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Effects of Vitamin E and Selenium on Some Blood Parameters of *Trypanosoma brucei brucei* Infected Rats

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Authors' contributions

Author DPY designed the study, wrote the protocol, author AD wrote the manuscript, managed the analyses of the study and author NAO managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To determine the effects of dietary supplementation of vitamin E and selenium on infection rate, pack cell volume (PCV) and white blood cell (WBC) of *T. brucei brucei* infected rats.
Study Design: Feeding of rats with varying levels of vitamin E and selenium to determine their effects on parasitaemia, packed cell volume and white blood cells of infected rats.
Place and Duration of Study: Animal House, Department of Pharmacology, University of Jos, Jos, Nigeria. January 2009.
Methodology: Thirty healthy albino rats were randomly divided into five groups (1-5) of six animals each. The PCV and WBC of the experimental animals were determined before they were inoculated with 0.2 ml of infected red blood cell containing 1.5 x 10^5 *T. brucei brucei* on the first day. The rats were fed with standard chick grower mash containing varied quantities of vitamin E and selenium for seventeen days. Group 1 (control) were given diet without vitamin E and selenium, group 2 were fed with diet containing vitamin E only, group 3 were fed with diet containing selenium only, group 4 were fed diet containing 0.3 mg vitamin E and 80 mg selenium and group 5 had diet containing 0.5 mg vitamin E and 100 mg selenium.
Results: There is a significant (P=.05) decrease in parasitaemia of rats fed varying levels of vitamin E and selenium. The PCV increased significantly (P=.05) in rats fed with diet

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containing vitamin E and selenium relative to the control. There was no significant (P =.05) change in WBC of rats fed with supplements and that of the control group.
Conclusion: The study showed that vitamin E and selenium supplementation significantly decreased parasitaemia resulting in delay of death in the rats, increased the PCV, while there was no significantly effect on WBC.

Keywords: Vitamin E; selenium; T. brucei brucei; blood; albino rats.

1. INTRODUCTION

Trypanosomiasis, also known as sleeping sickness in man, nagana in cattle or surra in camels, is a disease caused by flagellated protozoa belonging to the genus *Trypanosoma.* Trypanosomes are extracellular parasites which multiply in various body fluids e.g blood, lymph and spinal fluid. The disease is transmitted by tse tse flies (*Glossina* spp) and is characterised by anaemia, Oedema, Cachexia, intermittent fever and death. Sleeping sickness has been on the rise in recent years and is viewed as a major health problem in African countries, with 60 million people being at risk of infection in sub-Saharan Africa [1]. [2] reported that 46 million cattle are exposed to the risk of contracting nagana costing an estimated US $1340 million per year.

The degree of trypanotolerance is greatly affected by the nutritional status of the host animal. Nutrition is important in moderating the severity of pathophysiological effect of trypanosomiasis and also influences the rate of recovery.

Vitamin E has been used for the treatment of health condition, often based on its antioxidant properties and it also serves as a dietary supplement recommended for better health. [3] reported that the inhibition of alpha tocopherol transfer protein (α -TTP) a determinant of the Vitamin E concentration in host circulation confers resistance to *Trypanosoma congolense* infection, evidently owing to oxidative damage to parasite DNA.

Selenium is an essential trace element for all organism from bacteria to humans. One of its main functions is an anti-oxidant action, involved in protection against damage caused by free radical and oxidative stress [4]. Diet with selenium could be beneficial in the treatment of diseases correlated with considerable oxidative stress; particularly parasitoses.Dietary selenium levels modulate free thiol levels and specific signaling events during CD4+ T cell activation, which influence their proliferation and differentiation in mice [5]. [6] reported that supplementation of selenium enriched Japanese Radish sprouts (JRS) it 50 µg/kg or a combination of selenium enriched JRS and *Rhodobacter capsulatus* modulates leucocyte population in laying hens. It has been reported that a nutritional selenium deficiency can lead to a higher susceptibility of host to *Cryptosporidium parvum* infection and decrease immune responses in mice [7]. The dietary supplementation of selenium at low doses improved the antioxidant status in *Trichinella spiralis* infected rats [8].

Vitamin E and selenium are regarded as immunopotentiators as both appear to participate in similar nutritional and biochemical pathways which enhance humoral immunity. Selenium alone and its combination with vitamin E did not prolong the relapse interval of *T.brucei brucei* in rats [9]. This study examined the effect of dietary supplementation of Vitamin E and selenium on parasitaemia, PCV and WBC in *T. brucei brucei* infected rat.

