

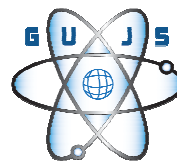
PAPER DETAILS

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Protective Role of Sodium Selenite and Vitamin E Against Rat Erythrocytes Induced by Mercury Chloride

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ABSTRACT

The aim of this study was to investigate the effect of subacute mercury chloride exposure on lipid peroxidation and activities of antioxidant enzymes and the possible protective effect of sodium selenite and/or vitamin E. For this purpose, male Wistar rats were divided into eight groups. It was shown that exposure to mercury chloride caused a significant increase in MDA, while significant decrease in SOD, CAT, GPx, GST activities in erythrocytes. Sodium selenite and vitamin E administration had a beneficial effect on the mercury chloride induced oxidative stress. Our results implicate mercury chloride-induced oxidative stress in erythrocytes protected by sodium selenite and vitamin E.

Keywords: Mercury chloride, Sodium selenite, Vitamin E, Erythrocytes, Oxidative stress

1. INTRODUCTION

Mercury is a widespread environmental and industrial pollutant, which induces severe alterations in the tissues [1]. Inorganic mercury compounds have been used in very extensive range of medical and cosmetic products such as antiseptics, teething powders, skin-lightening creams [1]. It is reported that various mechanisms, including lipid peroxidation, have been proposed for the biological toxicity of mercury chloride and it has been demonstrated that lipid peroxidation occurs in many tissues of experimental animals [2]. Researchers reported that mercury treatment leads to a reduction of effectiveness of the antioxidant enzyme defense system [3].

Erythrocytes contain antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [4]. Therefore, the decrease in the antioxidant capacity of erythrocytes may affect systemic antioxidant defense [5]. Erythrocytes are proposed as bioindicators and pathogenetic determinants of many chronic or acute diseases [6]. Furthermore, they are sensitive to a peroxidative process, since they are rich in nonconjugated polyunsaturated fatty acids in their membranes [7].

The enzymatic and nonenzymatic antioxidants are the natural defense against free radical mediated tissue damage in experimental animals [3,8]. Lipid peroxidation is an autocatalytic mechanism leading to oxidative stress. Cellular membrane destruction can

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lead to cell death and to the production of toxic free radicals [9]. ROS are implicated in damage to various cellular macromolecules including DNA, lipids, proteins, membrane fatty acids [5,10]. Mercury chloride exposure has also been shown to induce lipid peroxidation both *in vitro* and *in vivo* [10, 11].

Sodium selenite and vitamin E have been reported to be a potent free radical scavenger and an antioxidant [12]. Sodium selenite is not only a common dietary form of selenium [13], but also an essential element, and it protects cells against lipid peroxidation [14]. Vitamin E, which is located in cell membranes, has been known one of the most important antioxidant molecules [15]. It has been previously reported that vitamin E is protective against heavy metal induced toxicity [16]. Studies also reported that sodium selenite is known to attenuate tissue oxidative stress induced by heavy metals [17].

The present study was undertaken to investigate if the oral intake of mercury chloride sodium selenite and/or vitamin E could modify the oxidative stress.

2. MATERIALS AND METHODS

2.1. Chemicals

Mercury chloride (99% purity) and sodium selenite (99% purity) were supplied by Sigma Aldrich (Germany). Vitamin E was purchased by Merck (Germany).

2.2. Animals

Male wistar albino rats weighing approximately 300-320 g obtained from the Gazi University Laboratory Animals Growing and Experimental Research Center were used. All rats were housed in an animal holding room with controlled temperature 22 ± 3 and under a well-regulated light-dark (12 h: 12h). The animals fed with standard laboratory diet and top water *ad libitum*. They were quarantined for 10 days before beginning the experiment. All rats were handled in accordance with the standards guide for the care and use of laboratory animals. The Gazi University Committee on the Ethics of Animal Experimentation approved all animal experiments.

