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Effects of Sodium Selenite Supplementation on Lead Nitrate-induced Oxidative Stress in Lung Tissue of Diabetic and Non-diabetic Rats

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ABSTRACT

In this study, diabetic and non-diabetic male rats were given to sodium selenite, lead nitrate and sodium selenite plus lead nitrate through gavage. At the end of the 4th week, lipid peroxidation and antioxidant enzyme activities were investigated compared to control group. No significant differences were observed between control and sodium selenite treated groups. By the end of the fourth week, lead nitrate led to increase the levels of MDA, and decrease in antioxidant enzyme activities compared with the control group in diabetic and non-diabetic rats. As a result, sodium selenite significantly reduces lead nitrate induced lung toxicity in diabetic and non-diabetic groups.

Key Words: *Lead nitrate, sodium selenite, oxidative stress, diabetes, lung*

1. INTRODUCTION

Lead is one of the most common heavy metals in the environment [1,2]. It has many industrial and environmental pollutants [3]. It has number of harmful effects including neurological [4], immunological [5], hepatic [6], and especially hematological [7] dysfunctions. It was reported that lead increased level of lipid peroxidation [8] and also caused many histopathological alterations in several tissues like liver, kidney and brain [9]. It has been shown that lead exposure resulted in increased oxidative stress. Heavy metal toxicity has been related to generation of reactive oxygen species (ROS) [10,11].

Diabetes mellitus (DM) is a metabolic endocrine disorder. It is primary characterized by hyperglycemia and is related with the imbalance in protein, lipid and carbohydrate metabolism [12].

Selenium (Se) is well known antioxidant as its wide range of pleitropic effects and also an essential dietary trace element [3,10]. Additionally, it is known that

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sodium selenite is a common dietary form of selenium [13].

In the present study, we determined the possible adverse effects of lead nitrate on the lung tissue of diabetic and non-diabetic male rats and assess whether these effects can be ameliorate by co-administration with sodium selenite. To achieve this aim, diabetic and non-diabetic rats were given lead nitrate and sodium selenite by oral gavage for 4 weeks, then malondialdehyde (MDA) levels, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) activities were assessed.

2. MATERIAL AND METHODS

2.1. Animals

Adult male Wistar rats (weighing approximately 200-250 g) were procured from the Gazi University Laboratory Animals Growing and Experimental Research Center. Animals were housed in plastic cages, fed a standard laboratory diet and water ad libitum. Rats were exposed to a 12 h light/dark cycle, and maintained at 22±2 ºC. The animals were quarantined for 10 days before beginning the experiment. All rats were handles 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.

2.2. Chemicals

Lead nitrate (99% purity), streptozotocin (STZ, %99 purity) and sodium selenite (99% purity) were supplied by Sigma-Aldrich (Germany). All other chemicals used were analytical grade and also were obtained from Sigma-Aldrich (Germany).

2.3. Animal treatment schedule

The rats were divided into two main groups: non-diabetic (n = 24) and diabetic (n = 24).

Rats in the non-diabetic group were subdivided in four treatment groups, each consisting of six rats:

Group 1 - control rats

Group 2 – sodium selenite treated rats (1 mg/kg bw per day in distilled water)

Group 3 – lead nitrate treated rats (22,5 mg/kg bw per day in distilled water)

Group 4 – sodium selenite plus lead nitrate treated rats (1 mg/kg bw+ 22.5 mg/kg bw per day, respectively)

Diabetes was induced with single intraperitonal injection of STZ (55 mg/kg, freshly dissolved in 0,1M sodium citrate buffer, pH=4,5). Two days after injection, blood samples were collected via the tail vein, and glucose concentrations were measured by a strip-operated blood glucose sensor (Accu-Check Go, Blood glucose marker, Germany). Diabetes was confirmed by blood glucose level >300mg/dL and then subdivided in four treatment groups, each consisting of six rats:

Group 5 –control diabetic rats

Group 6 – sodium selenite treated diabetic rats (1 mg/kg bw per day in distilled water)

Group 7 – lead nitrate treated diabetic rats (22.5 mg/kg bw per day in distilled water)

Group 8 –sodium selenite plus lead nitrate treated diabetic rats (1 mg/kg+22,5 mg/kg bw per day, respectively).