2. MATERIALS AND METHODS

2.1 Study Area

The research was carried out in the animal House unit of the University of Jos, Jos, Nigeria.

2.2 Experimental Animals

Thirty healthy male and female albino rats weighing between 80-100 g were obtained from the animal house. They were housed in plastic cages with saw dust as bedding. The rats were used in accordance with the standard operation procedure for care and use of laboratory animals. *Trypanosoma brucei brucei* were obtained from the Nigerian Institute for Trypanosomiasis (NITR) in Vom and were maintained by serial passage in rats. Vitamin E and selenium were purchased from ECWA veterinary clinic in Jos, Plateau State, Nigeria.

The rats were divided into five groups (Group 1-5) of six rats all kept in one cage. Group one (control) were fed with diet which has no supplements with vitamin E and selenium. Group two were given diet which contained 0.3 mg vitamin E only per day. Group three were feed with diet containing 80 mg selenium only per day. Group four were fed with diet containing 0.3 mg vitamin E and 80 mg selenium per day, and group five were given diet containing 0.5 mg vitamin E and 100 mg selenium. The vitamin E and selenium supplements were added to 30 grams of the chow per day and given to the animals in each group to ensure full consumption of the supplemented feed before subsequent addition of the feed without supplements and water *ad libitum* for the rest of the day.

2.3 Determination of Parasitaemia

Rats were screened and certified negative for blood parasites before intraperitoneal inoculation on the first day with 1 ml of blood containing 1.5×10^5 *Trypanosoma brucei brucei* as estimated using the improved Neubauer haematocytometer parasitaemia was monitored daily from day four through day sixteen. Thin smears fixed in methanol stained with Giemsa stain were prepared from tail blood of each rat and examined under the microscope at x 40. Parasitaemia was obtained by counting the number of parasites per field.

2.4 PCV and WBC Determination

These were carried out as described by [10]. PCV and WBC were determined before the rats were infected with *T.b .brucei* on the first day. These values were examined after every two days. The blood was collected from the retro-orbital plexus of the eye. PCV was determined using the microhaematocrit centrifuge spun for 5 minutes at 1000 rpm before reading with a hawkey haematocrit counter. WBC was determined using the improved Neubauer counting chamber at 1:200 dilution of EDTA anticoagulant blood in Turks solution.

2.5 Statistical Analysis

The one way ANOVA test was used to analyse and compare supplemented groups with the control. Values of $P<0.05$ were considered significant.

3. RESULTS

3.1 Parasitaemia

The results of this study showed that all the infected rats developed parasitaemia that was detected by wet blood film examination four days post infection (Fig. 1). However the differences in the parasitaemia of the supplemented rats was significantly (P=.05) lower than that of the control (group 1). After an initial increase there was a drop in parasitaemia followed by a gradual increase in all treated groups. The unsupplemented control rats showed a sharp rise in parasitaemia till they all died eleven days post infection. Animals supplemented with vitamin E (0.3 mg) alone or with selenium (80 mg) alone died at an intermediate time (15-16 days post infection). Supplementation prolonged the survival of animals in group 4. Animals supplemented with both 0.3 mg vitamin E and 80 mg of selenium all died at a later time (17 days post infection). Animals in group 5 (treated with the highest levels of both vitamin E (0.5 mg) and selenium (100 mg)) all died by day 12. This indicates that supplementation with the higher levels of vitamin E and selenium is not very effective and actually reduces any beneficial effects.

