

Full Length Research Paper

Immunomodulatory effect of dietary selenium supplementation on serum antibody response and leucocytic profile of *Trypanosoma brucei brucei* infected rats

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Effect of selenium supplementation on the immune response of *Trypanosoma brucei brucei* infected rats was investigated. Twenty five (25) adult male albino rats divided into five groups of five rats each were used for this study. Groups A, B and C were fed 4, 8 and 16 part per million (ppm) selenium in their feed, respectively. Groups D and E were not given selenium supplementation. The supplementation started on day 0, followed by the infection of groups A, B, C and D with *T. brucei brucei* on day 14 post supplementation (PS). Immune response of the rats was assessed by determining the antibody response to sheep red blood cells (SRBC) using direct haemagglutination technique and total and differential leucocyte counts. The supplementation led to significant ($p < 0.05$) increase in antibody response to sheep red blood cell of the supplemented groups at pre- and post infection when compared with the control. The infection however, led to decrease in antibody titre but remained higher than the pre-supplementation titre. Also, the supplementation led to increase ($p < 0.05$) in leucocyte counts prior to infection on day 14 PS. The increase in total leucocyte count could be attributed to increase in lymphocyte and neutrophils. The mortality record showed that all rats (100%) in the infected, not supplemented group and 2 rats (40%) died from the 16 ppm group by day 42 PS. No rat died in 4, 8 ppm and not infected, not treated groups.

Key words: Selenium, antibody titre, leucocytes, trypanosomes, immunosuppression.

INTRODUCTION

Trypanosome infection of livestock and humans has been shown to result in: a reduced capacity to mount a primary humoral response to non trypanosome antigens; an inappropriate antibody response to trypanosome antigens; depressed T-cell proliferation to mitogen and trypanosome antigen; reduced cytokine production and inferior response to vaccination (Mansfield, 1995; Mackenzie et al., 1975; Taylor and Authié, 2004). Consequently, the infected host is rendered more susceptible to opportunistic or secondary infections (Onah and Wakelin, 2000; Ihedioha et al., 2003). This

immunosuppressive effect in trypanosome infection was first reported by Godwin (1970) and Godwin et al. (1972) and is thought to be responsible for the hosts' inability to clear trypanosomes after administration of trypanocidal drugs (Osma et al., 1992). Also, trypanosome-induced immunosuppression is considered one of the major mechanisms of evasion from the host's immune defences by the parasite (Uzonna et al., 1998). The ability of the host to produce or not produce sound immune responses are thought to determine the relative resistance and susceptibility of the host and the outcome of infection. The underlying cellular mechanism(s) responsible for immunosuppression could be due to trypanosome-derived B lymphocyte mitogen responsible for the polyclonal B lymphocyte responses occurring

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during the disease and possibly, for the ultimate suppression of B lymphocyte responses (Gómez-Rodríguez et al., 2009).

The immunosuppressive effect of trypanosome infection has made it necessary to investigate the effect of immunostimulants in overcoming trypanosome-induced immunosuppression. Restoration of normal immune functions may increase resistance to infectious diseases and reduce the severity of disease. Trials with immunostimulants resulted in a significant reduction in early anaemia, a reduction in the first parasitemia peak, the absence of acute splenomegaly and finally, a delayed mortality (Murray and Morrison, 1979; Whitlaw et al., 1983). Also, reduced weight loss, liver damage, acidosis and anaemia during infections by *Trypanosoma brucei brucei* were reported. For example, selenium, a trace mineral, is essential for good health but required only in small amounts (Goldhaber, 2003; Thomson, 2004).

