chloride [39].

**Table 1. The mean body temperature (°C) of *T. congolense* infected rats treated with vitamin C and choline chloride**

Days post infection	GROUPS				
	Uninfected untreated	Infected untreated	Infected treated with Choline chloride	Infected treated with vitamin C	Infected treated with Choline chloride and vitamin C
0	37.56 ± 0.24	36.94 ± 0.31	37.14 ± 0.38	37.14 ± 0.15	37.10 ± 0.3
7	36.57 ± 0.35	37.07 ± 0.31	37.15 ± 0.25	36.50 ± 0.62	36.20 ± 0.28
14	36.17 ± 0.42	36.65 ± 0.59	35.30 ± 0.37	36.10 ± 0.50	35.80 ± 0.42
21	36.72 ± 0.26 <sup>ab</sup>	37.12 ± 0.42 <sup>b</sup>	35.75 ± 0.52 <sup>a</sup>	36.90 ± 0.13 <sup>b</sup>	36.22 ± 0.25 <sup>ab</sup>
28	36.65 ± 0.25 <sup>ab</sup>	37.25 ± 0.41 <sup>b</sup>	36.00 ± 0.47 <sup>a</sup>	36.92 ± 0.14 <sup>ab</sup>	36.25 ± 0.25 <sup>ab</sup>

<sup>a,b</sup>Superscript in a row indicates significant difference between the means at the level of probability ( $p < 0.05$ )

**Table 2. The mean packed cell volume (%) of *T. congolense* infected rats treated with vitamin C and choline chloride**

Days post infection	GROUPS				
	Uninfected untreated	Infected untreated	Infected treated with Choline chloride	Infected treated with vitamin C	Infected treated with Choline chloride and vitamin C
0	39.80 ± 0.37	39.80 ± 1.06	40.80 ± 1.06	40.20 ± 1.62	39.40 ± 1.80
7	38.25 ± 1.31	38.50 ± 2.32	40.50 ± 1.25	39.25 ± 1.37	39.40 ± 1.70
14	38.50 ± 0.28	35.75 ± 2.09	39.75 ± 0.47	35.50 ± 2.25	38.00 ± 2.12
21	37.00 ± 2.38 <sup>b</sup>	32.50 ± 0.64 <sup>a</sup>	36.75 ± 3.70 <sup>b</sup>	37.25 ± 2.62 <sup>b</sup>	38.00 ± 4.08 <sup>b</sup>
28	37.25 ± 0.75 <sup>b</sup>	30.75 ± 0.75 <sup>a</sup>	36.50 ± 1.65 <sup>b</sup>	36.50 ± 2.39 <sup>b</sup>	38.75 ± 0.75 <sup>b</sup>

<sup>a,b</sup> superscript in the row indicates significant difference between the means at the level of probability ( $p < 0.05$ )

**Table 3. The mean haemoglobin concentration (g/dL) of *T. congolense* infected rats treated with Vitamin C and Choline chloride**

Days post infection	GROUPS				
	Uninfected untreated	Infected untreated	Infected treated with Choline chloride	Infected treated with vitamin C	Infected treated with Choline chloride and vitamin C
0	10.68 ± 0.18	10.56 ± 0.81	11.12 ± 0.59	10.16 ± 0.64	10.20 ± 0.32
7	14.30 ± 0.23 <sup>b</sup>	11.25 ± 0.47 <sup>a</sup>	13.60 ± 0.35 <sup>b</sup>	13.10 ± 0.42 <sup>b</sup>	13.70 ± 0.65 <sup>b</sup>
14	13.35 ± 0.39 <sup>b</sup>	11.30 ± 0.12 <sup>a</sup>	11.85 ± 0.78 <sup>a</sup>	11.55 ± 0.28 <sup>a</sup>	13.35 ± 0.39 <sup>b</sup>
21	13.15 ± 0.65 <sup>b</sup>	9.47 ± 0.32 <sup>a</sup>	12.65 ± 0.44 <sup>b</sup>	13.8 ± 0.50 <sup>a,c</sup>	14.60 ± 0.24 <sup>c</sup>
28	12.05 ± 0.41 <sup>b</sup>	9.25 ± 0.17 <sup>a</sup>	13.60 ± 0.57 <sup>b</sup>	12.80 ± 0.75 <sup>b</sup>	12.80 ± 0.46 <sup>b</sup>