2.3. Experimental design

Rats were divided into eight groups, each consisting of six rats (n=6). Control rats (treated with 1 mg/kg bw corn oil per day); sodium selenite treated rats (0.25 mg/kg bw per day in distilled water); vitamin E treated rats (100 mg/kg bw per day in corn oil); vitamin E plus sodium selenite treated rats (100 mg/kg bw+0.25 mg/kg bw per day, respectively); mercury chloride treated rats (1 mg/kg bw per day in distilled water); sodium selenite plus mercury chloride (0.25 mg/kg bw+1 mg/kg bw per day, respectively); vitamin E plus mercury chloride (100 mg/kg bw+1 mg/kg bw per day, respectively); sodium selenite plus vitamin E plus mercury chloride (0.25 mg/kg+100 mg/kg bw+1 mg/kg bw per day, respectively). The substances were administrated in the

morning *via* gavage (between 09:00 and 10:00 h) to non-fasted rats during 28 days. After the treatment, all animals were sacrificed and dissected.

2.4. Blood collection

Blood samples were collected via heart puncture after 28 days of treatment under anesthesia via hearth puncture. The blood was collected in heparinized tubes.

2.5. Erythrocytes preparation

Erythrocytes were sedimented by centrifugation at 1600 rpm at 4°C. The erythrocytes were washed three times with cold isotonic saline. Then, the supernatant and the buffy coat were carefully removed after each wash. The concentration of hemoglobin (Hb) was determined using the method of Drabkin [18]. The supernatants were then obtained and stored at -20 °C until analysis.

2.6. Biochemical measurements

MDA and antioxidant enzyme activities were measured spectrophotometrically by different methods.

2.6.1. Lipid peroxidation assay

MDA content was evaluated using the thiobarbituric acid (TBA) test as described by Ohkawa et al., [19]. Absorbance was measured at 532 nm to determine the MDA content. The specific activity is expressed as U/mg Hb.

2.6.2. Evaluation of antioxidant enzyme activity

SOD activity was measured according to Marklund and Marklund [20] by assaying the autooxidation and illumination of pyrogallol at 440 nm for 3 min. One unit of SOD activity was calculated as the amount of protein that caused 50% pyrogallol autooxidation inhibition. The SOD activity was expressed as U/mg Hb.

CAT activity was measured that is determined according to the method described by Aebi [21] by assaying the hydrolysis of H₂O₂ and the resulting decrease in absorbance at 240 nm over a 3 min period at 25 °C. CAT activity was expressed as U/mg Hb.

GPx activity was measured using H₂O₂ as substrate according to the method described by Paglia and Valentine [22]. The reaction was monitored indirectly as the oxidation rate of NADPH at 240 nm for 3 min. Enzyme activity was expressed as U/mg Hb.

GST activity was assayed by measuring the formation of GSH (Glutathione) and the 1-chloro-2, 4-dinitrobenzene (CDNB) conjugate [23]. Increases in absorbance were recorded at 340 nm for 3 min. The specific activity of GST was expressed as U/mg Hb.

2.7. Statistical analysis

The data were analyzed by using SPSS 11.0 for Windows. Statistical significance of difference was evaluated by using one-way analysis of variance (ANOVA) followed by Tukey's procedure for multiple

comparisons. $P < 0.05$ was considered statistically significant.

3. RESULTS

There is no difference between control, sodium selenite, vitamin E and sodium selenite plus vitamin E treated groups in terms of MDA and antioxidant enzyme activities.

3.1. Effects of treatments on lipid peroxidation

MDA level was significant increase in mercury chloride treated group with respect to control, sodium selenite, vitamin E and sodium selenite plus vitamin E treated groups. There was a significant decrease in MDA levels combined treatment with mercury chloride and sodium selenite, vitamin E, sodium selenite plus vitamin E compared to the only mercury chloride treated group in Figure 1.

