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Lead Nitrate Induced Oxidative Stress in Brain Tissues of Rats: Protective Effect of Sodium Selenite

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ABSTRACT

Lead used in many industrial applications and it has been recognized as a toxic metal. The aim of this study is to investigate the effects of lead nitrate on brain tissues of diabetic and non-diabetic rats and the protective role of sodium selenite. Four weeks later, changes in antioxidant enzyme activities and MDA levels determined in the brain tissues. At the end of the study, we showed that lead nitrate causes oxidative stress in diabetic and non-diabetic rat brain and the sodium selenite can ameliorate this toxicity, but not protect completely.

Key Words: *Lead Nitrate, Sodium selenite, Antioxidant Enzymes, Oxidative stress, Brain*

1. INTRODUCTION

Environmental pollution lead to health risks like as diseases nearly in all organ systems. Humans, at the top of the food chain, were exposed to different kind of environmental pollutants at different stages of life. Metals have an important place among other chemical pollutants because they are resistant to environment conditions and they can accumulate in the environment and they can pass to food chain [1]. Some of heavy metals are essential for life because they are found in some molecule's structure. On the other hand, some heavy metals such as lead, mercury and cadmium are very toxic for body [1].

Lead is a heavy metal that used in many industrial applications such as lead-acid batteries, cosmetics, hair coloring dyes, printing dyes and leaded crystal ware. Exposure to lead is damaging to the central nervous system and learning qualities. Recent studies remark that there may be neurotoxic effects of lead at lower levels of exposure [2].

Lead is considered to reduce the antioxidant levels and enhanced the free radical concentration and lipid peroxidation [3]. Free radicals contain one or more unpaired electrons [4]. They play a major role in biochemistry, plasma chemistry and a lot of other chemical processes in the human physiology [5]. In normal cells, generation of reactive oxygen species (ROS) is put in order by biological antioxidants and antioxidant enzymes. However, excessive production of these species causes oxidative damage to cellular molecules such as lipids, carbohydrates, proteins and

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DNA [4]. Cells are protected against oxidative stress by enzymatic and nonenzymatic antioxidant systems. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) are accounted among principal antioxidant enzymes [6].

Diabetes mellitus is the most common endocrine illness and it is characterized by increased blood glucose levels. This increasing is a result from defective insulin secretion and resistance to it [7]. This disease causes morbidity and mortality by way of the development of vascular complications like as nephropathy and cardiovascular diseases. It has been guessed that patients exceed over 200 million in the world and its extensity is quickly increasing. Diabetes mellitus is majorly spreading and it is regarded as a great health problem [8].

Selenium is usually identified to be a trace element of great importance for health. It is known that it protects cells from the unhealthy effects. Selenium neutralizes cancer damage together with increases our resistance to infections [9]. Selenoenzymes like as gluttahione peroxidase (GPx) and thioredoxin reductase, require one atom of selenium at their active site to be functional and this enzymes protect cells against damages related to oxidative stres [10]. Kalender et al. showed that sodium selenite ameliorated mercuric chloride-induced testicular toxicity in rats [6].
In the present study, we determined the possible adverse effects of lead nitrate on the brain tissues of diabetic and non-diabetic male rats and asses 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/or sodium selenite by oral gavage for 4 weeks, then malondialdehyde (MDA) levels and SOD, CAT, GPx and GST activities were assessed in their brain tissues.

2. MATERIAL AND METHODS

2.1. Animals and Chemicals

Lead nitrate (LN), sodium selenite, streptozotocin (STZ) and all the other chemicals were obtained from Sigma Aldrich. For this study 48 rats (weighting 200-250 g) were procured from the Gazi University Laboratory Animals Growing and Experimental Research Center. For 10 days rats were kept in quarantine before starting of the study. They were housed in plastic cages at 22±2 ^{0}C. Animal experiments were assented by the Gazi University Animal Experiments Local Ethics Commitee.

