Antioxidant Effects of Mixed Doses of Vitamins B12 and E on Male Wistar Albino Rats Infected with Trypanosoma Brucei Brucei

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Abstract The research was conducted to determine the antioxidant effects of the mixed doses of vitamins B12 and E on male Wistar albino rats infected with T. b. brucei. Fifty-four male Wistar albino rats were randomly divided into six groups of three rats each replicated three times. The rats were marked and kept in stainless wire cages labeled A-F. Groups A, B, and C were normal, negative, and standard controls respectively. Groups D, E and F were infected with 1.0 x 10^6 trypanosomes and treated with 0.2 + 0.5mg/kg (low-dose), 0.3 + 2.5mg/kg (medium-dose), and 0.4 + 5.0mg/kg (high-dose) of vitamins B12 + E per body weight per day respectively. The experiment lasted for twenty-one days from the day T. b. brucei infection was established. A sample of heart tissue homogenate was collected weekly across the groups and subjected to biochemical determination of malondialdehyde, hydrogen peroxide, nitric oxide, reduced glutathione, and glutathione peroxidase concentrations. There were significant differences in the effect of antioxidant biomarkers which was applied to the duration of the experiment. At post-treatment, the levels of the antioxidant parameters differed significantly (p<0.05) from the negative control. There was a significant increase in the levels of nitric oxide, hydrogen peroxide, and reduced glutathione, and glutathione peroxidase of cardiac tissue homogenate of the experimental rats following the treatments with vitamins B12 + E. The study also showed the pathogenesis of T. b. brucei significantly raised the malondialdehyde concentration and the treatment with vitamins B12 + E decreased the trypanosome-induced elevation of the malondialdehyde level. In conclusion, nitric oxide, hydrogen peroxide, reduced glutathione, and glutathione peroxidase levels significantly increased, while malondialdehyde concentration decreased.

Keywords Trypanosoma Brucei Brucei, Vitamin B12, Vitamin E, Malondialdehyde, Hydrogen Peroxide, Nitric Oxide, Reduced Glutathione, And Glutathione Peroxidase

1. Introduction

Trypanosoma brucei brucei is a species of salivarian trypanosome which causes African trypanosomiasis, known also as sleeping sickness in humans and nagana in animals. African trypanosomiasis is one of the most neglected tropical diseases, consisting of many important human and animal pathologies caused by parasitic protista of the order Kinetoplastida. Human African Trypanosomiasis (HAT), also called sleeping sickness, and Animal African Trypanosomiasis (AAT), known as nagana, are vector-borne diseases, which are primarily cyclically transmitted by the tsetse fly.

Trypanosomiasis has been associated with immunosuppression and induction of lipid peroxidation in the host [1]. The process of oxidative stress is a result of an imbalance between oxidant and antioxidant compounds due to the accumulation of free radicals and sufficient studies are suggesting that oxidative stress leads to cardiovascular diseases [2]. It is a common mediator in the pathogenicity of established cardiovascular factors [3]. Malondialdehyde (MDA) is a principal biomarker for lipid peroxidation. Serum MDA concentration is recognized as a biomarker of oxidative stress [4]. Da-Silva et al. [5] reported decreased glutathione (GSH) activities in T. envasi-infected rats. Reduced plasma GSH was also reported in blood of T. envasi-infected rats, but no change was recorded in glutathione-S-transferase (GST) activity
Studies conducted by El-Deeb and Esmoslemany (2015) with T. brucei-infected camels linked the elevated serum cardiac injury biomarkers with oxidative stress. Lipid peroxidation biomarker concentrations are significantly elevated in myocardial injuries [7].

Trypanosomosis is a debilitating as well as fatal tropical disease of livestock and man. It currently causes annual losses of about USD 1.5 billion and, over the long run, has had the effect of limiting Africa’s agricultural income to about USD 4.5 billion a year, below its potential level [8]. Besides, it is estimated that about USD 30 million per year is spent on prophylaxis and treatment. Thus, African livestock keepers are faced with serious challenges of controlling or reducing the impact of the disease. Controlling the disease has been directed towards vector control, chemotherapy, and chemoprophylaxis and the use of trypanotolerance breeds. Trypanocidal drugs remain the principal method of animal trypanosomiasis control in most African countries [9]. However, the therapeutic and prophylactic use of trypanocides is hampered by numerous limitations such as toxicity, prohibitive cost, and development of resistance by the parasites [9].