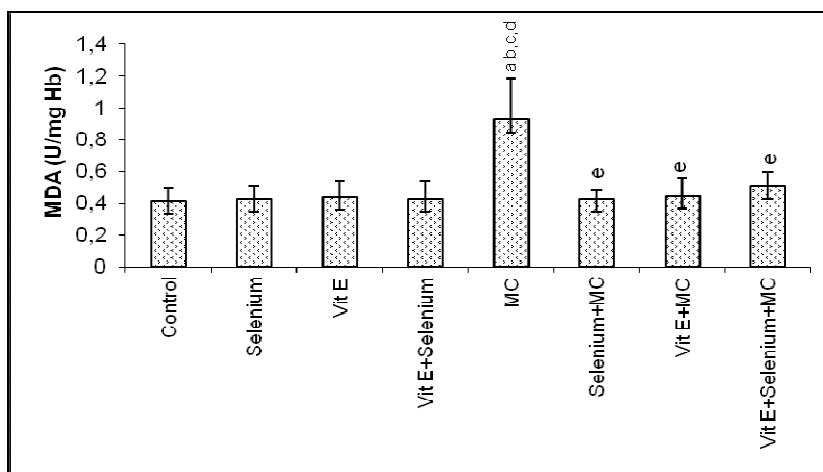


Fig.1. Effects of subacute treatment of mercury chloride on MDA content in rat erythrocytes. ^aComparison of control and other groups, ^bComparison of sodium selenite-treated group and other groups. ^cComparison of vitamin E-treated group and other groups. ^dComparison of Vitamin E+Sodium selenite-treated group and other groups. ^eComparison of mercury chloride-treated group and other groups. Data represents the means \pm SD of six samples. Significance at $P < 0.05$.

3.2. Effects of treatments on antioxidant enzyme activity

Antioxidant enzymes (SOD, CAT, GPx and GST) of rat erythrocytes were determined, and results are shown in Figures 2, 3, 4 and 5 respectively. All the antioxidant enzyme activities were reduced significantly in the

mercury chloride treated group compared to the control, sodium selenite, vitamin E and sodium selenite plus vitamin E treated groups. There was a significant increase in antioxidant enzyme activities treatment with mercury chloride and sodium selenite, vitamin E, sodium selenite plus vitamin E compared to the only mercury chloride treated group.

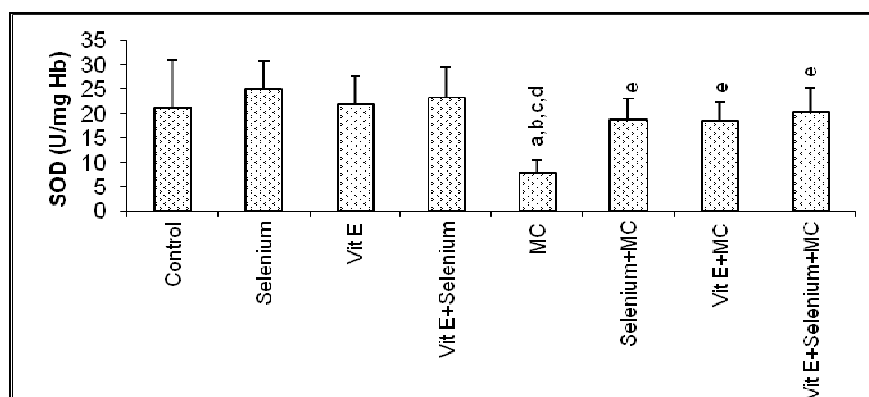


Fig.2. Effects of subacute treatment of mercury chloride on SOD content in rat erythrocytes. ^aComparison of control and other groups, ^bComparison of sodium selenite-treated group and other groups. ^cComparison of vitamin E-treated group and other groups. ^dComparison of Vitamin E+Sodium selenite-treated group and other groups. ^eComparison of mercury chloride (MC)-treated group and other groups. Data represents the means \pm SD of six samples. Significance at $P < 0.05$.