2.2. Animal Treatment Schedule

Eight groups (six rats in each groups) were constituted for this study. These are:

(Group 1) Control rats, (Group 2) sodium selenite-treated rats, (Group 3) lead nitrate-treated rats, (Group 4) lead nitrate and sodium selenite-treated rats, (Group 5) control diabetic rats, (Group 6) sodium selenite-treated diabetic rats, (Group 7) lead nitrate-treated diabetic rats, (Group 8) lead nitrate and sodium selenite-treated diabetic rats.
During 4 weeks, 1ml/ kg b.w (body weight) distilled water for control and diabetic control groups; 1ml/ kg b.w sodium selenite for sodium selenite, diabetic sodium selenite, LN+Sodium selenite and diabetic LN+Sodium selenite groups; 22,5 mg/kg b.w (1/100 LD_{50}) LN [11] for LN, diabetic LN, LN+Sodium selenite and diabetic LN+Sodium selenite groups was given to rats daily via gavage. At the end of the study, rats were dissected using combination of ketamin+xylazin than brain tissues were taken for investigations about MDA and antioxidant enzymes. These parameters were measured with spectrophotometer (Shimadzu UV 1700).

2.3. Induction of Diabetes Mellitus

STZ was used to produce type I diabetes. It was prepared in sodium citrate buffer (pH 4.5) and injected intraperitonally at a single dose of 55 mg/kg. Two days after STZ injection, progress of diabetes mellitus was affirmed by measuring blood glucose levels. Animals whose blood glucose levels of 300 mg/dl or higher were approved to be diabetic [12].

2.4. Measurement of Malondialdehyde

MDA level was measured by the method of Ohkawa et al. [13]. It was analysed using the thiobarbituric acid test and measured at 532 nm. Levels of MDA were stated as nmol/mg protein.

2.5. Measurement of Antioxidant Enzymes

The activity of SOD was analysed using Marklund and Marklund's method [14]. Data was expressed as nmol/mg protein. CAT activity was obtained accordingto the method of Aebi [15]. The activity of CAT was defined as μmol/mg protein. GPx activity was measured in brain tissues by a method previously described by Paglia and Valentine [16]. Specific activity of GPx was given as nmol/mg protein. Acording to method of Habig et al. GST activity was obtained [17]. Data was given as nmol/mg protein.

2.6. Statistical Analysis

The data were eveluated by using SPSS 11.0 for Windows. All statistical analyses were performed by a one way analyses of variance (ANOVA) followed by Tukey. Statistically differences were considered when $P<0.05$.

3. RESULTS

MDA levels and SOD, CAT, GPx, GST activities of brain tissues were measured in all groups of rats. These parameters were not different in sodium selenite treated and control group animals, and in diabetic control and diabetic sodium selenite treated animals. At the end of the treatment (after four weeks), LN treated and LN+sodium selenite treated groups showed increasing of MDA level (Figure 1) and decreasing of SOD, CAT, GPx, GST activities when compare with control group

(Figures 2, 3, 4, 5) (P<0,05). However, when compared with LN+sodium selenite treated group with LN treated group there were significantly decreasing in MDA levels and significantly increasing in antioxidant enzyme activities. Similar results were observed in diabetic control, diabetic sodium selenite, diabetic LN and diabetic LN+sodium selenite treated groups.

When compared control with diabetic control group, sodium selenite with diabetic sodium selenite group, LN with diabetic LN group, LN+sodium selenite with diabetic LN+sodium selenite group, a significant increment in MDA levels and a significant reduction in SOD, CAT, GPx, GST activities were determined in diabetic groups.