Vitamin B₁₂ is a water-soluble vitamin that either occurs in some foods naturally or fortified to others and available as a dietary supplement and a prescription for therapy. The main dietary sources of vitamin B₁₂ are animal products such as meat, fish, eggs, and dairy products [10]. Other sources may include plant products including cereals, plant-based milk, soy products, and yeast [11]. Vitamin B₁₂ functions in red blood cell formation, neurological function, and DNA synthesis [12]. It also functions in nervous regulation and for the metabolism of carbohydrates, protein, and fat. Vitamin B₁₂ is also a cofactor for methionine synthase conversion of homocysteine to methionine [13]. Methionine is essential for the formation of S-adenosylmethionine, a universal donor for almost 100 different substrates, including DNA, RNA, hormones, proteins, and lipids.

Deficiencies in vitamin B₁₂ can result in ineffective erythropoiesis and megaloblastic anemia [14]. Neurological disorders such as neuropathy, myelopathy, memory impairment, dementia, depression, and brain atrophy may result in patients with vitamin B₁₂ deficiency [10]. Pernicious anemia is an autoimmune disease that affects the gastric mucosa and causes gastric atrophy. This type of anemia results in the destruction of parietal cells, achlorhydria, and failure to produce intrinsic factor, resulting in malabsorption of vitamin B₁₂. If pernicious anemia is left untreated, it results in vitamin B₁₂ deficiency, leading to megaloblastic anemia and neurological disorders, even when dietary vitamin B₁₂ intake is adequate.

Although various studies have been performed to confirm the biochemical involvement of vitamin B₁₂, studies dealing with the effect of vitamin B₁₂ on trypanosomes are limited. Ciccarelli et al. [15] reported that cyanocobalamin produced a significant reduction in motility and growth rate of Trypanosoma cruzi epimastigotes. Cytotoxic action of vitamin B₁₂ on the parasite is through the generation of reactive oxygen species.

Vitamin E exists in eight chemical forms (alpha–, beta–, gamma–, and delta-tocopherol and alpha-beta–, gamma–, and delta-tocotrienol) that have distinctive biological functions [16]. Alpha- and gamma-tocopherol are the two major forms in which vitamin E occurs, with the relative proportions of these depending on the source. Alpha– (or α–) tocopherol is the only form that has met human requirements. Among the tocopherols, the alpha- and gamma-tocopherols are present in the serum and the red blood cells, with alpha-tocopherol having the highest concentrations.

The mechanisms by which vitamin E might promote healthy living include its antioxidant property and its function in anti-inflammatory processes, inhibition of platelet aggregation, and improvement of immunity. According to the Institute of Medicine Food and Nutrition Board (IMFNB) [17], the major problem of characterizing vitamin E functions in health is the lack of acceptable biomarkers for vitamin E intake and status to help relate intakes to acceptable clinical outcomes of trusted precision. People with an inherited or acquired conditions, such as cystic fibrosis, short bowel syndrome or bile duct obstruction and those with fat-malabsorption disorders are more vulnerable to vitamin E deficiency than people without such disorders, this is because the alimentary canal requires fat to absorb vitamin E.

Some people with abetalipoproteinemia, a rare inherited disorder resulting in poor absorption of dietary fat, require enormous doses of supplemental vitamin E (approximately 100mg/kg or 5-10g/day [16]. Abetalipoproteinemia linked vitamin E deficiency can lead to poor transmission of nerve impulses, muscle weakness, and blindness. Ataxia and vitamin E deficiency (AVED) is another rare genetic disorder in which the liver’s alpha-tocopherol transfer protein is missing. People with vitamin E deficiency precipitated cellular atrophy and diminished dendritic branching of Purkinje neurons [18].

