Effects of Selenium and Vitamin E on some Biochemical Parameters in Wistar Rats Subjected to Water Immersion Restraint stress

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ABSTRACT
The aim of the present study was to determine the effects of selenium and vitamin E supplementation on some trace elements and biochemical parameters in Wistar rats, subjected to water immersion restraint stress (WRS). Wistar rats of both sexes (n = 35) weighing 200-220 g were divided into five groups of seven rats each: (i) passive control (non-stress rats) (ii) active control (WRS + distilled water) (iii) WRS + vitamin E (iv) WRS + selenium (v) WRS + vitamin E + selenium. The WRS procedure lasted for 3.5 hours. Blood samples were collected through cardiac puncture for trace element and biochemical analysis. The results demonstrated that acute WRS significantly (P < 0.001) increased MDA and plasma Zn concentrations. Pretreatment with selenium and/or vitamin E significantly (P < 0.001) lowered by pre-treatment with selenium and/or vitamin E, especially in rats co-administered with selenium and vitamin E. Exposure to WRS significantly (P < 0.001) decreased activities of CAT, SOD, GPx and Mg concentration. Pre-treatment with selenium and/or vitamin E resulted in significant (P < 0.001) increased in the activities of the enzymes. Pre-treatment with selenium and/or vitamin E ameliorated some adverse effects of WRS. Co-administration of selenium and vitamins E exerted potentiating effects on restoration of WRS-induced changes. It was concluded that WRS exposure causes significant (P < 0.001) alteration in biochemical parameters and increase in serum antioxidant balance.

Key Words: Biochemical, Parameters, Trace element, Stress, Selenium, Vitamin E, Water immersion

INTRODUCTION
Chronic restraint stress causes a significant alteration in physiological, biochemical and lipid peroxidative parameters and, consequently a shift in oxidant and antioxidant balance[1]. During stress, there is increased generation of reactive oxygen species (ROS) [2]. The ROS damage cell membranes via lipid peroxidation [2]. Lipid peroxidation generates a variety of relatively stable decomposition end-products; mainly α, β-unsaturated reactive aldehydes, such as malondialdehyde (MDA). Physiological studies have shown that stress from any source may influence the endocrine, haemopoietic and immune systems [3]. Water-immersion restraint stress (WRS) is an easy and convenient method to induce both psychological and physical stress [4].

Selenium (Se) is an essential trace and antioxidant element and its low status in human has been linked to increased risk of various diseases [5]. Vitamin E (α-tocopherol) is a lipid–soluble antioxidant and a well-accepted first-line defense mechanism against lipid peroxidation. It functions as a chain-breaking antioxidant for lipid peroxidation in cell membranes and as a scavenger of ROS, including superoxide anion, hydrogen peroxide and single oxygen [6]. There is paucity of information on the effect of selenium and/or vitamin E on water-immersion on biochemical changes in rats exposed to water-immersion restraint stress.

MATERIALS AND METHODS
Experimental Animals
Thirty five (35) Wistar rats of both sexes weighing 200-220 g and purchased from the Department of Pharmacology, Ahmadu Bello University, Zaria were used in the study. They were fed standard laboratory diet and given access to drinking water ad libitum. Forty eight hours before the induction of water-immersion restraint stress, the animals was deprived of food to allow for complete gastric emptying but they were given access to water ad libitum. The procedure has been proven to be non-ulcerogenic and sufficient for absolute emptying of the stomach [7, 8]. During fasting, rats were housed each in separate cage with a wide-raised, wire mesh bottom to prevent coprophagy [9]. Ethical approval was obtained from the Department of Human Physiology, Ahmadu Bello University, Zaria. The study was conducted in accordance with internationally-accepted principles for laboratory animal use and care.

**Chemicals**
The antioxidants, sodium selenate pentahydrate (Na$_2$SeO$_3$·5H$_2$O), and α-tocopherol (vitamin E) in the study were of analytical grade, and were purchased from Sigma Aldrich (Chemical Co. St. Louis, MO, USA).