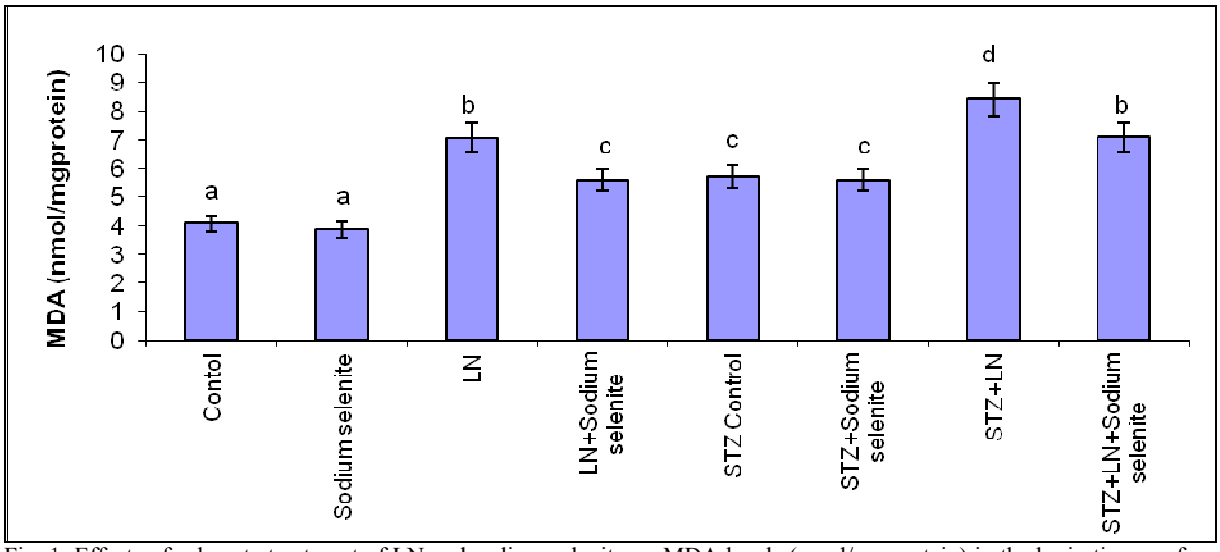

Fig. 1. Effects of subacute treatment of LN and sodium selenite on MDA levels (nmol/mg protein) in the brain tissues of rats. Each bar represents mean±SD of six animals in each group. Bars superscripts with different letters are significantly different. Significance at $P < 0.05$.

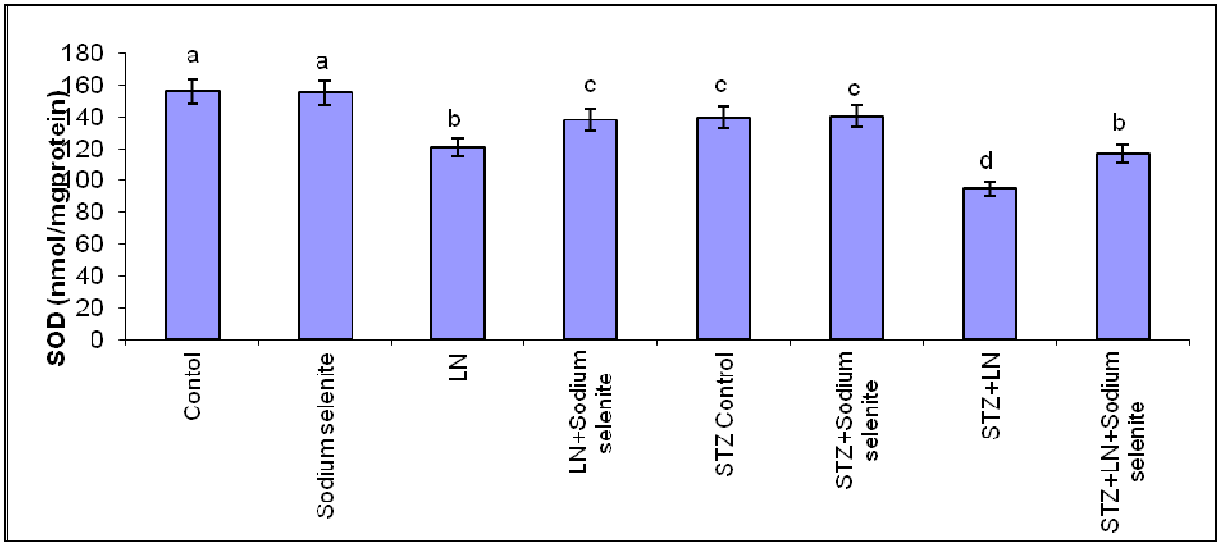

Fig. 2. Effects of subacute treatment of LN and sodium selenite on SOD levels (nmol/mg protein) in the brain tissues of rats. Each bar represents mean±SD of six animals in each group. Bars superscripts with different letters are significantly different. Significance at $P < 0.05$.