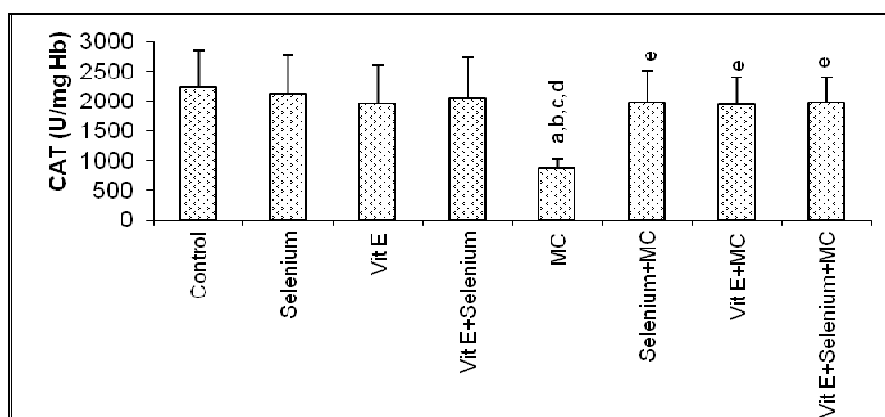


Fig.3. Effects of subacute treatment of mercury chloride on CAT content in rat erythrocytes. ^aComparison of control and other groups, ^bComparison of sodium selenite-treated group and other groups. ^cComparison of vitamin E-treated group and other groups. ^dComparison of Vitamin E+Sodium selenite-treated group and other groups. ^eComparison of mercury chloride-treated group and other groups. Data represents the means±SD of six samples. Significance at $P < 0.05$.

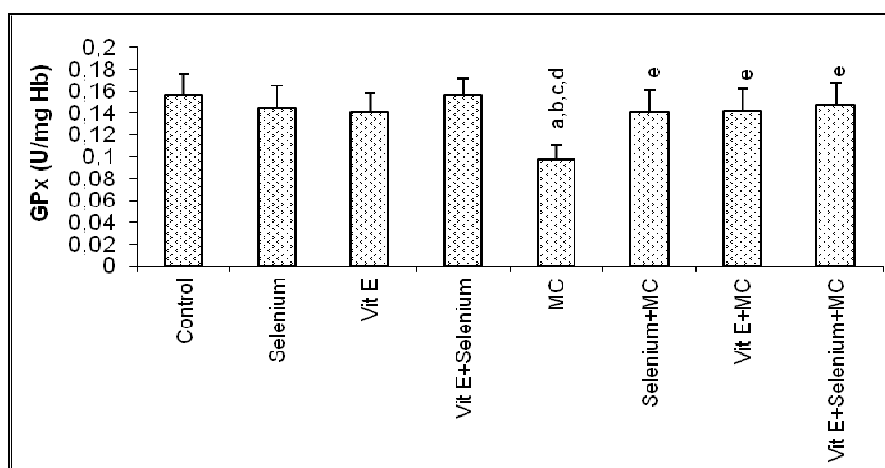


Fig. 4. Effects of subacute treatment of mercury chloride on GPx content in rat erythrocytes. ^aComparison of control and other groups, ^bComparison of sodium selenite-treated group and other groups. ^cComparison of vitamin E-treated group and other groups. ^dComparison of Vitamin E+Sodium selenite-treated group and other groups. ^eComparison of mercury chloride-treated group and other groups. Data represents the means±SD of six samples. Significance at $P < 0.05$.