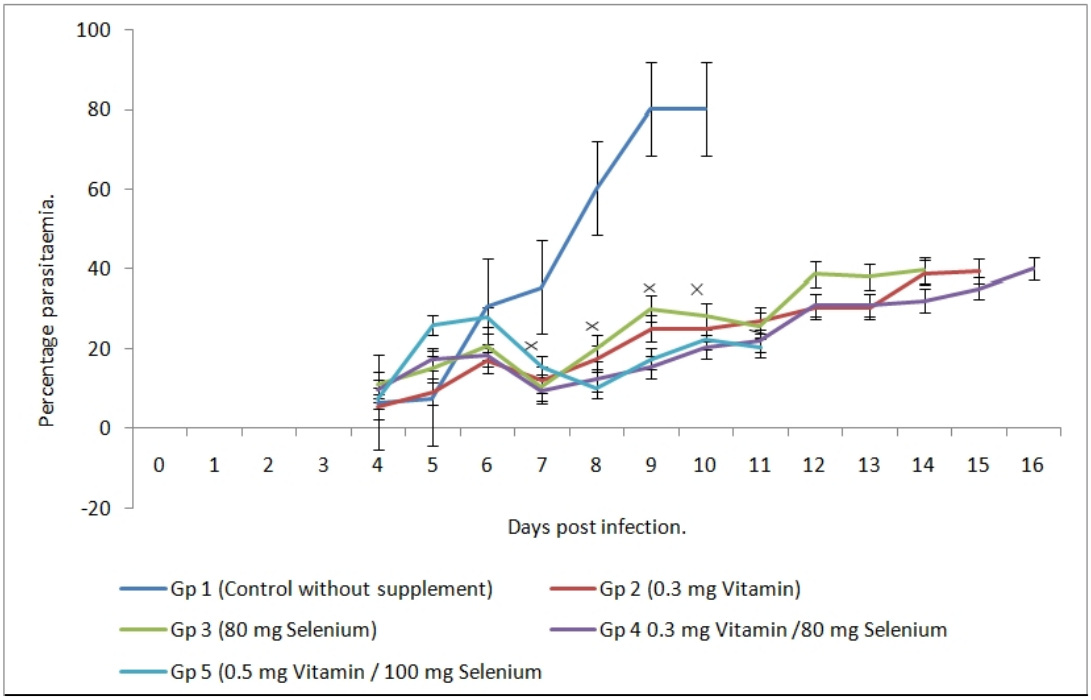

Fig. 1. Percentage parasitaemia of rats infected with *Trypanosoma brucei* and fed with diet containing varying levels of vitamin E and Selenium. X P=.05 compared with control. Mean showing±standard error.

Gp=Group, mg= milligram.

3.2 PCV and WBC

The rats fed diet containing vitamin E and selenium had significantly (P=.05) higher PCV compared with that of the control animals (Fig. 2). The PCV of the control group declined from 28.05% on day zero pre infection to 18.00% on day eight post infection. The PCV values of rats in groups given vitamin E and selenium rose on day three post infection and thereafter fell down in groups two, three and five while a steady increase was observed in group four post infection.