The doses used in this study were chosen on the basis of previous studies [14,15]. The substances were administrated in the morning (between 09:00 and 10:00 h) to non-fasted rats. The first day the animals were treated was considered experimental day 0. At the end of the 4th week (28 days) of treatment, the rats in each group were sacrificed and dissected. The lung tissues were quickly taken to assess antioxidative enzyme activities (SOD, CAT, GPx and GST activities), and malondialdehyde (MDA) levels.

2.4. Biochemical estimation

The lung tissues were dissected and washed in sodium phosphate buffer (pH 7.2). After washing, samples were taken and store at -80 ºC until analysis. The tissues were homogenized using a Teflon homogenizer (Heidolph Silent Crusher M), and then the homogenates were centrifuged at 10,000g for 15 min at +4 ºC. MDA content and antioxidant enzyme activities were determined by measuring the absorbance of the samples in a spectrophotometer (Shimadzu UV 1700, Kyoto, Japan). Protein content of supernatant was determined by the method of Lowry [16] using bovine serum albumin as standard.

2.4.1. Lipid peroxidation assay

MDA is the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems and is commonly used as an indirect index of lipid peroxidation. MDA content was assayed using the thiobarbituric acid (TBA) test as described by Ohkawa [17]. After incubation of lung homogenate with TBA at 95°C, MDA reacts to form a pink colored complex. Absorbance was measured spectrofotometrically at 532 nm to determine the MDA content. The specific activity is expressed as nmol/mg protein.

2.4.2. Measurement of superoxide dismutase (SOD)

Total SOD activity was determined according to the method described by Marklund and Marklund [18] 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 is expressed as U/mg protein.

2.4.3. Measurement of catalase (CAT)

Before determination of the CAT activity, lung homogenates were diluted 1:9 with 1% (v/v) Triton X-100. CAT activity was measured according to the method described by Aebi [19] by assaying the hydrolysis of H_2O_2 and the resulting decrease in absorbance at 240 nm over a 3 min period at 25 ^oC. CAT activity is expressed as mmol/mg protein.

2.4.4. Measurement of glutathione peroxidase (GPx)

GPx activity was measured using H_2O_2 as substrate according to the method described by Paglia and

Valentine [20]. The reaction was monitored indirectly as the oxidation rate of NADPH at 240 nm for 3 min. Enzyme activity was expressed as nmol/mg protein.

2.4.5. Measurement of glutathione-S-transferase (GST)

GST activity was assayed by measuring the formation of GSH (Glutathione) and the 1-chloro-2, 4-dinitrobenzene (CDNB) conjugate [21]. Increases in absorbance were recorded at 340 nm for 3 min. The specific activity of GST is expressed as μmol/mg protein.

2.5. Statistical analysis

The data were analyzed using SPSS 11.0 for Windows. The statistically significance of differences 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

3.1. Evaluation of biochemical parameters

At the end of the 4th week, there were no statistically significant changes in MDA levels, SOD, CAT, GPx and GST activities between the non-diabetic sodium selenite treated group compared to the control group and the diabetic sodium selenite treated group compared with the diabetic control group. At the same time, there were no significantly differences in the diabetic control and diabetic sodium selenite treated groups compare to non-diabetic control and sodium selenite treated group in SOD, CAT, GPx and GST activities ($P < 0.05$, Figs. 1-5).

3.1.1. Malondialdehyde (MDA) levels

MDA levels in lung tissues significantly increased in the lead nitrate and sodium selenite plus lead nitrate treated groups compared to the control group, while there were decreased in the sodium selenite plus lead nitrate treated group compared to the only lead nitrate treated in non-diabetic group. When diabetic lead nitrate and diabetic sodium selenite plus lead nitrate treated groups compared with the diabetic control group, there were significantly increased in MDA levels. Whereas, a significant decrease in MDA levels was observed in diabetic sodium selenite plus lead nitrate treated group compared to the diabetic control group. Besides, there was statistically increased in the all diabetic groups compared to non-diabetic groups in MDA levels ($P < 0.05$, Fig. 1).

3.1.2. Antioxidant activities

There is a significantly decrease in SOD, CAT, GPx and GST activities in the lead nitrate and sodium selenite plus lead nitrate treated groups compared to the control group in the lung tissues. However, SOD, CAT, GPx and GST activities increased in the sodium selenite plus lead nitrate treated group compared to the only lead nitrate treated group. Compared to the diabetic control and non-diabetic control groups, there were statistically significantly decreased in the SOD, CAT, GPx and GST activities in the diabetic lead nitrate and diabetic sodium selenite plus lead nitrate treated groups at the end of the 4th week, while there was increased in the diabetic sodium selenite plus lead nitrate treated group compared with the diabetic lead nitrate treated group in lung tissues ($P < 0.05$, Fig. 2-5).

