Full Length Research Paper

Influence of high dietary vitamin C and E oral administration on anemia and organ damage in wistar rat infected with *Trypanosoma brucei brucei* (Federe strain)

Ajakaiye Joachim Joseph¹, Kugu Bashir Adamu*¹, Shuaib Yahya¹, Bizi Ramatu Lawan², Benjamin Sunday Martina², Muhammad Asma’u Asabe¹, Mohammad Bintu¹ and Mazadu Melemi Richard

***¹Extension Services Unit, Consultancy and Extension Services Division, Nigerian Institute for Trypanosomiasis Research, No. 1 Surame Road, U/Rimi, P. M. B. 2077, Kaduna, Nigeria.***

**¹Corresponding author: Mobile Phone: +2348067029512; E-mail: bashkugu@yahoo.com**

Abstract

The influence of vitamin C and E in experimental *Trypanosoma brucei brucei* (Federe strain) infected Wistar rats was investigated. The rats were infected intraperitoneally with the same parasite load. All infected animals developed terminal Parasitemia and percentage PCV, its severity in the untreated infected animals was significantly (P<0.05) higher than the three infected groups treated with the vitamins. The group given vitamin C and E (combined) developed a significantly (P<0.05) less severe Terminal Parasitemia and percentage PCV than those given vitamin C and E (separately). The positive control showed higher increase in Alanine transaminase, aspartate transaminase, alkaline phosphatase, urea and creatinine. The vitamins significantly (P<0.05) prevented the disease-induced increase in these parameters. The two vitamins combination prevented, to a significant degree, the disease-induced elevation of serum urea and creatinine. The organ-body weight ratio significantly (P<0.05) increased in all the infected groups. Treatment with the vitamins however, significantly (P<0.05) decreased the weight ratio with higher effect in the group treated with combined vitamins. It was concluded that oral administration of both vitamins C and E ameliorated the degenerative changes in tissues, blood and organs associated with *Trypanosoma brucei brucei* (Federe strain) infection in Wistar rats.

**Keywords:** *Trypanosoma brucei brucei*, vitamin C, vitamin E, anemia, organ damage, Wistar rat.

INTRODUCTION

African trypanosomiasis is one of the most neglected Tropical diseases, consisting of a number of important human and animal pathologies caused by parasitic protista of the order Kinetoplastida. Human African Trypanosomiasis (HAT), or sleeping sickness, and Animal African Trypanosomiasis (AAT), or nagana, are vector-borne diseases, which are primarily cyclically transmitted by tsetse fly. The animal trypanosomiasis challenge, caused by several species of trypanosome, e.g. *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma brucei brucei* cause about 3 million deaths annually in cattle and has a marked impact on African agriculture (Kalu et al., 2001; Njokou et al., 2004). There is considerable variation in the pathogenicity of different strains and the susceptibility of different host species. *T. brucei brucei*, like other pathogenic trypanosomes is covered by a dense protein layer consisting of a single protein called the variable surface glycoprotein (VSG), which acts as a major immunogen and elicits the formation of specific antibodies. The parasites are able to evade the consequences of these immune reactions by switching the VSG, a phenomenon known as antigenic
variation (Damian, 1997). The hematological and biochemical abnormalities induced by trypanosomes arose from their direct effect via their products on host cells such as red blood cell (RBC), white blood cell (WBC), platelets and tissues such as liver, kidney, bone marrow and lymphoid organs, resulting in cell destruction and organ malfunction as well as extractions from and additions to host chemistry associated with parasite metabolism (Anosa, 1988; Ekanem and Yusuf 2008; Akanji et al., 2009). The oxidative stress which occurs in trypanosomiasis host is as a result of systematic ascorbic acid depletion due to increased ascorbic acid consumption in infected animals; this oxidative stress leads to peroxidative tissue damage, which elevates erythrocyte free radicals, oxidative haemolysis and depletion of erythrocyte and liver glutathione by free radicals generated by the trypanosome. As a result membrane Phospholipids and Proteins are attacked leading to alteration in membrane structure, which also affects the membrane fluidity. Vitamin C (Ascorbic acid) is a water-soluble antioxidant capable of protecting against oxidative injuries in the aqueous compartments of cell membrane while Vitamin E is a fat-soluble compound comprising tocopherols and tocotrienols (Brigelius-Flohe, 1999). As a fat-soluble antioxidant, it stops the production of reactive oxygen species (e.g. Oxygen ion and peroxides) formed when fat undergoes oxidation (Packer, 2001; Devasagayam et al; 2004). This work was carried out to investigate the Influence of High Dietary Vitamin C and E Oral administration on Anemia and Organ damage in Wistar Rats infected with Trypanosoma brucei brucei (Federe strain).