Enhanced immunity had been reported for dietary supplementation of selenium in animals (Walter and Jensen, 1963; Sidhu et al., 1993). Selenium influences both the innate “nonadaptive” and the acquired “adaptive” immune system (Kiremidjian-Shumacher and Roy, 1998; McKenzie et al., 2001; Bhaskaran, 2002; Beckett et al., 2003). It appears to affect non-specific immune indices, humoral immunity, cellular immunity and cytotoxicity. Spallholz et al. (1973) demonstrated that high dietary selenium enhanced serum immunoglobulin G (IgG) and immunoglobulin M (IgM) antibody titres in mice challenged with sheep red blood cells. Also, selenium supplementation increased survival time of mice infected with *Candida albicans* (Boyne and Arthur, 1986), in mice infected with *Trypanosoma cruzi* (Davies et al., 1991) and when combined with vitamin E increased survival interval of *Trypanosoma congolense* infected rats (Mgbenka and Ufele, 2004). In summary, selenium supplementation leads to increases in antibody response, T-cell proliferation and killing by macrophages, lymphocyte proliferation, expression of interleukin (IL)-2 receptor, and IL-2 production and also to an augmented cellular immune response to live vaccines (Roy 1994; McKenzie et al., 1998; Levander, 2000; Pagmantidis et al., 2008).

Immune response to sheep red blood cells has been widely used in the study of animals' immune status (Ikeme and Adelaja, 1990; Ihedioha et al., 2003). The use of sheep red blood cells (SRBC) challenge is based on the fact that, responses to it is T-dependent and T-cells (T-helper cells specificity) coordinate the battle against infection by activating macrophages, B-cells and other T-cells and thus, indirectly control immunoglobulin production (ILRAD, 1992; Sherman and Halliquet, 1990). The method has been used successfully to investigate trypanosomal immunosuppression by many researchers (Godwin et al., 1972; Hudson et al., 1976; Baltz et al., 1981; Ekejindu et al., 1985; Ikeme and Adelaja, 1990; Ihedioha et al., 2003).

This study was an attempt to investigate the immuno-

modulatory effect of selenium supplementation on leucocytic profile and antibody responses of *T. brucei brucei* infected rats.

MATERIALS AND METHODS

Experimental animals

25 adult male outbreed albino rats weighing between 278 to 302 g were used for this study. The rats were acquired from the Laboratory Animal unit of the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka. The rats were housed in a fly proofed house and given feed and water *ad libitum*. A period of 10 days was allowed for acclimatization of the rats.

Trypanosome

T. brucei brucei (Federe strain) used for this study was obtained from the National Institute for Trypanosomiasis Research (NITR), Vom Nigeria. The strain was isolated from N'dama cattle from Federe village in Plateau State, Nigeria and has been maintained in liquid nitrogen at the NITR Vom. The strain was passage in rats from where the experimental animals were infected.

Selenium

Selenium as sodium selenite was manufactured by Biorganics Nigeria Limited, Ikeja-Lagos, Nigeria.

Sheep red blood cells (SRBC)

Fresh sheep blood was obtained from sheep in the Animal house of Department of Veterinary Parasitology and Entomology, University of Nigeria through their jugular vein. Before use, the red blood cells were washed three times with about 1 part of blood to 9 parts of phosphate-buffered saline (PBS), pH 7.2, by centrifugation at 3000 rpm for 10 min on each occasion. After the final wash, the SRBCs were suspended in PBS as a 2% suspension (based on packed cell volume) for the serological tests and as a 10% suspension for immunization of the rats. A 1 ml amount of the 2% suspension contained approximately 5×10^8 red blood cells.

Experimental design

The rats were divided into five (5) groups (A, B, C, D and E) of 5 rats each and each group received treatment as follows; groups A, B and C received 4, 8 and 16 part per million (ppm) selenium in their feed from day 0 till termination of the experiment. The selenium content of the feed was assayed for and made up to 4, 8 and 16 ppm of the feed. On day 14 of post supplementation (PS), groups A, B, C and D rats were infected with 0.5 ml of saline diluted trypanosome infected rat blood containing 1×10^6 trypanosomes intraperitoneally. All the rat groups A, B, C, D and E were given 0.3 ml of sheep red blood cells (SRBC) per rat 7 days before supplementation with repeats every other 14th day. The immune responses of the rats were assessed using serum antibody response to sheep red blood cells (SRBC), total leucocyte count and differential leucocyte count.

Immunization of rats with SRBCs

Immunization was achieved by an initial intraperitoneal injection of

Table 1. Mean antibody titre of rat groups supplemented with different levels of selenium in their feed and controls.