**Experimental Design**
The rats were anaesthetized with chloroform by inhalation and were kept conscious during the experiment. Thereafter no additional anesthetic agent was applied. The four limbs of each rat were bound on a wooden board (25 cm x 19 cm) with upper limbs anchored at horizontal position, and the lower limbs extended downwards as reported by Shu [9]. In WRS group, the rats were restrained after recovery from the anaesthesia, and the anchored plate was immersed to the level of xiphoid process vertically (head up) in water bath, thermostatically-controlled at 23 ± 0.5°C [9]. The stress procedure lasted for 3.5 hours. The rats were subdivided into five groups of seven rats each as follows:-

- **Group I**: Passive control rats (unstressed control).
- **Group II**: Rats received distilled water as the vehicle at 5 ml/kg body weight orally for 3 days + WRS (active control).
- **Group III**: WRS + vitamin E (250mg/kg body weight) orally for 3 days [10]
- **Group IV**: WRS + selenium pentahydrate (100µg / kg body weight) orally for 3 days [11].
- **Group V**: WRS + vitamin E + selenium pentahydrate for 3 days.

At the end of this experimental procedure, blood samples were collected through cardiac puncture [3].

**Assessment of Lipid Peroxidation**
Lipid peroxidation as evidenced by the formation of thiobarbituric acid-reactive substances (TBARS) was measured by the modified method of Niehaus and Samuel [16] and as described by Akanji [12]. Briefly, to 150 µL (0.15 mL) of serum were treated with 2mL (1:1:1 ratio) of thiobarbituric acid, trichloroacetic acid, and hydrochloric acid (TBA-TCA-HCl), reagent (thiobarbituric acid 0.37%, 15% trichloroacetic acid and 0.25N hydrochloric acid) and placed in water bath for 1 hour at 90°C. The mixture was cooled and centrifuged at 1000 x g for 5 minutes at 4°C. The absorbance of the pink supernatant (2.0 mL) was measured against reference blank using spectrophotometer (BTB Bench centrifuge machine. Baird and Tatlock, London No 68/3856/23) at 535 nm.

The MDA concentration was calculated using the molar extinction coefficient of 1.56x 10$^5$ cm$^{-1}$M$^{-1}$. MDA concentration = absorbance/1.56 x 10$^5$ cm$^{-1}$M$^{-1}$ x 1

**Assay of Reduced Glutathione Concentration**
Reduced glutathione (GSH) concentration measurement was done according to Ellman [13] and as described by Rajagopalan [14]. Briefly, 0.2 M phosphate buffer: 8.40 g of NaH$_2$PO$_4$ and 9.94 of Na$_2$HPO$_4$ were dissolved in distilled water and made up to 1000 mL mark in volumetric flask. The buffer was adjusted to pH 8. To 150 µL of serum, 1.5 mL of 10% TCA was added and centrifuged at 1500 g for 5 minutes (BTI Bench centrifuge machine. Baird and Tatlock London No 68/3856/23). The supernatant (1mL) was treated with 0.5 mL of Ellman’s reagent (19.8 mg of 5,5-dithiobis (nitro benzoic acid) (DTNB) in 100 mL of 0.1% sodium nitrate), and 3 mL of phosphate buffer (0.2 M, pH 8). The absorbance was read at 412 nm.

**Determination of Superoxide Dismutase Activity**
Superoxide dismutase (SOD) activity was determined by the method described by Fridovich [15]. The ability of SOD to inhibit auto-oxidation of adrenaline at pH 10.2 forms the bases, of this assay. Briefly, 0.1 mL of microsome was diluted in 0.9 mL of distilled water to make 1:10 dilution of microsome. An aliquot mixture of 20 mL of the diluted microsome was added to 2.5 mL of 0.05 M carbonate buffer. The reaction was started with addition of 0.3 mL of 0.3 mM adrenaline and 0.20 mL of distilled water. Absorbance was measured 30 seconds up to 150 seconds at 480 nm. Increase in absorbance per minute = (A$_2$ – A$_1$) / 2.5

Percentage inhibition = 100 - Increase in absorbance of substrate/Increase in absorbance of blank x 100.

One unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adrenochrome in 1 minute.
Determination of Catalase Activity

Catalase activity was determined using the method described by Sinha [16]. The method is based on the reduction of dichromate in acetic acid to chromic acetate, when heated in the presence of hydrogen peroxide with formation of perchromic acid as unstable intermediate. Chromic acetate so produced was measured colorimetrically using spectrophotometer at 570 nm. This is because dichromate has no absorbance at 570 nm and does not interfere with the determination of catalase activity. Distilled water (0.9 mL) was added to 0.1 mL of microsome (serum) and mixed thoroughly. Phosphate buffer (2.5 mL) was placed in a small conical flask, 0.5 mL of microsme was added, and 2.0 mL of H₂O₂ added. The stop-watch was started. The reaction mixture was thoroughly mixed and was stopped after every 60 seconds for 3 minutes with 1 mL dichromate/acetic acid solution. It was heated in water bath for 10 minutes at 80°C. Absorbance was read at 570 nm. Standard curve was obtained using absorbance recorded at various H₂O₂. The quantity of H₂O₂ consumed was obtained from the graph of the catalase standard curve, and it determined the catalase activity.