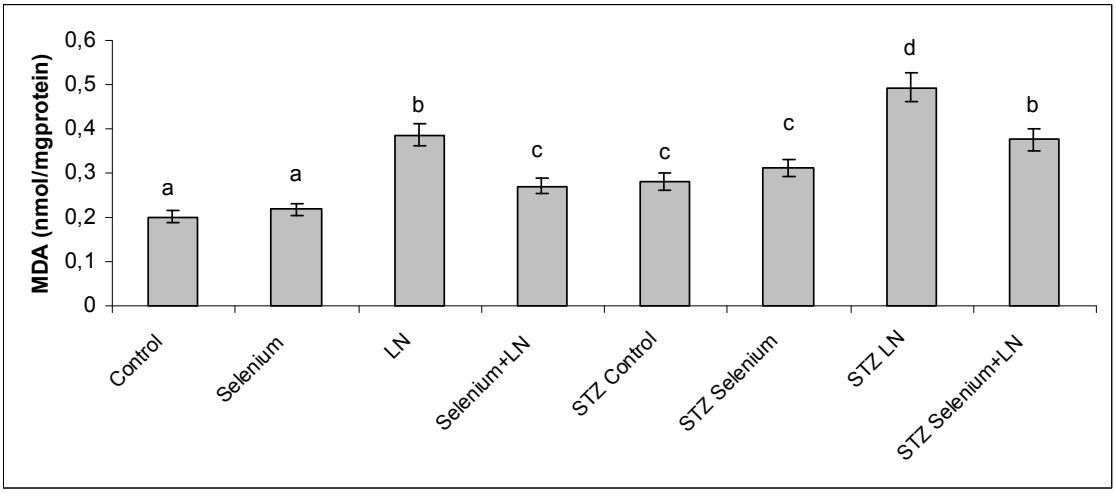

Figure 1. Effects of lead nitrate and sodium selenite (selenium) on MDA levels (nmol/mg protein) in the lung tissues of rats. Each bar represents mean+SD of six animals in each group. Bars superscripts with different letters are significantly different. Significance at $P < 0.05$.

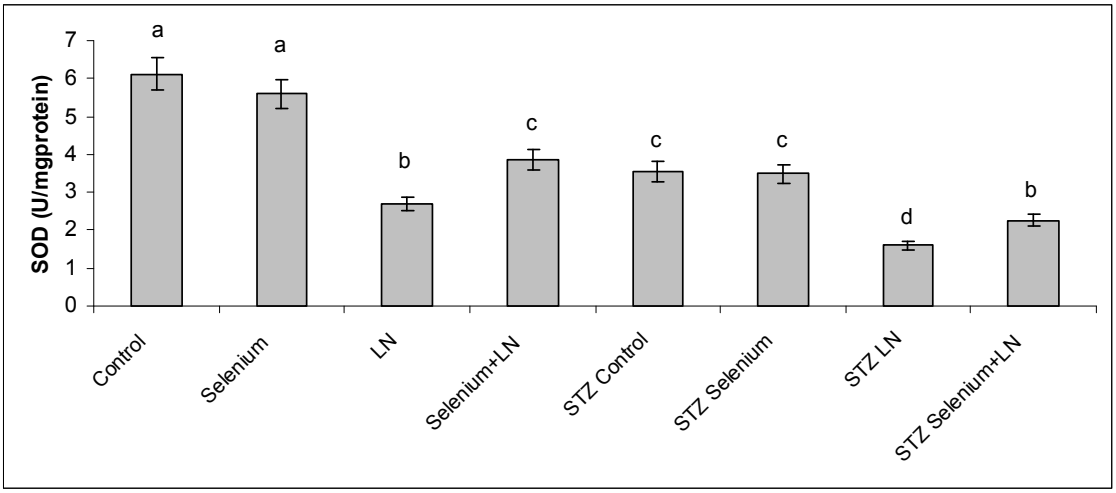

Figure 2. Effects of lead nitrate and sodium selenite (selenium) on SOD levels (nmol/mg protein) in the lung tissues of rats. Each bar represents mean+SD of six animals in each group. Bars superscripts with different letters are significantly different. Significance at $P < 0.05$.