Experimental day	4 ppm	8 ppm	16 ppm	Infected not supplemented	Not infected, not supplemented
0	25.60 (10.6)	30.40 (9.60)	25.6 (3.92)	28.8 (10.3)	22.4 (3.92)
14	32.00 ^a (8.76)	38.40 ^a (10.9)	57.60 ^a (18.7)	22.40 ^b (3.92)	20.80 ^b (4.80)
28	102.4 ^a (15.7)	153.6 ^a (43.4)	166.4 ^a (38.4)	32.00 ^b (8.76)	36.80 ^b (8.76)
42	89.90 ^a (15.6)	102.4 ^a (15.7)	128.0 ^a (35.1)	-	22.4 ^b (3.92)

Day 0 was 7 days after the first immunization and the day supplementation started, day 14 was day of infection. a and b, Means within same row with different superscripts are significantly different ($p < 0.05$).

0.3 ml of a 10% sheep red blood cells suspension in normal saline. This was followed by booster doses every other 14th day during the experimental period.

Collection of blood sample from rats

About 0.5 ml of blood was collected from the retro bulbar plexus of the medial canthus of rats. 0.2 ml of the blood was put into anticoagulant bottle for leucocyte count, while the remainder was put in Eppendorf tube, allowed to clot and later centrifuged at 15,000 revolutions per minute (rpm) for 10 min to separate the serum for determination of antibody response to SRBC and enzyme.

Determination of total and differential leucocyte counts

The total leucocyte count was determined using the procedures described by Schalm et al. (1975) whereas the differential leucocyte count was determined by putting a drop of blood on a clean slide and thin smear made. The smear was air dried and stained with Leishman stain. The slides were examined under oil stained immersion objective light microscope. 100 cells were counted by the longitudinal counting method. The number of each cell type (neutrophil, lymphocyte, monocyte, eosinophil and basophil) counted among the 100 cells represent the percentage of each cell type. The percentage values were changed to absolute value by dividing the percentage value by 100 and multiplying by the total leucocyte count.

Determination of antibody response to sheep red blood cells (SRBC)

The antibody response to SRBC antigen was assayed for by direct haemagglutination technique (Ikeme and Adelaja, 1990). The test serum was initially diluted (1:8) to exclude non-specific isoagglutination of SRBC by normal rat serum. The microtitre plates were labelled according to the rat group names (A, B, C, D and E). 25 microtitre of normal saline was pipetted into all the wells of each row except the first and penultimate wells. A 25 ml volume of the diluted test serum was pipetted into the first well. Another 25 ml of the test serum was pipetted into the second well this was used for doubling dilutions. The last well in each row contained 25 ml of normal rat serum control. To each well was added 25 ml of a 2% SRBC suspension. The content of the wells were mixed for one minute by gentle rocking of the plate. This was incubated at room temperature (27 to 30°C) for three hours after which the resulting agglutination titres were read and expressed as geometric mean titre.

Statistical analysis

The data obtained from this study were summarized as means \pm standard error of means. Statistical comparisons between the treatment groups were made by one way analysis of variance (ANOVA). Means were considered significant at $P < 0.05$ and the means separated using Duncan's multiple range test (DMRT).

RESULTS

The pre-infection supplementation with selenium prolonged the pre-patent interval from 4.30 ± 0.12 days in group D to 5.23 ± 0.18 days in group B, though, the difference was not statistically significant ($p > 0.05$) when compared with the control. The supplementation led to significant increase in antibody titre of supplemented groups when compared with the infected not supplemented and not infected not supplemented groups (Table 1). The 8 ppm group had significantly higher ($p < 0.05$) antibody response on day 28 post supplementation when compared with other supplemented groups. The decrease in antibody titre of the supplemented groups by day 42 PS was higher than the pre-infection titre.

The pre-infection supplementation with selenium led to significant increase in total leucocyte count by day 14 PS (Figure 1). Following infection on day 14 PS, the total leucocyte count decreased slightly but remained higher than the pre-supplementation level. The leucocyte count of the 8 and 16 ppm groups were significantly ($p \leq 0.05$) higher than the controls on days 21 and 35 PS.

The differential leucocyte counts are shown in Figure 2a, b, c, d and e. The lymphocyte count (Figure 2) increased significantly ($p \leq 0.05$) in all the supplemented groups from day 28 PS and remained high till the termination of the experiment on day 42 PS. Also, the supplementation increased neutrophil counts significantly ($p \leq 0.05$) in groups supplemented with 8 and 16 ppm when compared with other groups. The basophils, eosinophils and monocyte counts did not show any consistent pattern in their changes. However, the supplemented groups maintained higher counts than the infected not supplemented and not infected not supplemented groups.