Assessment of Mineral Changes

Atomic absorption spectrophotometry (AAS) is the most effective method for trace mineral element analyses in plasma [17]. Diluted plasma was aspirated directly into the AAS flame (AAS: Factory name Varian, model number: AA240FS), used to determine magnesium, zinc and copper concentration.

Statistical Analyses

Results were computed for mean values ± S.E.M. Statistical comparisons between variables were carried out using analysis of variance, and Tukey’s post hoc test was used to compare the differences between the means. Values of P < 0.05 were considered significant.

RESULTS

Malondialdehyde Concentration

Figure 1 show the malondialdehyde (MDA) concentrations obtained in rats. There was a significant (P < 0.001) increase in MDA concentration of the active controls, compared with the passive controls. The MDA concentration significantly (P < 0.001) decreased in all the selenium and vitamin E pre-treated groups, when compared with the active controls. The decrease was more pronounced in the group co-administered with selenium and vitamin E.

Antioxidant Enzymes

The activities of catalase, superoxide dismutase (SOD) and reduced glutathione (GPx) are shown in Table 1. There was a significant (P < 0.001) decrease in the catalase, SOD and GPx activities in the active control group as compared with the passive controls. In all the selenium and vitamin E pre-treated groups, there was a significant (P < 0.001) increase in the mean activities of catalase, SOD, and GPx, when compared with those of the active controls. The increase was more marked in the vitamin E group, followed by the
selenium group, while the least increase in the activities was obtained in the group co-administered with vitamin E and selenium.

Table 1: Effect of selenium and vitamin E on the activities of antioxidant enzymes in rats subjected to water-immersion restraint stress (mean ± SEM, n = 7)

<table>
<thead>
<tr>
<th>Group</th>
<th>Catalase (µmoles of H$_2$O$_2$ consumed/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>GPx (µg of GSH/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passive control</td>
<td>32.00 ± 1.99a</td>
<td>1.84 ± 0.13a</td>
<td>8.33 ± 0.33a</td>
</tr>
<tr>
<td>Active control</td>
<td>13.43 ± 0.82b</td>
<td>0.81 ± 0.04b</td>
<td>5.84 ± 0.28b</td>
</tr>
<tr>
<td>WRS + Se</td>
<td>28.30 ± 1.38a</td>
<td>1.53 ± 0.08a</td>
<td>7.53 ± 0.46a</td>
</tr>
<tr>
<td>WRS + VE</td>
<td>30.97 ± 1.93a</td>
<td>1.46 ± 0.13a</td>
<td>8.51 ± 0.30a</td>
</tr>
<tr>
<td>WRS + Se + VE</td>
<td>23.33 ± 2.33a</td>
<td>1.65 ± 0.13a</td>
<td>8.26 ± 0.19a</td>
</tr>
</tbody>
</table>

b, a = means with different superscript letters are significantly (P < 0.001) different compared with active control (n = 7).


Mineral Concentration

The serum concentrations of Cu, Zn and Mg in rats subjected to WRS are shown in Table 2. There was no significant (P > 0.05) difference in the Cu concentration of active controls when compared with that of passive control. Similarly there was no significant difference (P > 0.05) in Cu concentrations in all the selenium and vitamin E pre-treated groups. The Zn concentration in active controls was 0.515 ppm ± 0.06, which was significantly (P < 0.05), higher when compared with that of the passive controls, 0.215 ppm ± 0.06. Pre-treatment with selenium or vitamin E did not alter Zn concentration significantly (P > 0.05), when the concentration of any of the pre-treated groups was compared with that of the active controls. There was a significant (P < 0.05) decrease in the concentration of Mg, when the concentration of the active controls was compared with that of the passive controls. Pre-treatment with selenium and vitamin E before induction of WRS resulted in a significant (P < 0.05) increase in the serum Mg concentration, when compared with that of the active controls. Pre-treatment with either selenium or vitamin E increased Zn concentration greater than in any other pre-treatment group.