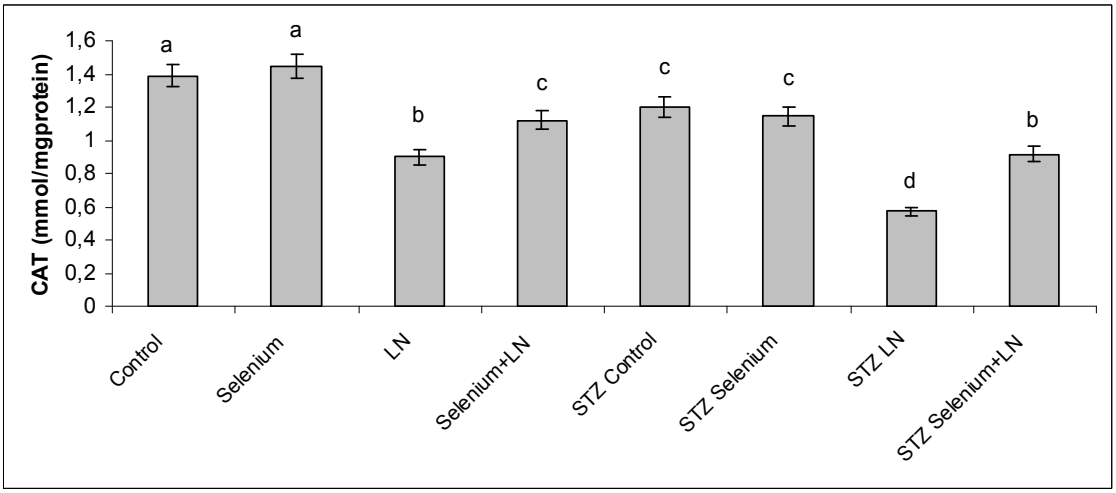

Figure 3. Effects of lead nitrate and sodium selenite (selenium) on CAT levels (μmol/mg protein) in the lung tissues of rats. Each bar represents mean+SD of six animals in each group. Bars superscripts with different letters are significantly different. Significance at $P < 0.05$.

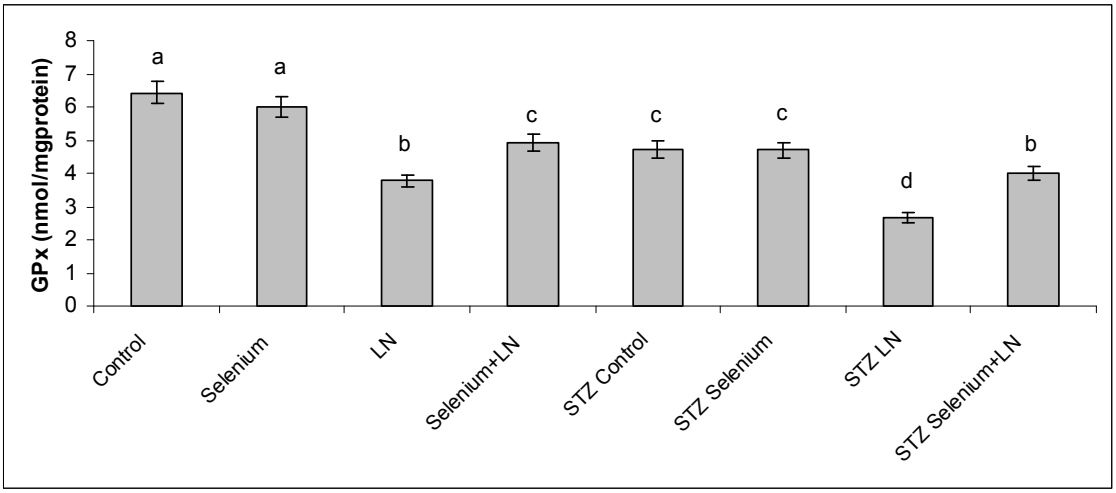

Figure 4. Effects of lead nitrate and sodium selenite (selenium) on GPx levels (nmol/mg protein) in the lung tissues of rats. Each bar represents mean+SD of six animals in each group. Bars superscripts with different letters are significantly different. Significance at $P < 0.05$.