Several observations have been documented on the antitypansomal effects of vitamin E. The works of Ammouche et al. [19] and Kiron et al. [20] also reported that dietary vitamin E supplement increases the activities of antioxidant enzymes. Vitamin E supplementation significantly enhanced the antioxidant enzymes (superoxide dismutase and catalase) activities of T. b. brucei-infected rats when compared with non-supplemented groups [20]. However, vitamin E significantly reduced serum MDA concentrations of the supplemented T. b. brucei-infected rats [21]. The objective of the study is to determine the antioxidant effects of mixed doses of vitamins B₁₂ and E on male Wistar albino rats infected with Trypanosoma brucei brucei.
2. Material and Methods

2.1. Animal Model and Experimental Protocol

Fifty-four (54) male albino Wistar rats (Rattus norvegicus) aged 3 months, weighing between 180-220g were procured, housed, and allowed to acclimatize for two weeks at the Pharmacy Animal House, Madonna University Elele, Rivers state. The rats were grouped into six (6) cages labeled A-F comprising three (3) rats that were replicated three (3) times from each group. The animals were kept under normal room temperature with ad libitum access to feed and water. The cages were cleaned daily to prevent infection of the animals and to minimize extraneous variables.

The groups (A-F) were as thus: Group A (Normal Control) were neither infected with trypanosomes nor treated with vitamins; Group B (Negative Control) were infected with 1.0 x 10^6 trypanosomes but not treated; Group C (Standard Control) were infected with 1.0 x 10^6 trypanosomes and treated with 0.2 mg/kg diminazene aceturate body weight); Group D (Low-dose of vitamins B12 + E) were infected with 1.0 x 10^6 trypanosomes and treated with 0.2 mg/kg + 0.5 mg/kg body weight of vitamin E per day; Group E (Medium-dose of vitamins B12 + E) were infected with 1.0 x 10^6 trypanosomes and treated with 0.2 mg/kg + 0.5 mg/kg body weight of vitamin E per day; Group F (High-dose of vitamins B12 + E) were infected with 1.0 x 10^6 trypanosomes and treated with 0.4 mg/kg + 5.0 mg/kg body weight of vitamins B12 + E.

The experiment lasted for twenty-one days after T. b. brucei infection was established. A sample of serum was collected weekly from the three (3) rats across the groups and taken to Divine Chemicals and Analytical Laboratory, Nsukka for the biochemical determinations of the antioxidant parameters.

2.2. Procurement and Inoculation of Trypanosomes

T. b. brucei was obtained from an experimentally infected rat previously inoculated with the parasite from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Each experimental rat was administered 0.1ml of infected blood in 0.3ml normal saline containing 1.0 x 10^6 trypanosomes using the rapid matching method [22] to determine the level of parasitemia. Inoculation was done intraperitoneally.

2.3. Determination of Parasitaemia

Wet blood preparations were covered with a coverslip on a slide and examined under X40 and oil immersion using a microscope at X100 magnification. The identification of parasites was done using morphological description [23].

2.4. Formulation and Administration of Vitamin B12

Vitamin B12 was procured at Science Line, New Parts, Onitsha, Anambra State, Nigeria in a powdered bottle. The working concentrations were determined at the Departmental of Biochemistry, Madonna University, Elele Campus Line from the result of acute oral toxicity (LD50) test of vitamin B12 as thus:
- Mild dose: 40mcg = 0.2 mg/kg b.w.d.
- Medium dose: 60mcg = 0.3 mg/kg b.w.d.
- High dose: 80mcg = 0.4 mg/kg b.w.d.

The working concentrations were administered via intubation using 2ml of distilled water as a vehicle.

2.5. Formulation and Administration of Vitamin E

Vitamin E was procured at Science Line, New Parts, Onitsha, Anambra State, Nigeria in a powdered bottle. The working concentrations were weighed at the Departmental of Biochemistry, Madonna University, Elele from the result of acute oral toxicity (LD50) test of vitamin B12 as thus:
- Mild dose: 0.5 mg/kg b.w.d.
- Medium dose: 2.5 mg/kg b.w.d.
- High dose: 5.0 mg/kg b.w.d.

The working concentrations were dissolved in 2% ethanol as a vehicle and administered via intubation.

2.6. Standard Drug

Diminazene aceturate was procured from the faculty of Veterinary Medicine Clinic, University of Nigeria, Nsukka, Nigeria in a 2.36g granules. The working dosage was 0.2m/kg. The administration was intravenous.

2.7. Preparation of Cardiac Tissues Homogenate

The heart was weighed and homogenized with a potter-Elvenhjem tissue homogenizer in a potassium phosphate buffer 10 Mm pH (7.4). The crude tissue homogenate was centrifuged at 10,000 revolutions per minute, for 15 minutes in a cold centrifuge, and the resultant supernatant was used for the different estimations of antioxidant parameters.