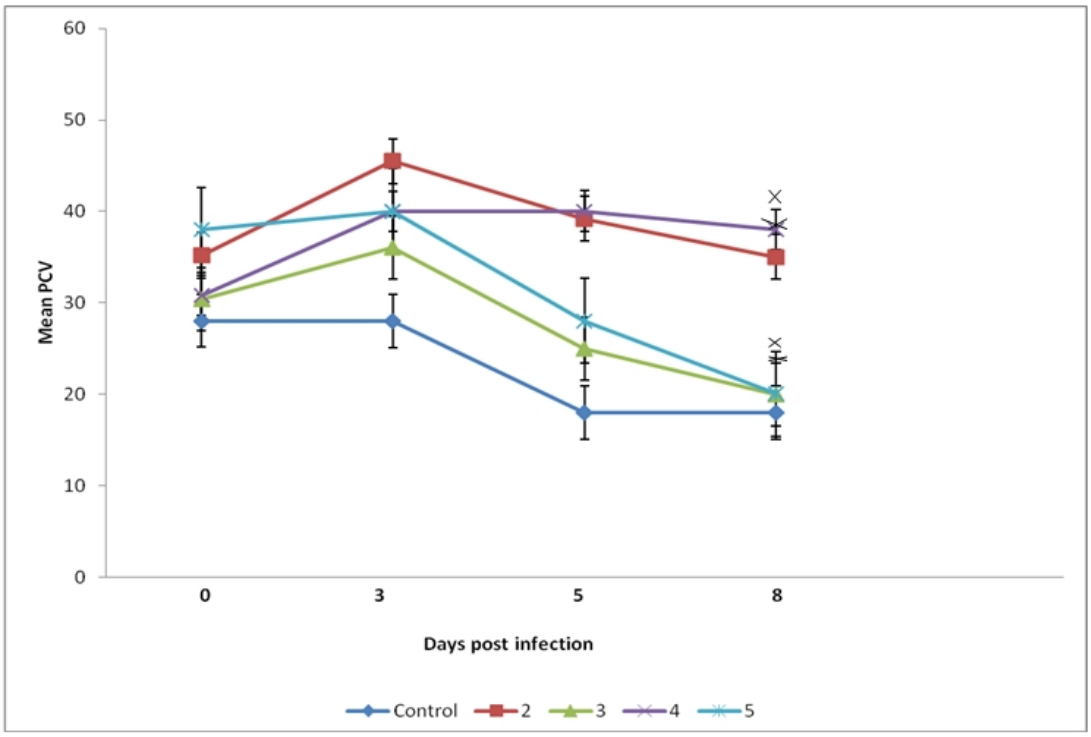

Fig. 2. Pack cell volume (PCV) rats infected with *Trypanosoma brucei* and fed with diet containing varying levels of vitamin E and Selenium. X P=.05 compared with control group.

Mean showing ± standard error.

The WBC in the control rats fell from 7.63 on day zero to 7.23 x 10^3/mm^3on day three post-infection and subsequently rose to 9.25 x 10^3/mm^3 on day eight, (Fig. 3). There was a significant (p=.05) increase in WBC of rats from groups three to five (10.50 x 10^3/mm^3, 10.60 x 10^3/mm^3 and 9.23 x 10^3/mm^3 respectively) on day three post infection while a decrease was observed in group two compared with the control. The WBC decreased in groups three and four on day eight while that of group two and five were relatively similar to the control. However these changes in WBC of the supplemented rats were not significant (P=.05) compared with that of the control.

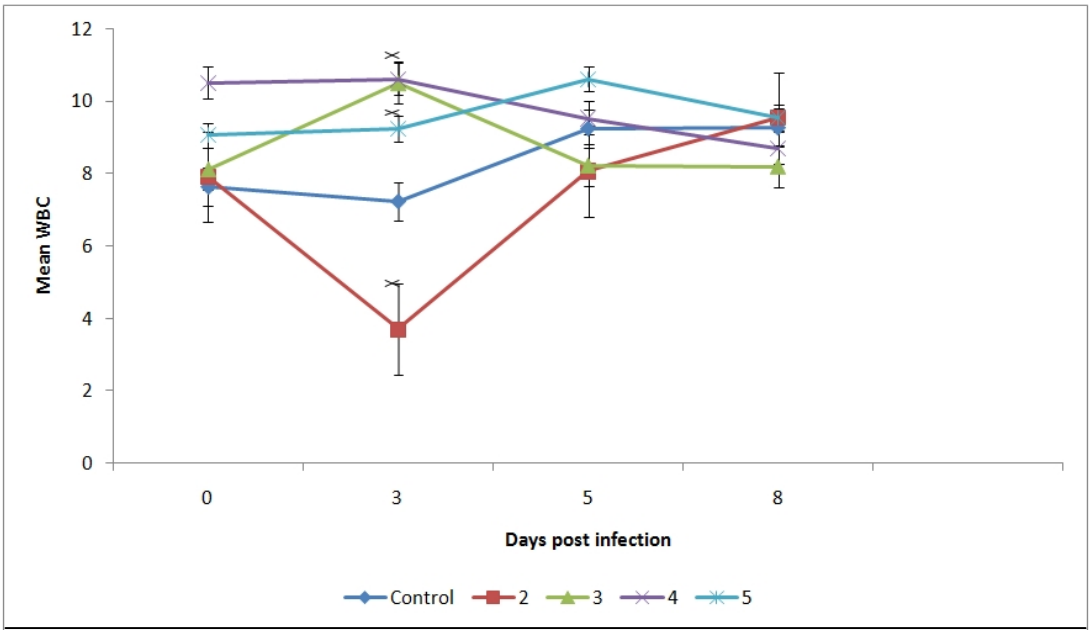

Fig. 3. White blood cell (WBC) count rats infected with *Trypanosoma brucei* and fed with diet containing varying levels of vitamin E and Selenium. X P=.05 compared with control group.

Mean showing ± standard error.

