

## EFFECT OF VITAMIN C TREATMENT ON SERUM PROTEIN, ALBUMIN, BETA-GLOBULIN PROFILES AND BODY WEIGHT OF *Trypanosoma brucei*-INFECTED *Rattus norvegicus*

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### ABSTRACT

*The effect of vitamin C supplements on serum protein profile and body weight of Trypanosoma brucei-infected rats was investigated. The rats were inoculated with trypanosomes intraperitoneally and samples were collected on fourth, eighth, twelfth and sixteenth days of post infection (pi). Sixty (60) parasite free-albino rats were used, which were divided into four groups. Group A (control) was left uninfected with trypanosomes, group B and C were infected with Trypanosomes and treated with 40mls and 60mls of ascorbic acid (Vitamin C), respectively. Trypanosoma brucei infection caused significant ( $p < 0.01$ ) decreases in serum total proteins, albumin, beta globulin and body weight levels in untreated rats. Consumption of Vitamin C, however, prevented these disease-induced anomalies in the treated infected rats. Analyses of the sera using Bradford method and cellulose acetate electrophoresis showed that Vitamin C infected the state of serum protein, albumin and gamma globulin in the trypanosome-infected treated rats. It was concluded that consumption of the Vitamin C ameliorated the pathological changes in serum protein and body weight of T. brucei – infected rats.*

**Keywords:** *Trypanosoma brucei*, *Rattus norvegicus*, Ascorbic acid, Body weight, Serum protein

### INTRODUCTION

Trypanosomiasis is still a major factor retarding the growth of livestock in Africa. It is one of the most important livestock diseases in sub-Saharan Africa (Morrison *et al.*, 1981). Economic loss due to this disease runs into hundreds even higher in other parts of Africa (Doko *et al.*, 1991). Trypanosome is known to attack red blood cells and vascular endothelium. It concentrates more in the peripheral circulation (Jackson, 1979). The first wave of parasitaemia is accompanied by depressed packed cell volume, neutropenia and thrombocytopenia (Schoral *et al.*, 1981).

Trypanosomiasis is characterized by tissue and organ degenerative changes. One factor implicated in the pathogenesis of the disease is oxidative stress (Vray *et al.*, 1991) imposed by trypanosome and macrophageal activities. Oxidative stress has been alleviated in experimental infections with various species of trypanosomes (Umar *et al.*, 1999a; 2000) by administration of exogenous antioxidants, such as ascorbic acid and/or Vitamin E to infected rats and rabbits (Umar *et al.*, 1999b; 2000; 2008). This vitamin therapy considerably reduced the degree and rate of degeneration of tissues and organs and in some instances

significantly reduced the parasitaemia and anaemia in the trypanosome-infected animals (Umar *et al.*, 1999b). This study was designed to evaluate to what extent this ascorbic acid can influence the state of serum protein profile and body weight in trypanosome-infected rats.

## MATERIALS AND METHODS

Sixty adult male albino rats (*Rattus norvegicus*) weighing approximately  $145 \pm 2.13\text{g}$ , were used for this experiment. The rats were marked for identification and held in stainless wire rats cages in clean experimental animal house. The cages were labeled A to D corresponding to four (4) groups, replicated thrice with each replicate having five (5) rats. Rats in cage A were not infected while rats in cages B, C and D were infected with *Trypanosoma brucei*. One rat was first inoculated with trypanosome of NITR type from Veterinary Medicine Faculty, University of Nigeria, Nsukka. It was isolated from other animals and after 14 days of inoculation, the blood of that rat was used to inoculate others. Each experimental rat was intraperitoneally infected with about  $10^6$  cells of *Trypanosoma brucei* in 0.5 ml of cold saline diluted blood from the donor rat, using a matching chart to determine the level of parasitaemia (Herbert and Lumsden, 1976). Infected animals were monitored for 7 days for the establishment of *Trypanosoma brucei*. Rats in cages A and D served as control groups. Diet 1 given to rats in cage A contained 1 kg of chick mash without Vitamin C (control). Diet 2 was given to rats in cage B and contained 1 kg of chick mash mixed with 40 ml of Vitamin C. Diet 3 was used to feed rats in cage C and had 1 kg of chick mash mixed with 60 ml of Vitamin C and Diet 4 was used to feed rats in cage D which contained 1 kg of chick mash without any Vitamin C. Each experimental set up was replicated three times. The rats had unlimited supply of clean water. Five (5) ml of the blood of the rats were collected at four days intervals for sixteen days experimental period to determine the total serum albumin and gamma globulin concentrations. The collected blood was allowed to clot for about 30 minutes at room temperature.