The mortality record showed that all rats (100%) with mean survival interval of 28.33 ± 5.13 days in the infected,

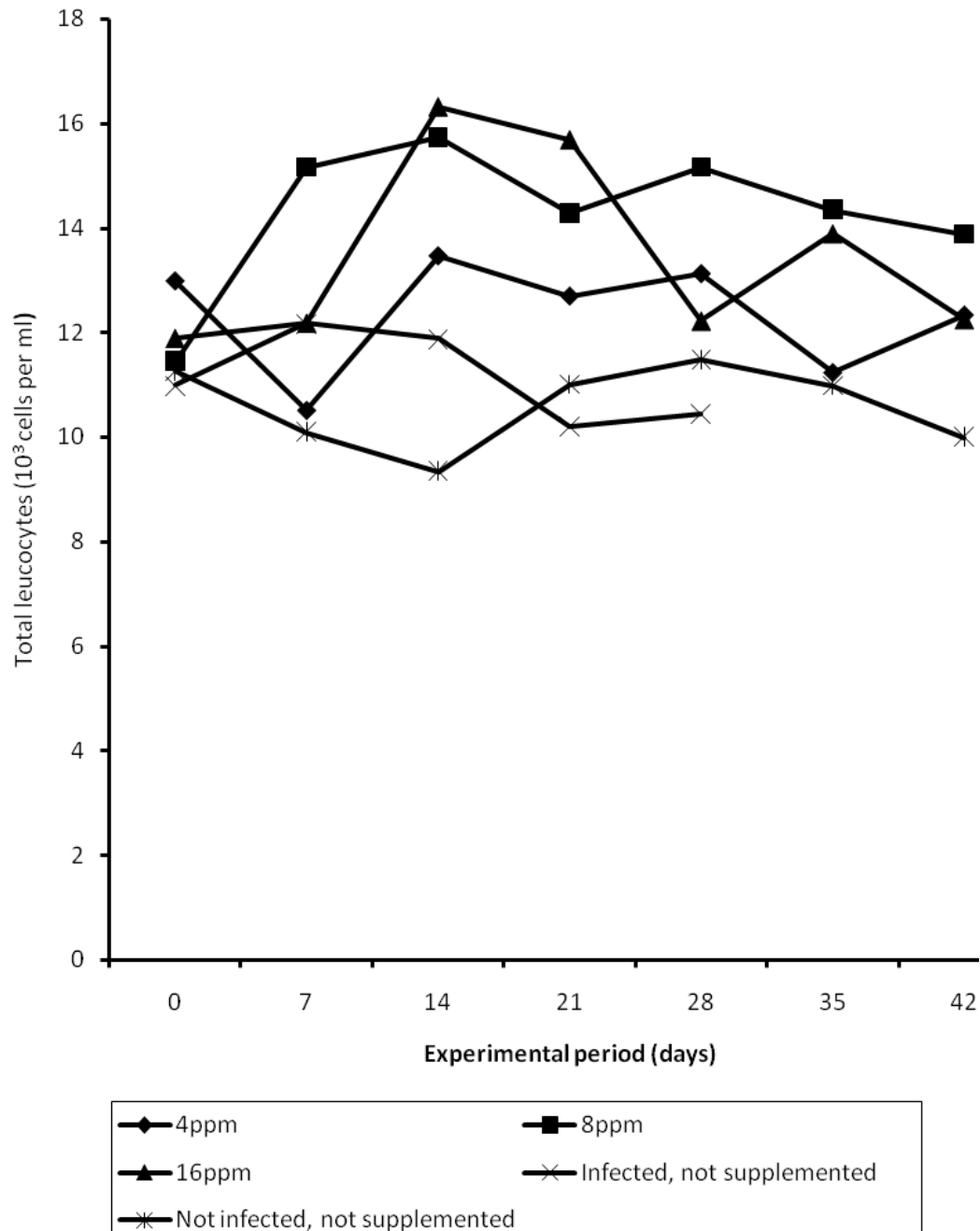


Figure 1. Mean total leucocyte count (10^3) of *T. brucei* infected rats supplemented with varying doses of selenium.

not supplemented group died before day 42 PS, whereas 2 rats (40%) died with mean survival interval of 39.00 ± 4.69 days from the 16 ppm group by day 42 PS. No rat died in 4, 8 ppm and not infected, not treated groups (Figure 3).