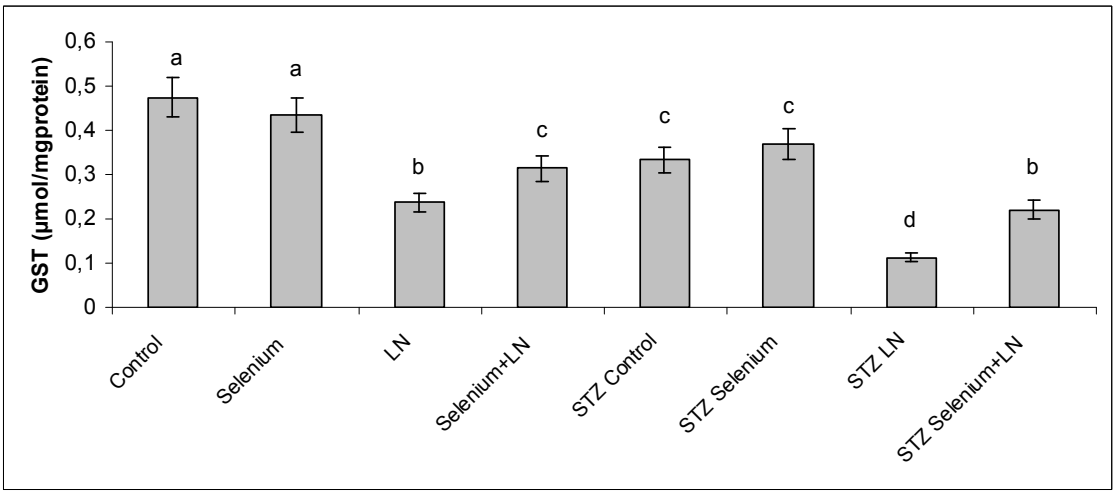

Figure 5. Effects of lead nitrate and sodium selenite (selenium) on GST levels (nmol/mg protein) in the lung tissues of rats. Each bar represents mean+SD of six animals in each group. Bars superscripts with different letters are significantly different. Significance at $P < 0.05$.

4. DISCUSSION

Lead is an important environmental toxicant that induces a broad range of dysfunctions [22]. For example previous studies, it is reported that lead cause alter serum testosterone level and also sperm quality in rats [23].

Diabetes mellitus remains one of the leading cause syndromes [24]. The production of reactive oxygen species (ROS) is central to the pathogenesis of diabetes [24]. The lung has a rich network of antioxidant defenses to protect itself from oxidative stress [25]. Alireza et al., reported in their study that diabetes induces lung pathology in rats [26]. Although various studies have shown that oxidative stress mediates diabetes-induced alterations in various tissues [27].

A complex antioxidant defense system (SOD, CAT, GPx and GST) and non-enzymatic antioxidants, which are the line of defense against oxidants [28,29]. Oxidative stress is caused by an imbalance between the productions of reactive oxygen species [30]. Oxidative damage may occur when antioxidant potential is changed and oxidative stress is increased. SOD is an important in biological body and widely distributed in all kinds of organisms. SOD has special physiological activity as a primary tool to scavenge free radicals [31]. It reduces superoxide radicals to water and molecular oxygen. CAT reduces hydrogen peroxide. GPx is an antioxidant enzyme, which modifies the poisonous peroxide to a non-toxic hydroxyl compound in order to protect membrane structure and function [29]. GST, GST catalyzes the conjugation of several electrophilic substrates to the thiol group of GSH so it produces less toxic forms [32]. Lipid peroxidation (LPO) is known to have deleterious effects on structure and function of cell membranes [33]. MDA is an end-product by peroxide decomposition of unsaturated fatty acid. It is an indicator of tissue damages [30]. Previous studies researchers showed that parallel

results like this study [11, 32]. Our study, oxidative stress has also been implicated to contribute to lead-associated lung tissue injury.

Liu et al., reported that Se supplements could alleviate toxic effect of lead in their study [3]. Furthermore, some researchers shows that selenium inhibit the absorption of lead in intestinal system [34]. Selenium may therefore play a protective role on toxicity caused by lead.

In the present study, it is evident that lead nitrate caused oxidative stress in lung tissues including, LPO and disturbances in antioxidant enzyme activities both diabetic and non-diabetic rats. However, sodium selenite has manifested beneficial effects against oxidative stress.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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