2.7.1. Lipid Peroxidation (LPO) assay

The method described by Ohkawa et al. [24] was employed in the determination of cardiac tissue malondialdehyde concentration.

2.7.2. Hydrogen peroxide (H2O2) scavenging assay

Hydrogen peroxide was determined according to the method of Ruch et al. [25].

2.7.3. Nitric oxide scavenging activity

The method illustrated by Marcocci et al. [26] was used for the determination of nitric oxide.

2.7.4. Reduced glutathione (GSH) estimation

The method illustrated by Ellman [27] was used to
determine reduced glutathione concentration.

2.7.5. Glutathione Peroxidase (GPx)

The procedure according to Wood [28] was employed in the estimation of glutathione peroxidase concentration.

2.7.6. Statistical Analysis

Data resulting from the experiments were subjected to a 2-way analysis of variance (ANOVA) using the SPSS software (version 21) and the difference between means were separated using Duncan’s multiple range tests. The test for significance was set at 0.05 probability level.

3. Result

3.1. Cardiac tissue malondialdehyde concentration

The result of mean malondialdehyde (MDA) level (u/mg) at short and long duration were compared in various groups. A similar pattern occurred at the various duration of the study. On the fourteenth day of post-infection (week 2), the result indicated a significant increase in the negative control (4.966 ± 0.038) when compared to the normal control (3.518 ± 0.095) (p<0.05) (Table 1). There was no significant difference in the mean cardiac tissue MDA between the normal and the standard controls at weeks 1, 2, and 3 post-infection (p>0.05).

There was a significant difference in the negative control in mean MDA concentration between the duration of the study (p<0.05). This showed that the mean cardiac tissue MDA level increased as the parasitemia increased. A comparison of mean MDA levels in vitamins B12 + E treated groups showed there was a significant reduction in the level of MDA when compared to the negative control at weeks 1, 2, and 3 post-infection and treatment.

3.1.1. Cardiac tissue malondialdehyde concentration

Table 1. Effects of mixed doses of vitamins B12 + E on cardiac tissue malondialdehyde (MDA) level (U/mg)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.518±0.095&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.435±0.062&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.555±0.126&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>4.966±0.038&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.843±0.088&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.990±0.075&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>3.676±0.077&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.676±0.049&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.490±0.073&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>3.752±0.153&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.854±0.169&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.619±0.025&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>3.962±0.035&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.966±0.067&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.915±0.014&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>3.983±0.081&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.897±0.152&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.671±0.101&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

In a column, mean value with the same letter as superscript is not significantly different (p>0.05). In a row, mean value with the same number as superscript is not significantly different (p>0.05).

3.1.2. Cardiac tissue hydrogen peroxide (H2O2) concentration

The result of mean hydrogen peroxide (H2O2) level (u/mg) was compared in the various groups. A similar pattern was followed at the various duration of the study. On the seventh day of post-infection (week 1), the result indicated a significant increase in the negative control (0.413±0.007) when compared to the normal control (0.308±0.100) (p<0.05) (Table 2).

There was no significant difference in the mean cardiac tissue H2O2 between the normal and the standard controls at weeks 1, 2, and 3 post-infection (p>0.05). There was a significant difference in the negative control in mean H2O2 concentration between the duration of the study (p<0.05). This showed that the mean cardiac tissue H2O2 level increased as the parasitemia increased. A comparison of mean H2O2 levels in vitamins B12 + E treated groups showed there was a significant reduction in the level of H2O2 when compared to the negative control at weeks 1, 2, and 3 post-infection and treatment.

3.1.3. Cardiac tissue nitric oxide concentration

The result of mean nitric oxide (NO) level (u/mg) at short and long duration were compared in various groups. On the seventh day of post-infection (week 1), the result of cardiac tissue NO indicated a significant reduction in the level of the negative control (18.065±0.240) when compared to the normal control (20.827±0.211) (p<0.05) (Table 3). There was no significant difference in the mean cardiac tissue NO between the normal and the standard controls at weeks 1, 2, and 3 post-infection (p>0.05).

Table 2. Effect of mixed doses of vitamins B12 + E on cardiac tissue hydrogen peroxide level (U/mg)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.308±0.100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.306±0.021&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.332±0.010&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>0.413±0.007&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.450±0.012&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.506±0.010&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>0.320±0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.318±0.012&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.337±0.012&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>0.342±0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.346±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.362±0.008&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>0.338±0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.323±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.358±0.005&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>0.33±0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.349±0.012&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.378±0.006&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

In a column, mean value with the same letter as superscript is not significantly different (p>0.05). In a row, mean value with the same number as superscript is not significantly different (p>0.05).