DISCUSSION

Immunosuppression has been described in both natural

and experimental infections of humans and domestic mammals as well as laboratory rodents with trypanosomes (De Baetselier, 1996; Taylor, 1998) and is suggested to be responsible for the poor responsiveness of trypanosome-bearing hosts to vaccinations (Rurangirwa et al., 1978) as well as renders the infected host more susceptible to secondary infections (Holmes, 1980). It appears to be a nearly universal feature of infection with African trypanosomes and thus, may represent an essential element of the host-parasite rela-

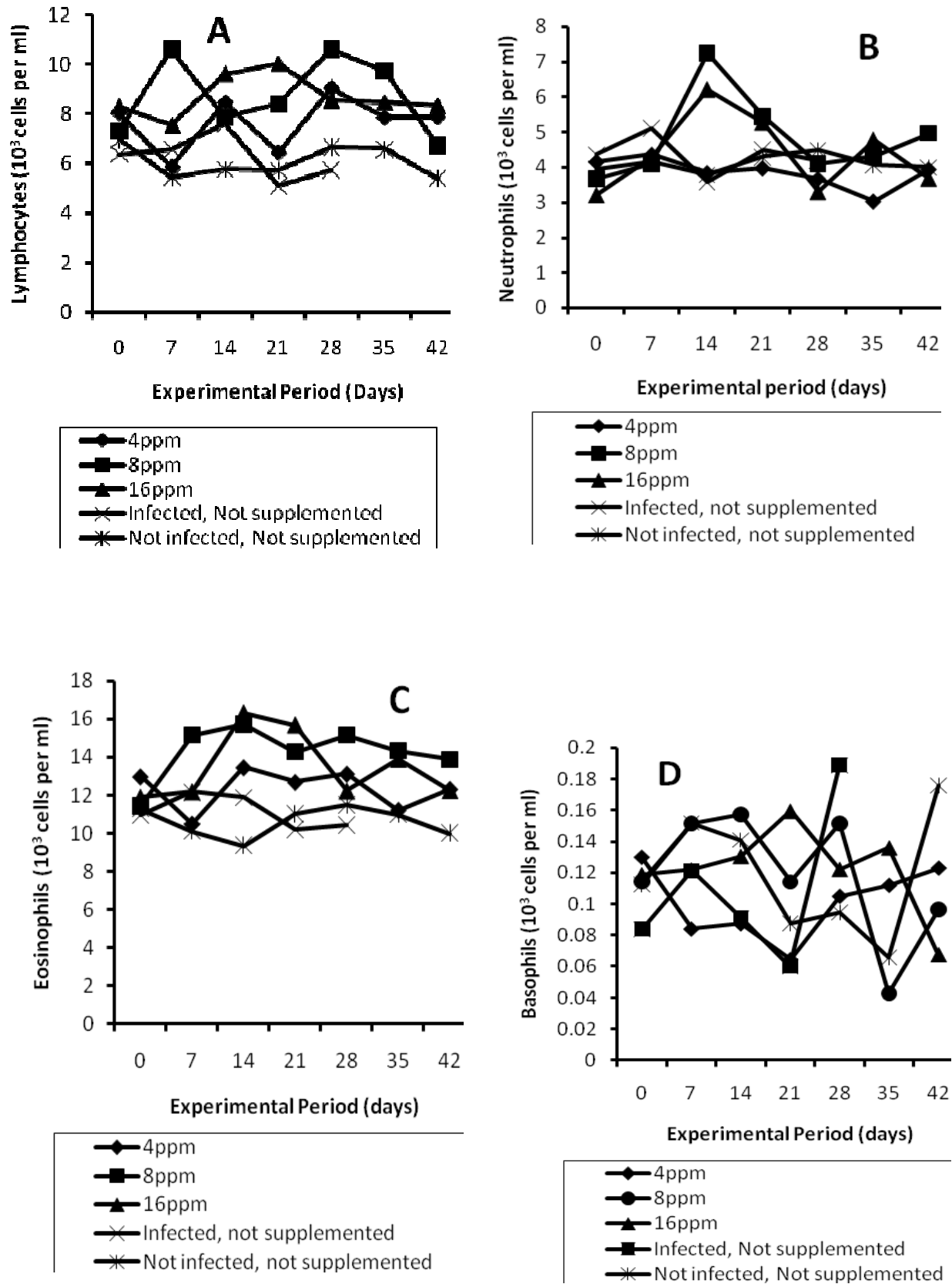


Figure 2. Showing the mean lymphocyte (A), neutrophils (B), eosinophils (C) and basophils (D) of *T. brucei* infected rats receiving levels of dietary selenium supplementation.

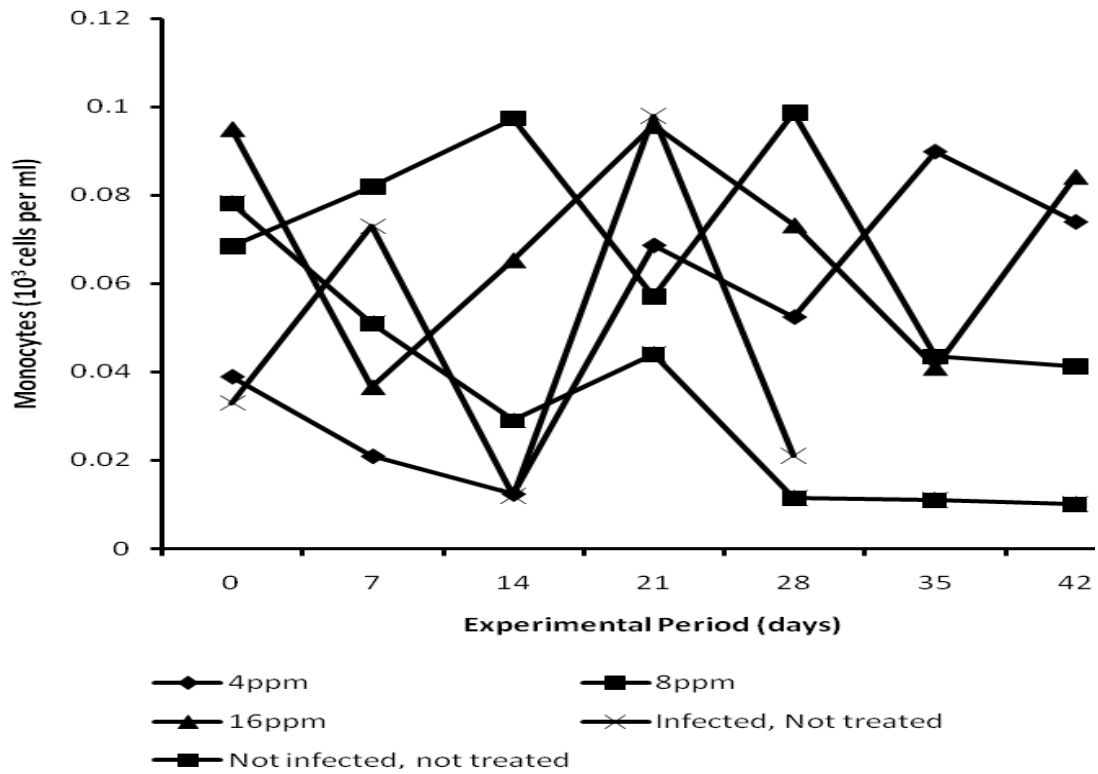


Figure 2e. Mean monocyte count of rat groups receiving different levels of selenium in their feed.