**MATERIALS AND METHODS**

**Experimental site**

The study was conducted at the Nigerian Institute for Trypanosomiasis and (Onchocerciasis) Research (NITR), and located in Kaduna North Local Government Area of Kaduna State, at latitude 10° 30´ 00´´ N and longitude 7° 25´ 50´´ E of Nigeria.

**Experimental animals**

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 m³ dimension equipped with wire mesh lids. The rats were acclimatised for two weeks and duly dewormed with standard drugs before commencement of the experiment. Group A was neither treated nor infected (positive control), group B was intraperitoneally infected with 1 × 10⁶ innoculum containing T. brucei brucei (Federe strain) parasites only (negative control), while groups C, D and E were given the same dose of innoculum 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 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).

(a) Vitamin supplement per (kg) diet: Vitamin A, 6000 IU, vitamin D₃, 5000 IU, vitamin E; 23.0 mg; vitamin k₃, 4.0 mg; thymine, 11.0 mg; riboflavin, 4.0 mg; vitamin B₁₂, 0.005 mg; pyridoxine, 1.8 mg; pantothenic acid, 20.0 mg; nicotinic acid, 35 mg; folic acid, 2.5 mg; choline chloride, 615

(b) 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 serves 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× 10⁶ parasites was injected intraperitoneally into each rat in the infected groups.

**Blood Sample collection, organs collection and Serum Analysis**

Tail blood was collected daily for monitoring parasitemia as described by Herbert and Lumsden (1976) and PCV by the micro-haematocrit method. On 28 DPI, the rats were sacrificed by humane decapitation prior anesthesia with sterile cotton impregnated chloroform, and blood was collected in plain vacutainers, serum was harvested and used for estimation of alanine amino-transferase
Table 1. Composition and calculated bromatological analysis of basal diet

<table>
<thead>
<tr>
<th>Nutrients/constituents</th>
<th>Quantity in g/kg diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>480.0</td>
</tr>
<tr>
<td>Soya cake</td>
<td>175.0</td>
</tr>
<tr>
<td>Wheat offal</td>
<td>160.0</td>
</tr>
<tr>
<td>Fishmeal</td>
<td>100.0</td>
</tr>
<tr>
<td>Brewer’s dried grain</td>
<td>20.0</td>
</tr>
<tr>
<td>Vegetables oil</td>
<td>25.0</td>
</tr>
<tr>
<td>Limestone</td>
<td>5.0</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>10.5</td>
</tr>
<tr>
<td>Dry molasses</td>
<td>15.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Pre-mix Vitamins(a) and Minerals(b)</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Calculated analysis /Kg

- 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).

Table 2. Serum chemistry of Wistar rats infected with *T. brucei brucei* (Federe strain) and administered with vitamins C and E (Means ± SEM, n = 5)

<table>
<thead>
<tr>
<th></th>
<th>Not infected not treated</th>
<th>Infected not treated</th>
<th>Infected + Vit. C</th>
<th>Infected + Vit. E</th>
<th>Infected + Vit. C and E</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>19.80±0.68</td>
<td>32.20±0.70</td>
<td>31.40±0.31</td>
<td>30.30±0.63</td>
<td>28.50±0.85</td>
</tr>
<tr>
<td>AST</td>
<td>31.70±0.78</td>
<td>42.90±0.94</td>
<td>36.90±1.01</td>
<td>36.70±1.13</td>
<td>35.40±1.10</td>
</tr>
<tr>
<td>ALP</td>
<td>78.40±0.67</td>
<td>211.20±0.70</td>
<td>228.30±1.67</td>
<td>230.10±2.52</td>
<td>229.70±1.19</td>
</tr>
<tr>
<td>UREA</td>
<td>174.50±2.01</td>
<td>319.30±2.79</td>
<td>149.80±0.83</td>
<td>147.50±0.92</td>
<td>145.20±0.76</td>
</tr>
<tr>
<td>CREATININE</td>
<td>59.40±0.56</td>
<td>106.00±1.9</td>
<td>63.80±0.63</td>
<td>67.60±1.02</td>
<td>61.70±0.94</td>
</tr>
</tbody>
</table>

Values with different superscripts within a row are statistically different (P<0.05)

(ALT), aspartate amino-transferase (AST) and alkaline phosphatase (ALP) activities using the method described by Bergmeyer et al. (1978) with the aid of commercial reagent kit (Gaselich alt fur Biochemica und Diagnostica, Wiesbgden, Germany). The serum samples were also used for the estimation of Urea and Creatinine by the Diacetylmonoxime and Jaffe’s reactions, respectively as described by Kaplan et al. (1988). Organs were removed aseptically from all the groups and kept in 10 % buffered formalin.