Table 3. Effects of mixed doses of vitamins B12 + E on cardiac tissue nitric oxide level (U/mg)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20.827±0.211&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.024±0.427&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.811±0.187&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>18.065±0.240&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.691±0.217&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.879±0.174&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>20.627±0.188&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.927±0.389&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.724±0.193&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>20.182±0.119&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.205±0.215&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.747±0.089&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>20.270±0.464&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.529±0.233&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.058±0.056&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F</td>
<td>20583±0.081&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.757±0.404&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.453±0.0874&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

There was a significant difference in the negative control in mean NO concentration between the duration of the
study (p<0.05). This showed that the mean cardiac tissue NO level decline as the parasitemia increased. A comparison of mean NO levels in vitamin B12 + E treated groups showed there was a significant rise in the level of NO when compared to the negative control at weeks 1, 2, and 3 post-infection and treatment. A similar pattern occurred at the various duration of the study.

In a column, mean value with the same letter as superscript is not significantly different (p>0.05). In a row, mean value with the same number as superscript is not significantly different (p>0.05).

3.1.4. Cardiac tissue reduced glutathione (GSH) concentration

The result of cardiac tissue reduced glutathione level (u/mg) at the seventh day of post-infection in the negative control was significantly lower (26.598±0.235) than the normal control (31.545±0.407) (p<0.05) (Table 4). The standard control group decreased significantly (26.598±0.235) than the normal control (group A) (p<0.05). The groups treated with vitamins B12 + E were not significantly different (28.271±0.335, 28.304±0.351, 28.353±0.271) when compared to the negative control group (26.598±0.235) (p>0.05).

The reduced glutathione concentrations in vitamins B12 + E treated groups at weeks 2, and 3 differed significantly with the controls. There was a significant difference in cardiac tissue GSH levels in the negative control between the durations of the study (p<0.05). This showed that the cardiac tissue GSH levels decreased following the proliferation of the parasites.

Table 4. Effects of mixed doses of vitamins B12 + E on cardiac tissue reduced glutathione (GSH) level (U/mg)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>31.545±0.407</td>
<td>31.676±0.359</td>
<td>31.830±0.271</td>
</tr>
<tr>
<td>B</td>
<td>26.598±0.235</td>
<td>25.509±0.058</td>
<td>24.203±0.067</td>
</tr>
<tr>
<td>C</td>
<td>28.715±0.058</td>
<td>29.436±0.076</td>
<td>30.024±0.106</td>
</tr>
<tr>
<td>D</td>
<td>28.271±0.335</td>
<td>28.546±0.088</td>
<td>28.939±0.150</td>
</tr>
<tr>
<td>E</td>
<td>28.304±0.351</td>
<td>28.589±0.063</td>
<td>28.958±0.063</td>
</tr>
<tr>
<td>F</td>
<td>28.353±0.271</td>
<td>28.689±0.016</td>
<td>29.124±0.150</td>
</tr>
</tbody>
</table>

In a column, mean value with the same letter as superscript is not significantly different (p>0.05). In a row, mean value with the same number as superscript is not significantly different (p>0.05).

3.1.5. Cardiac tissue glutathione peroxidase (GPx) concentration

The result of cardiac tissue glutathione peroxidase (GPx) level (u/mg) at the seventh day of post-infection in the negative control was significantly lower (20.414±0.333) than the normal control (24.381±0.116) (p<0.05). The standard control group decreased significantly (21.056±0.099) than normal control (p<0.05). The groups treated with vitamins B12 + E were not significantly different (22.855±0.256, 22.898±0.175, 22.924±0.183) when compared to the negative control and standard control groups (20.414±0.333, and 21.056±0.099) (p<0.05) (Table 5).

The cardiac tissue GPx concentrations in vitamin B12 + E treated groups at week 2 differed significantly with the controls. The result of cardiac tissue glutathione peroxidase at week 3 followed a similar pattern with that of week 1. There was a significant difference in cardiac tissue GPx level in the negative control at weeks 1, 2, and 3 of the study (p<0.05). This showed that the cardiac tissue GPx levels decreased with increased parasitemia.