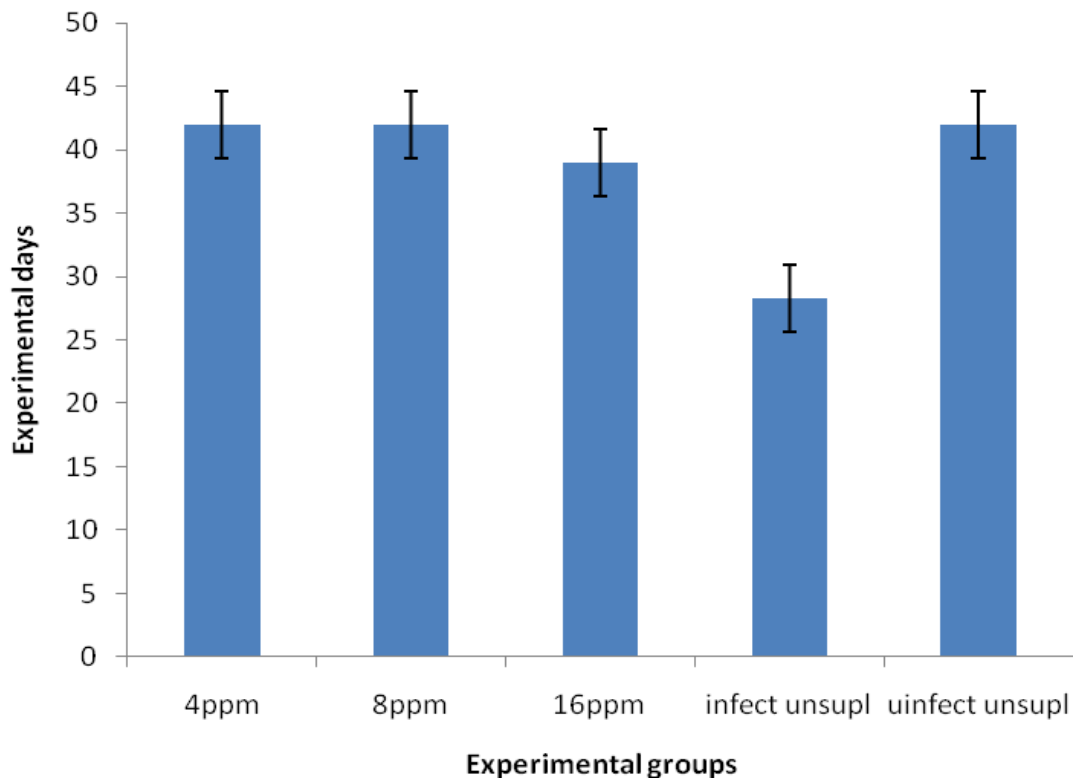


Figure 3. Survival intervals of rat groups supplemented with different levels of selenium.

tionship, possibly by reducing the host's ability to mount a protective immune response. Antibody, T cell and macrophage/monocyte responses of infected hosts are depressed (Taylor and Mertens, 1999). Lymphocytes from infected hosts give a lower response to T cell mitogens or allogeneic cells *in vitro* (Sileghem et al., 1994). Low levels of T-cell independent, antitrypanosomal antibodies are adequate to clear the bloodstream of parasites during DFMO therapy (Bitonti et al., 1986).

From this study, pre-infection supplementation with selenium prolonged the pre-patent period of the supplemented groups. This may be attributed to the immunomodulatory and antioxidant activities of selenium (Bhaskaran, 2002; Beckett et al., 2003; Arthur et al., 2003) which are necessary for the optimum function of both cellular and humoral immune processes. Earlier researchers demonstrated that, dietary supplementation with selenium and vitamin E enhanced immune response in white rats (Mgbenka and Ufele, 2004), enhanced the efficacy of diaminazine aceturate and isometamidium chloride in chemotherapy of murine trypanosomiasis (Eze et al., 2009), reduced parasitaemia and prolonged survival interval in *T. cruzi* infected mice (Davis et al., 1998). Insufficient intake of selenium is thought to contribute to the pathogenesis of various degenerative and inflammatory diseases, aging and cancer (Lee et al., 1996).