**Statistical Analysis**

All the datas obtained from this experiment are presented as mean ± SEM. Data were analyzed by the one-way analysis of variance (ANOVA) and the significance of differences between mean values computed for particular levels of experimental factors was determined by (Duncan, 1955) post-hoc test and means that differs at p < 0.05 were considered significant.

**RESULTS**

Table 2 presents the result of the serum biochemical indicators in this experiment. Infected groups showed significant (P<0.05) increase in the levels of ALT, AST, ALP and creatinine when compared to the uninfected group. Group II showed significant (P<0.05) increase in
Table 3. Organ: body weight ratios of Wistar rats infected with *T. brucei brucei* (Federe strain) and administered with vitamins C and E (Means ± SEM, n = 5)

<table>
<thead>
<tr>
<th>Organ</th>
<th>Not infected not treated</th>
<th>Infected not treated</th>
<th>Infected + Vit. C</th>
<th>Infected + Vit. E</th>
<th>Infected + Vit. C and E</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEART</td>
<td>1.47± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.16± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.87± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.79± 0.06&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.63± 0.05&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>LIVER</td>
<td>3.10± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.82± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.43± 0.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.44± 0.10&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.30± 0.06&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>SPLEEN</td>
<td>0.59± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.13± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.71± 0.02&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.83± 0.02&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.27± 0.20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>KIDNEY</td>
<td>0.83± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.06± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.7± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.80± 0.01&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.73± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with different superscripts within a row are statistically different (P<0.05).

Table 4. Terminal Parasitemia and % change in PCV of Wistar rats infected with *T. brucei brucei* (Federe strain) and administered with vitamins C and E (Means ± SEM, n = 5)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Not infected not treated</th>
<th>Infected not treated</th>
<th>Infected + Vit. C</th>
<th>Infected + Vit. E</th>
<th>Infected + Vit. C and E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal Parasitemia</td>
<td>152.30±1.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102.20±2.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>101.90±2.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.50±0.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Initial PCV (%)</td>
<td>48.90±1.12</td>
<td>48.90± 1.03</td>
<td>48.60± 1.2</td>
<td>48.70± 1.13</td>
<td>49.00± 1.22</td>
</tr>
<tr>
<td>Final PCV (%)</td>
<td>49.20±1.14</td>
<td>29.20± 0.95</td>
<td>40.50± 1.60</td>
<td>39.70± 1.97</td>
<td>44.10± 1.27</td>
</tr>
<tr>
<td>% change in PCV</td>
<td>(+)30±1.78</td>
<td>(-)19.70±1.09</td>
<td>(-)8.10± 1.35</td>
<td>(-)9.00± 2.04</td>
<td>(-)4.90±1.92</td>
</tr>
</tbody>
</table>

Values with different superscripts within a row are statistically different (P<0.05). *Positive signs (+) indicates increases and negative signs (-) indicates decreases.

DISCUSSION

In this experiment, there is increase in levels of Alanine amino-transaminase(ALT), aspartate amino-transaminase(AST), alkaline phosphatase(ALP), urea and Creatinine due to infection. This agrees with the findings of Hudson (1944), Kalu et al. (1989), Adah et al. (1992), Ismaila et al. (2000) and Umar et al. (2008) who reported increase in serum levels in experimental trypanosomiasis. Increases in the levels of these enzymes are indications of damage to liver, brain, and cardiac muscles (Kaplan, 1988) and several workers have reported hepatocellular damage and generalized degenerative changes in other tissues and organs in trypanosomiasis (Anosa et al., 1984; Bruijn, 1987). The decrease observed in the levels of ALT, AST, ALP, Creatine and Urea in the treated groups might be a result of Vitamins C and E supplementation.

The enlargement of the organs (Heart, Liver, Kidney and Spleen) Otherwise known as cardiomegaly, Hepatomegaly and Splenomegaly respectively as observed in the result, is presumably due to membrane damage caused by the large amount of free radicals and other oxidative species being generated and the
The vitamins C and E aided in reducing the free radicals being generated by the *Trypanosoma brucei brucei* (Federe strain), ameliorated anemia and organ damage. Nevertheless, the combined administration of vitamins was more effective than single administration. The data lend further support to the significant roles of oxidative stress and depletion of endogenous antioxidant reserves in the organ pathogenesis of African trypanosomiasis.

CONCLUSION

The vitamins C and E aided in reducing the free radicals being generated by the *Trypanosoma brucei brucei* (Federe strain), ameliorated anemia and organ damage. Nevertheless, the combined administration of vitamins was more effective than single administration. The data lend further support to the significant roles of oxidative stress and depletion of endogenous antioxidant reserves in the organ pathogenesis of African trypanosomiasis.

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