Then each sample was centrifuged at 3,000 rpm for 15 minutes and then serum was removed. The sera were used for total serum protein and serum fractions determination using Bradford method and cellulose acetate electrophoresis (Herbert and Lumsden, 1976). The absorbance of the solutions was read at 520 nm-wavelengths using spectrophotometer. As an index of the physical status of the animals, the weight of each experimental rat was monitored over the period of study. Initial weights and interval weights of all experimental rats were taken at day 0, 4, 8, 12 and 16. The data were analyzed by one-way ANOVA, significant differences of treatment means were established using F-LSD at  $p < 0.05$ .

## RESULTS AND DISCUSSION

The result obtained indicated that administration of Vitamin C positively influenced the serum profile and body weight of trypanosome infected rats. The lowest level of total serum protein of  $49.12 \pm 5.22\text{g/l}$ , albumin of  $17.77 \pm 3.52\text{g/l}$  and beta-globulin of  $3.46 \pm 0.82\text{g/l}$  was observed in infected untreated rats (cage D rats). Followed by  $50.79 \pm 4.01\text{g/l}$  total serum protein,  $20.76 \pm 3.52\text{g/l}$  albumin,  $5.16 \pm 0.54\text{g/l}$  beta-globulin observed in infected rats treated with 40 ml of Vitamin C per kg of chick mash (cage B rats). Infected rats treated with 60 ml of Vitamin C per kg of chick mash had  $52.71 \pm 4.02\text{g/l}$  albumin and  $5.38 \pm 0.63\text{g/l}$  beta-globulin (cage C rats). The highest serum biochemical level was seen in cage A rats which had  $60.99 \pm 0.48\text{g/l}$  total serum protein,  $32.24 \pm 0.43\text{g/l}$  albumin and  $8.26 \pm 0.23\text{g/l}$  beta-globulin (Table 1). The body weights were  $120 \pm 7.07$ ,  $129.75 \pm 7.05$ ,  $133.50 \pm 4.35$  and  $153.00 \pm 5.12$  grams corresponding to treatments D, B, C and A, respectively (Table 2).

Several scientific researches have been done on trying to identify and standardize active food supplement that would be active in treatment of trypanosomiasis. Trypanosomiasis is characterized by tissue and organ degenerative changes. One factor implicated in the pathogenesis of the disease is oxidative stress imposed by trypanosome and

**Table 1: Total serum protein, albumin and beta-globulin concentrations in *Trypanosoma brucei* - infected *Rattus norvegicus* administered vitamin C for 16 days**

| Duration of experiment (days) | (Cage A)                                       | (Cage B)                        | (Cage C)                        | (Cage D)                                     |
|-------------------------------|--|---------------------------------|---------------------------------|--|
|                               | Uninfected rats + 0 ml Vitamin C (-ve control) | Infected rats + 40 ml Vitamin C | Infected rats + 60 ml Vitamin C | Infected rats + 0 ml Vitamin C (+ve control) |
| <b>Serum protein (g/l)</b>    |  |                                 |                                 |  |
| 4                             | 60.60 ± 0.46 <sup>a</sup>                      | 59.60 ± 0.78 <sup>a</sup>       | 59.84 ± 0.43 <sup>a</sup>       | 58.80 ± 0.45 <sup>a</sup>                    |
| 8                             | 59.82 ± 0.46 <sup>a</sup>                      | 55.86 ± 0.24 <sup>a</sup>       | 56.24 ± 0.46 <sup>a</sup>       | 54.20 ± 0.50 <sup>a</sup>                    |
| 12                            | 61.68 ± 0.49 <sup>a</sup>                      | 50.88 ± 0.45 <sup>a</sup>       | 53.46 ± 0.84 <sup>a</sup>       | 48.82 ± 0.45 <sup>a</sup>                    |
| 16                            | 61.86 ± 0.48 <sup>a</sup>                      | 36.80 ± 0.53 <sup>a</sup>       | 41.31 ± 0.71 <sup>a</sup>       | 34.64 ± 0.46 <sup>a</sup>                    |
| <b>Group Mean</b>             | 60.99 ± 0.48 <sup>a</sup>                      | 50.79 ± 4.01 <sup>b</sup>       | 52.71 ± 4.02 <sup>c</sup>       | 49.12 ± 5.22 <sup>d</sup>                    |
| <b>Albumin (g/l)</b>          |  |                                 |                                 |  |
| 4                             | 32.70 ± 0.48 <sup>a</sup>                      | 30.02 ± 0.26 <sup>a</sup>       | 30.22 ± 0.46 <sup>a</sup>       | 28.00 ± 0.28 <sup>a</sup>                    |
| 8                             | 30.94 ± 1.06 <sup>a</sup>                      | 20.40 ± 0.01 <sup>a</sup>       | 23.90 ± 0.47 <sup>a</sup>       | 15.62 ± 0.01 <sup>a</sup>                    |
| 12                            | 32.68 ± 0.50 <sup>a</sup>                      | 19.74 ± 0.48 <sup>a</sup>       | 22.06 ± 0.46 <sup>a</sup>       | 15.70 ± 0.48 <sup>a</sup>                    |
| 16                            | 32.64 ± 0.01 <sup>a</sup>                      | 12.88 ± 0.23 <sup>a</sup>       | 17.08 ± 0.46 <sup>a</sup>       | 11.74 ± 0.01 <sup>a</sup>                    |
| <b>Group Mean</b>             | 32.24 ± 0.43 <sup>a</sup>                      | 20.76 ± 3.52 <sup>b</sup>       | 23.32 ± 2.70 <sup>c</sup>       | 17.77 ± 3.52 <sup>d</sup>                    |
| <b>Beta-globulin (g/l)</b>    |  |                                 |                                 |  |
| 4                             | 7.88 ± 0.01 <sup>a</sup>                       | 6.44 ± 0.01 <sup>a</sup>        | 6.92 ± 0.01 <sup>a</sup>        | 5.84 ± 0.00 <sup>a</sup>                     |
| 8                             | 8.66 ± 0.00 <sup>a</sup>                       | 5.20 ± 0.01 <sup>a</sup>        | 5.78 ± 0.01 <sup>a</sup>        | 3.20 ± 0.01 <sup>a</sup>                     |
| 12                            | 8.18 ± 0.01 <sup>a</sup>                       | 4.22 ± 0.01 <sup>a</sup>        | 4.88 ± 0.02 <sup>a</sup>        | 2.68 ± 0.02 <sup>a</sup>                     |
| 16                            | 8.33 ± 0.01 <sup>a</sup>                       | 4.78 ± 0.02 <sup>a</sup>        | 3.92 ± 0.00 <sup>a</sup>        | 2.11 ± 0.02 <sup>a</sup>                     |
| <b>Group Mean</b>             | 8.26 ± 0.23 <sup>a</sup>                       | 5.16 ± 0.54 <sup>a</sup>        | 5.38 ± 0.63 <sup>a</sup>        | 3.46 ± 0.82 <sup>a</sup>                     |