Table 5. Effects of mixed doses of vitamins B12 + E on cardiac tissue Glutathione Peroxidase level (U/mg)

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>24.381±0.116</td>
<td>24.585±0.264</td>
<td>24.585±0.168</td>
</tr>
<tr>
<td>B</td>
<td>20.414±0.333</td>
<td>20.619±0.170</td>
<td>19.879±0.074</td>
</tr>
<tr>
<td>C</td>
<td>23.05±0.099</td>
<td>23.11±0.036</td>
<td>23.518±0.060</td>
</tr>
<tr>
<td>D</td>
<td>22.855±0.256</td>
<td>22.767±0.218</td>
<td>21.397±0.169</td>
</tr>
<tr>
<td>E</td>
<td>22.898±0.175</td>
<td>22.882±0.156</td>
<td>21.428±0.199</td>
</tr>
<tr>
<td>F</td>
<td>22.924±0.183</td>
<td>22.946±0.179</td>
<td>23.285±0.059</td>
</tr>
</tbody>
</table>

In a column, mean value with the same letter as superscript is not significantly different (p>0.05). In a row, mean value with the same number as superscript is not significantly different (p>0.05).

4. Discussion and Conclusions

4.1. Discussion

The present study inferred that the administrations of mixed doses of vitamins B12, and E altered the pathogenesis of T. b. brucei in rats. This was shown by the fall in the level of the cardiac MDA of the infected rats following the treatment with the interventions. This aligns with the work of Verhagen et al. [29] who reported the antioxidants can ameliorate the destructive effects of free radicals. It also indicated that the lipid peroxidation biomarker (MDA) significantly elevated due to the increased proliferation of T. b. brucei infection. The accumulation of MDA in the heart tissue is indicative of the extent of free radical generations, and damage [30]. The study also showed that the administrations of mixed doses of vitamins B12 and E changed the pathogenesis of T. b. brucei in the experimental rats. This was evidenced by the reduction in the concentration of the cardiac tissue hydrogen peroxide of rats infected with T. b. brucei following the treatments with different doses of vitamins B12 and E.

This is consistent with the work of Birch et al. [31] who reported that cobalamins can inhibit intracellular hydrogen peroxide production. The study also aligns with the
findings of Verhagen et al. [29] which described antioxidants can ameliorate the destructive effects of reactive oxygen species. The result indicated the pathogenesis of T. b. brucei decreased the nitric oxide concentration of the experimental rats and the administration of vitamin B₁₂ raised the trypanosome-induced reduction of nitric oxide. The significant and progressive decrease in nitric oxide (NO) concentration observed in the infected groups as compared to the concentration obtained in the normal control is in agreement with the work of Buguet et al. [32].

They attributed the changes to an impaired iNOS activity that was evident in peritoneal macrophages collected from the same animals. It is also in line with the observation of Saha and Pahan [33] that linked the reduced concentration of nitric oxide to iNOS activity in peripheral compartments. The significant reduction in reduced glutathione concentration shown in the infected groups agreed with the observation of Pratt [34] who reported a decreased T. b. brucei glutathione synthetase. Expectedly, the interventions returned the trypanosome-induced glutathione reduction close to the level of the standard control. This conforms to the findings of Glynn et al. [35] which found that antioxidant vitamins can stop heart attack and venous thromboembolism. It also aligns with the work of Niki [36] which described vitamin E as the most efficient scavenger of lipid peroxyl radicals.

The experiment showed that the proliferating parasites caused a reduction in the level of glutathione peroxidase in the experimental groups. This is consistent with the observation of Anschau et al. [6] which reported decreased glutathione peroxidase activities in the blood of T. evansi-infected rats. In the groups infected and treated with the mixed doses of vitamins B₁₂ + E, there were significant increases in the trypanosome-induced glutathione peroxidase reduction. This is consistent with the findings of Verhagen et al. [29] which observed the antioxidant capacity of the vitamins in the prevention of the free radicals damaging potentials.

4.2. Conclusions

The findings of the present study indicated that the pathogenesis of T. brucei brucei on albino rats caused a significant increase in the levels of MDA, H₂O₂, and a profound reduction in the concentration of nitric oxide, GSH, and GPx. This research will help scientific community to manage the patients with Trypanosoma brucei brucei infection with the use of the therapeutic vitamins.

REFERENCES