Table 2: Effects of selenium and vitamin E on the concentration of copper, zinc and magnesium in rats subjected to water immersion restraint stress (mean ± SEM, n = 7)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cu (ppm)</th>
<th>Zn (ppm)</th>
<th>Mg (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pass. cont.</td>
<td>0.046 ± 0.03</td>
<td>0.215 ± 0.06*</td>
<td>3.546 ± 0.15*</td>
</tr>
<tr>
<td>Act. Cont.</td>
<td>0.054 ± 0.01</td>
<td>0.515 ± 0.33</td>
<td>1.950 ± 0.44</td>
</tr>
<tr>
<td>WRS + Se</td>
<td>0.043 ± 0.01</td>
<td>0.287 ± 0.04</td>
<td>3.043 ± 0.15*</td>
</tr>
<tr>
<td>WRS + VE</td>
<td>0.096 ± 0.07</td>
<td>0.292 ± 0.12</td>
<td>2.822 ± 0.21*</td>
</tr>
<tr>
<td>WRS + Se + VE</td>
<td>3.252 ± 0.01</td>
<td>0.430 ± 0.04</td>
<td>3.043 ± 0.15*</td>
</tr>
</tbody>
</table>

* = Significant (P < 0.05) differences between active and passive controls, and active controls compared with selenium and vitamin E groups


DISCUSSION

The MDA is a product of lipid peroxidation, which is an indicator of ROS in tissues [18]. It is also a basic indicator of tissue damage in the heart, lungs, small intestine and stomach [11]. In the present study the MDA concentration increased significantly in the active controls, when compared with the passive controls. Thus, the selenium and vitamin E pre-treatments reduced the concentration of the MDA significantly in comparison to the active controls. The results of the study clearly revealed that selenium and vitamin E supplementation may inhibit tissue damage in rats, WRS through prevention of lipid peroxidation. Co-administration of selenium and vitamin E produced considerable decrease in the MDA concentration, compared to selenium or vitamin E alone in the present study.

The findings of the present study is in agreement with result obtained by Jeong-Hwan [11], reported that pre-treatment of 100 µg/kg selenium for three days significantly decreased MDA concentration in ethanol-induced gastric mucosal lesion in rats. The result of the present study agreed with the findings of
Naime[18], who showed that a combination of selenium (a co-factor of GSH-Px) and vitamin E significantly decreased gastric mucosal damage and lipid peroxidation-induced MDA concentrations in rats, subjected to cold restraint stress. The enzymatic antioxidant system, CAT, SOD and GPx, has been shown to counteract the ROS and reduce the oxidative stress [19]. The enzyme SOD accelerates the conversion of superoxide radical to hydrogen peroxide, while CAT or GPx converts hydrogen peroxide to water. The present study also investigated the effect of selenium and vitamin E on the activities of ROS scavenging enzymes, CAT, SOD and GPx in the blood serum as shown in Table 1. The WRS significantly decreased the activities of CAT, SOD and GPx, recorded when the activities of the enzymes in active controls were compared with those of passive controls. Selenium and vitamin E pre-treatment increased significantly the activities of the enzymes, when their activities in pre-treated groups were compared with those of the active controls. The findings of the present study were in agreement with the result of Gulgun [20], who demonstrated that vitamin E inhibits oxidant damage in acetic acid-induced ulcerative colitis in rats. Jeong-Hwan [11], also found that selenium markedly attenuated ethanol-induced lipid peroxidation in gastric mucosa and increased activities of ROS scavenging enzymes, SOD, CAT and GPx. Similar results were obtained by Surajit and Sudipta [21], who showed the considerable beneficial effects of selenium and vitamin E co-administration against arsenic-induced changes in biochemical parameters, indicating that supplementation of vitamin E along with micronutrient, selenium exerts anti-oxidative effects against arsenic-induced cardio-toxicity. Trace elements such as Cu and Zn are required in small amount, usually less than 100 mg/kg dry matter [22], and are present in very minute quantities in animal serum. The present study found no significant difference in serum concentration of Cu between the active controls and passive controls, but a significant increase in Zn concentration in active controls was obtained in comparison to the passive controls. Pre-treatment with selenium and vitamin E had no effects on both Cu and Zn concentrations (Table 2). The result of the present study showed a significant decrease in serum Mg concentration in active controls, when compared with that of passive controls. However, pre-treatment with selenium and vitamin E significantly elevated serum Mg concentration, when the active control was compared with the pre-treated groups. The above findings were in accordance with the result obtained by Antonyuk [23], and Huang [24], that trace elements act as co-factors of enzymes like SOD, GPx and CAT. The elements contribute in antioxidant functions playing vital roles in many physio-biochemical processes, like protein, hormone and enzyme synthesizes [24]. It was concluded that WRS exposure causes significant (P < 0.001) alteration in biochemical parameters and increase in serum antioxidant balance.

REFERENCES