The increase in antibody titres of selenium supplemented groups is in agreement with works done by Larsen et al. (1988), Turner and Finch (1991) and Arshad et al. (2005). It has also been reported that feeding of animals with selenium deficient feed lowers the antibody response to antigen (Mulhern et al., 1985). Studies with small animals supplemented with 0.7 or 2.8 ppm selenium resulted in a 7 or 30-fold increase in antibody titers (sheep red blood cell antigen), respectively, over the non-supplemented group (Spallholz et al., 1975). The trypanosomes infection on day 14 PS did not cause decrease in antibody titres on day 28 PS, but by day 42 PS, there was a decrease which is an indication that immunosuppression is a manifestation of chronic infection with trypanosomes. The decrease in antibody titre was however, higher than the pre-supplementation values. According to Hughes and Kelly (2006), prevention of infection relies predominantly on barrier function and innate immunity, whereas clearance of an established infection requires either a successful humoral response (for example, trypanosomiasis) or a successful cell-mediated immune response (for example, schistosomiasis). Also, specific antibodies directed against the trypanosome VSG mediate the destruction and clearance of parasites in successive parasitemic waves and hence, contribute to antibody-mediated trypanotolerance.

The pre-infection supplementation with selenium led to increase in total leucocyte count which remained high

following infection with trypanosomes on day 14 PS. The increase in total leucocyte may be attributed to proliferation of neutrophils and eosinophils. Increase in total leucocyte count is an indication of improved immune response. In humans, selenium supplementation has been reported to lead to increases in lymphocyte proliferation, augmented cellular immune response to live attenuated polio vaccine virus and a greater clearance of the virus (Roy et al., 1994; Broome et al., 2004; Pagmantidis et al., 2008). The increase in leucocytes may be attributed to the fact that, antioxidant enzymes like selenium-containing glutathione peroxidase and thioredoxin reductase protect neutrophils, macrophages and other tissues from the free radicals damage and could lower the level of immunosuppressing lipid peroxides, alter arachidonic acid metabolism (Brambilla et al., 2008). Free radicals are mediators of cellular injury and are involved in the onset of cellular damage. Neutrophils with reduced glutathione peroxidase due to selenium deficiency are less able to defend themselves against the free radicals they release onto pathogens.

The reports on the effect of selenium supplementation on total and differential leucocyte are not consistent. An experiment with aged mice showed that, sodium selenite supplementation above the normal levels of selenium could restore lymphocyte proliferation due to antigens to the levels of young adult mice. The different and contrasting effects that Se supplementation had on white blood cell and platelet selenoenzyme activities and the fact that, these were seen in only 60±70% of subjects may be indicative of a difference in metabolic need regulated at the level of Se dependent cell function (Brown and Arthur, 2001). The results indicated that the immunoenhancing effects of selenium in humans require supplementation above the replete levels produced by normal dietary intake (Kiremidjian-Schumacher et al., 1994). It is also evident from clinical studies that, increasing Se intake, decreases infection rate and susceptibility to viral mutation, which might increase the virulence of the pathogen.

The death of rats in the infected unsupplemented group is consistent with other reports that trypanosomiasis is pathogenic to rats (Ihedioha et al., 2009; Eze et al., 2009). Also, 2 rats died in the group that received 16 ppm selenium in their feed. The death in the later may be due to the toxicity of selenium at that level. However, Davis et al. (1998) did not record any death in mice that received 16 ppm selenium in drinking water. The 16 ppm group recorded the highest antibody response which may be an indication that there is more to trypanosome pathology than immunosuppression. Increasing selenium above normal levels in young adult mice increased the bacteria-killing activity of spleen lymphocytes by 22.3% (Roy et al., 1990). Also, immune reactions in trypanosomiasis do not always lead to protection and are also involved in immunopathology disorder (Vincendeau and Bouteille, 2006). Although, antibody has been shown to be respon-

sible for clearing the African trypanosomes from the blood of infected animals, recent evidence suggests that the survival time of infected mice does not necessarily correlate with the ability of the animal to produce trypanosome-specific antibody. In other words, resistance as measured by survival time may not solely involve the specific humoral immune system. Selenium supplementation can quickly augment and/or restore effective immune functions, due to its stimulatory effect on white blood cells and activity of the thymus gland, thus enhancing the body's resistance to infection (Broome et al., 2004).

In conclusion, selenium supplementation at 4 and 8 ppm improved the immune status of the supplemented rats and at higher dose of 16 ppm produced highest antibody titre and leucocytic proliferation, but led to death in 2 rats at the termination of the experiment. Thus, higher dose of selenium supplementation seem to provoke higher immune response but are toxic to animals.

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