**Table 2: Body weight (g) of *Trypanosoma brucei* - infected *Rattus norvegicus* administered vitamin C for 16 days**

| Duration of experiment (days) | (Cage A)                                       | (Cage B)                        | (Cage C)                        | (Cage D)                                     |
|-------------------------------|--|---------------------------------|---------------------------------|--|
|                               | Uninfected rats + 0 ml Vitamin C (-ve control) | Infected rats + 40 ml Vitamin C | Infected rats + 60 ml Vitamin C | Infected rats + 0 ml Vitamin C (+ve control) |
| 4                             | 142.00 ± 0.94 <sup>a</sup>                     | 150.00 ± 0.94 <sup>a</sup>      | 146.00 ± 1.70 <sup>c</sup>      | 138.00 ± 1.25 <sup>d</sup>                   |
| 8                             | 147.00 ± 0.82 <sup>a</sup>                     | 128.00 ± 2.62 <sup>b</sup>      | 132.00 ± 1.25 <sup>c</sup>      | 122.00 ± 1.25 <sup>d</sup>                   |
| 12                            | 164.00 ± 1.70 <sup>a</sup>                     | 123.00 ± 1.70 <sup>b</sup>      | 130.00 ± 1.63 <sup>c</sup>      | 116.00 ± 2.50 <sup>d</sup>                   |
| 16                            | 159.00 ± 1.25 <sup>a</sup>                     | 118.00 ± 3.40 <sup>b</sup>      | 126.00 ± 1.25 <sup>c</sup>      | 104.00 ± 1.25 <sup>d</sup>                   |
| <b>Group Mean</b>             | 153.00 ± 5.12 <sup>a</sup>                     | 129.80 ± 7.05 <sup>b</sup>      | 133.50 ± 4.35 <sup>c</sup>      | 120.00 ± 7.07 <sup>d</sup>                   |

macrophageal activities. Oxidative stress has been alleviated in experimental infections with various species of trypanosomes by administration of exogenous antioxidants, such as ascorbic acid to infected rats and rabbits (Umar *et al.*, 1999b; 2000; 2008). The observed effect of vitamin C supplement from this study on the serum protein of trypanosome infected

rats was attributed to its effect on the haemopoietic system (Vray *et al.*, 1991). Its effect on the infected treated rats when compared with the infected untreated rats showed that vitamin C had positive influence on the defense capacity of infected treated rats (Schoral *et al.*, 1981). Therefore, this study has provided evidence that vitamin C has potential for influencing the state of hypoproteinaemia in

the trypanosome-infected rats. Even if vitamin C cannot destroy the trypanosomes, it can ameliorate the stress of trypanosomiasis and boost the host's immune system to fight the invaded pathogens.

**Conclusion:** From the research, we thus conclude that consumption of dietary vitamin C enhances the immune system of animals. It is advisable to include vitamin C in the feed of livestock because it boosts the immune system of animals. Also in the treatment of trypanosomiasis, it is advisable to add vitamin C to infected animals' feed for quick recovery.

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