4. DISCUSSION

All the rats developed parasitaemia by the fourth day post infection which progressed to a high peak culminating in the death of all the animals by day seventeen. The supplemented groups had less of an increase in parasitaemia, a subsequent drop and then gradual increase compared with the control group fed with chow that contained vitamins that their composition was not indicated by the manufacturers and was not determined during the feeding since the study was concerned with investigating the effect of supplementation of the fed given to animals. Similarly, the feed was given to six animals together per cage since the mean values of each animal were considered in each group. The death of the animals may be attributed to the enormous increase in the number of circulating trypanosomes and their by–products causing haemolysis of the red blood cells. The significant (P=.05) decrease in the level of parasitaemia supported by prolonged survival in vitamin E and /or selenium supplemented rats indicate that these micronutrients influence the immune response of *T. brucei* infected rats compared with the non supplemented rats. Vitamin E and selenium supplementation kept the parasitaemia lower than those of the non supplemented group. This could be attributed to their enhanced antioxidant action following infection with *T. brucei.* The result of this study is similar to the findings of [11] who reported that oral supplementation with manganese chloride in rats appeared to delay the onset of parasitaemia and reduce parasitaemia, anaemia and organ damage in *T. congolense* infections. The rat groups two and three which received vitamin E and selenium supplemented food respectively showed slightly higher parasitaemia than group four and five which received different levels of both vitamin E and selenium. This shows that supplementation of food with either vitamin E or selenium alone probably lowered the

resistance of rats to *T. brucei brucei* infections. This finding is supported by [12] who in a study on diet induced deficiencies in selenium or vitamin E in *Heligmosomoides polygyrus* infected mice, reported that single deficiencies in selenium or vitamin E resulted in impaired ability to clear infection while mice doubly deficient in selenium and vitamin E had a significantly impaired clearance of a secondary infection.

The nutritional status determines the susceptibility and inhibition to trypanosomes infections. Rats given moderate levels (Group four) survived longer than rats with low levels (Groups one two). However, rats with higher levels (Group five) did not last longer compared with group two to four. This might be due to the fact that higher amounts of vitamin E and selenium may be beneficial to the parasite- resulting in the higher peak of parasitemia compared to group 4 and increased mortality. Investigating the effect of elevated levels of selenium on the course of *T. cruzi* in mice, [13] reported that mice that received 4 ppm and 8 ppm sodium selenate in drinking water, exhibited 60% survival while those without selenate exhibited zero percent survival. The effect of starvation in *T. brucei* infected guinea pigs is also evident from the varying degree of tolerance to trypanosomes. [14] showed that parasitaemia rose more sharply among starved animals than underfed and normally fed. [15] reported that selenium supplementation does not lead to general protection during the course of *T. cruzi* infection in mice ,but may help protect the heart from inflammatory damage. The effects of supplementation with modest levels may reflect a balance between benefits to the host immune system and any benefits to the parasite since excess vitamin E may provide antioxidant effects to the parasite [3].

The decreased PCV of all the *T. brucei* infected rats except group four which had an increase, might be attributed to the haemolytic activities of parasites leading to the destruction of red blood cells. The observed significant increase in PCV is at variance with [16], who reported a decrease in the PCV of *T. congolense* infected rats fed with food vitamin E and selenium compared to the supplemented rats. [9] also reported that selenium supplementation did not significantly improve PCV and RBC values. [11] showed that no significant variations existed in the PCV of the manganese chloride supplemented and unsupplemented *T. congolense* infected rats both within and between the groups during the period of the experiments .The increase in the PCV might be due to the boosting effects of moderate levels of vitamin E and selenium on the immune system which enhance the process of the production of red blood cells in the body while decline in the PCV with increased vitamin E and selenium could probably be due to inhibitory constituents of the fed which was not determined in this case.

The slight increase in the WBC of rats fed with vitamin E and/or selenium during the early days (day zero to five) except group two compared with the control probably indicated that vitamin E and selenium supplementation enhances the immune response of *T. brucei brucei* infected rats. However, there was a slight increase in the WBC of all the rats on day eight except group four which had a slight decrease compared with the values on day zero. This general increase may probably be due to immune response to the presence of *T. brucei* in the rats, while the low WBC on day eight compared with the control could probably be due to the effect of supplements which arrested the development of the parasites. This finding agrees with [17] who reported high mean values of total WBC count, mononucleated cells, and polymorphonucleated cells in rats fed with balanced diet with moderate protein level. According to [16], the increase in mean weekly of total WBC, of rats given diet with selenium and vitamin E than the non supplemented rats indicated that selenium and vitamin E had impact on the immune system of the rats.

5. CONCLUSION

The study showed that vitamin E and selenium reduced the parasitaemia, increased the PCV and enhances the immune system which is evidenced by the increase in the WBC compared with the non supplemented control rats.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

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COMPETING INTERESTS

Authors do not have any competing interests.

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