<sup>a,b,c</sup> superscript in a row indicate significant difference between the means at the level of probability ( $p < 0.05$ )

**Table 4. The mean red blood cell count ( $10^6/\mu\text{l}$ ) of *T. congolense* infected rats treated with Vitamin C and Choline chloride**

Days post infection	GROUPS				
	Uninfected untreated	Infected untreated	Infected treated with Choline chloride	Infected treated with vitamin C	Infected treated with Choline chloride and vitamin C
0	7.66 ± 0.70	7.66 ± 0.20	7.37 ± 0.25	7.71 ± 0.30	6.78 ± 0.63
7	7.33 ± 0.23	7.39 ± 0.43	7.77 ± 0.22	7.51 ± 0.28	7.56 ± 0.36
14	7.07 ± 0.11	7.10 ± 0.48	7.64 ± 0.09	6.75 ± 0.40	7.31 ± 0.40
21	7.86 ± 0.18	6.18 ± 0.10	7.60 ± 0.72	7.19 ± 0.50	7.30 ± 0.78
28	7.39 ± 0.53 <sup>b</sup>	5.96 ± 0.33 <sup>a</sup>	7.04 ± 0.31 <sup>ab</sup>	7.10 ± 0.46 <sup>ab</sup>	7.38 ± 0.11 <sup>b</sup>

<sup>a,b</sup> superscript in a row indicates significant difference between the means of the level of probability ( $p < 0.05$ )

The lower total white blood cell counts across the infected groups on day 28 post infection may be attributed to the immunosuppressive actions of trypanosome infection [40,41]. Leucocytosis observed on day 21 post infection across the infected groups has been implicated in trypanosomosis and these conditions are usually as a result of the wax and wear syndrome on the changing variable surface glycoprotein of the infecting trypanosomes [40].

Vitamin C and Choline chloride supplementation in this study are the only exogenous factors that might have accounted for the difference in observations of the parameters assessed. Infective dose of trypanosome species have been reported to influence variations in other parameters in trypanosomosis [42,43], which were not applicable in this study since all the infected rats received the same dose of infection.

**Table 5. The mean total white blood cell count ( $10^3/\mu\text{L}$ ) of *T. congolense* infected rats treated with vitamin C and Choline chloride**

Days post infection	GROUPS				
	Uninfected untreated	Infected untreated	Infected treated with Choline chloride	Infected treated with vitamin C	Infected treated with choline chloride and vitamin C
0	6.57 ± 0.71	7.07 ± 1.27	4.85 ± 1.03	4.85 ± 1.03	4.14 ± 0.85
7	8.16 ± 2.57	4.53 ± 1.20	8.30 ± 0.92	5.25 ± 0.40	4.91 ± 0.97
14	9.12 ± 1.98 <sup>ab</sup>	5.28 ± 0.70 <sup>a</sup>	9.51 ± 0.70 <sup>b</sup>	6.72 ± 0.41 <sup>ab</sup>	8.00 ± 1.19 <sup>ab</sup>
21	9.55 ± 2.881 <sup>a</sup>	11.37 ± 2.74 <sup>ab</sup>	18.33 ± 2.17 <sup>b</sup>	15.52 ± 1.79 <sup>ab</sup>	16.66 ± 1.31 <sup>ab</sup>
28	12.70 ± 1.29 <sup>b</sup>	9.88 ± 2.20 <sup>ab</sup>	8.65 ± 1.50 <sup>ab</sup>	8.05 ± 0.23 <sup>a</sup>	12.87 ± 1.26 <sup>b</sup>

<sup>a,b</sup> superscript in a row indicates significant difference between the means of the level of probability ( $p < 0.05$ )

## 5. CONCLUSION

The results of this study indicated that Vitamin C and Choline chloride supplementation appeared to have ameliorated the effect of trypanosomes on the haematological values of albino rats. It can be speculated that the combined administration of vitamin C and Choline chloride will be more effective as a nutritional supplement in control and prevention of trypanosomiasis in endemic areas than single administration of vitamin C or Choline chloride. We therefore recommend that more research be carried out to evaluate the effects of combined administration of vitamin C and Choline chloride with a standard trypanocide in the management of African animal trypanosomiasis.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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