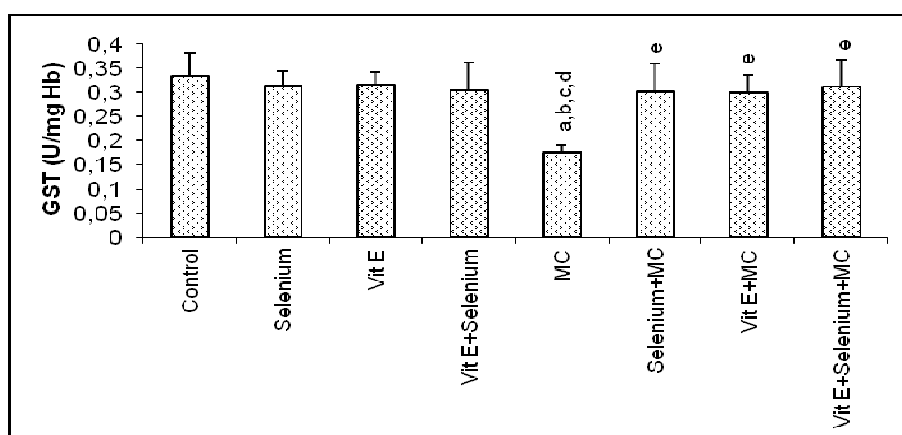


Fig. 5. Effects of subacute treatment of mercury chloride on GST content in rat erythrocytes. ^aComparison of control and other groups, ^bComparison of sodium selenite-treated group and other groups. ^cComparison of vitamin E-treated group and other groups. ^dComparison of Vitamin E+Sodium selenite-treated group and other groups. ^eComparison of mercury chloride-treated group and other groups. Data represents the means±SD of six samples. Significance at $P < 0.05$.

4. DISCUSSION

Exposures to metals especially mercury are frequently related to the development of toxicity and pathological conditions like neurodegenerations [24]. It is well known that heavy metals have adversely effects on erythrocytes [25].

The change in erythrocytic behavior has been observed in a number of pathological conditions as well as exposure to xenobiotic [6]. The erythrocytes are susceptible to oxidative damage due to high percentages of polyunsaturated fatty acids in their membranes [26]. Similarly, it is reported that mercury causes oxidative stress [27].

MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an end product of membrane lipid peroxidation [28]. In this study, exposure to mercury chloride was shown to increase lipid peroxidation of rats. This can be explained by the excessive production of free radicals. Our results show that sodium selenite and vitamin E treatment significantly inhibits MDA production, implying a reduction on lipid peroxidation and cellular injury that protect the tissues against HgCl₂-induced oxidative damage.

It has been reported that Hg (II) can inactivate a number of enzymes by blocking the functional sites through binding to sulphhydryl groups [2]. In this study, a significant decrease in the antioxidant enzymes was observed. This could be due either to a loss of the cells expressing these enzymes, to direct effects of ROS on the enzymes, or due to direct inhibition from Hg (II) causing impairment of the antioxidant function [2]. In previous studies, it is reported that heavy metals cause decrease of antioxidant enzyme activities [25]. SOD-CAT system provides the first line of defense system against oxygen toxicity [29]. Our results revealed that subacute exposure to mercury chloride caused a statistically significant decrease in SOD activity in rat erythrocytes. This decrease may be owed to the consumption of this enzyme in converting the O₂⁻ to H₂O, because the dismutation of O₂⁻ to H₂O is catalyzed by SOD which contains both copper and zinc. Catalase is found as a soluble protein in erythrocytes. CAT is important in eliminating the potentially dangerous formation of H₂O₂ in cells. In our study, we have noted that subacute treatment with mercury chloride caused a significant decrease in CAT activity in rat erythrocytes. Our results supported by previous finding which demonstrated an inductor effect of some chemicals on erythrocyte SOD and CAT activities in rat [29]. Similarly, GPx and GST are also important sensitive indicators of increased oxidative stress [7,29]. GPx modifies the poisonous peroxide to a non-toxic hydroxyl compound for to protect cell membrane structure. For GPx, the decrease in its activity may be the result of O₂⁻ production or a direct action of mercury chloride on the synthesis of the enzyme has been shown to be important indicators of increased oxidative stress and also decreased activity of GST may lead to decrease the protection against oxidants [8,29]. In this study, the

decrease in the SOD, CAT, GPx and GST activities and increase in lipid peroxidation could explain in the induction of free radicals in mercury chloride treated rats.

Vitamin E and selenium complement each other in their function as antioxidants. They prevent free radical production and neutralize the free radicals that are present in the biological matrix [30]. Selenium has been shown to detoxification effects on various heavy metals [31]. Moreover, selenium and vitamin E protect various tissues against damages induced by free radicals [3]. In this study, vitamin E and sodium selenite may have ameliorated effects due to their antioxidative properties.

In conclusion, our results showed that mercury induced a decrease in RBC SOD, CAT, GST and GPx activities and an increase in MDA level in erythrocytes. Vitamin E and sodium selenite supply on exposure to protect against mercury.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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