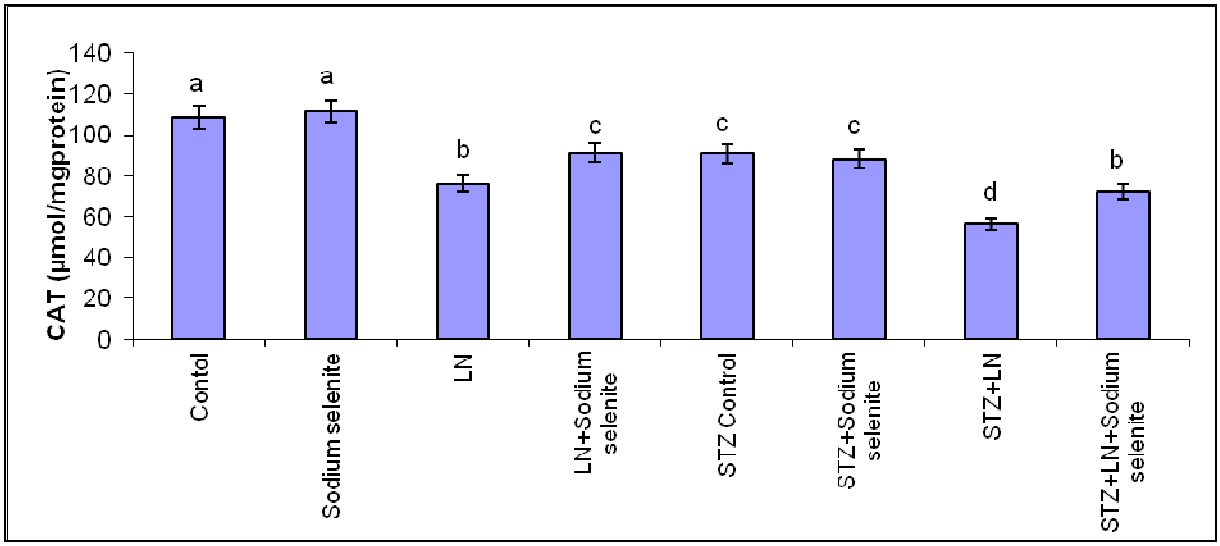

Fig. 3. Effects of subacute treatment of LN and sodium selenite on CAT levels (µmol/mg protein) in the brain tissues of rats. Each bar represents mean±SD of six animals in each group. Bars superscripts with different letters are significantly different. Significance at $P < 0.05$.

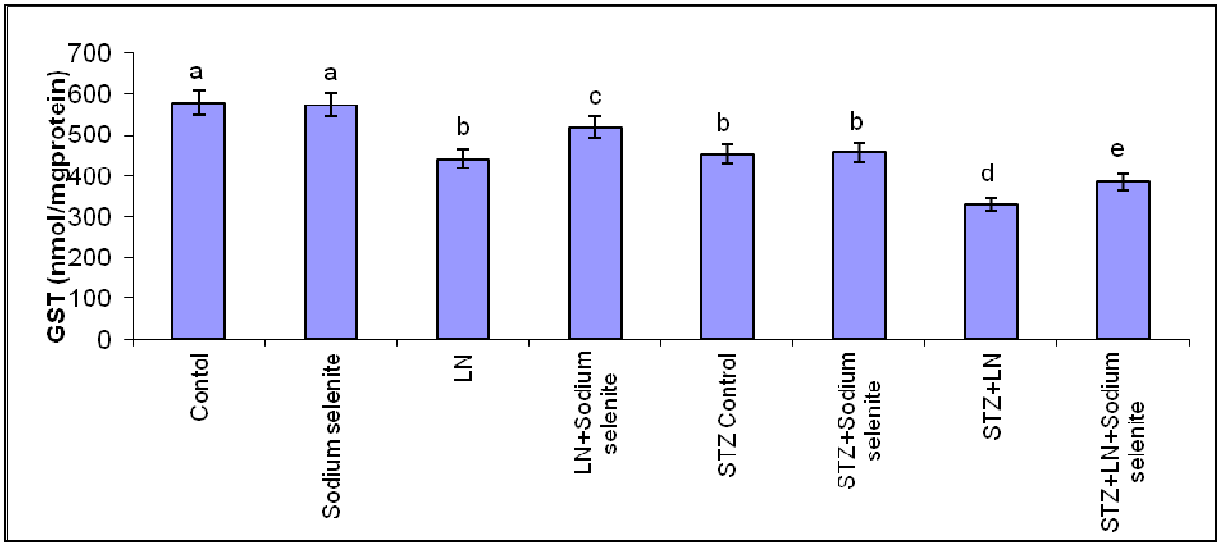

Fig. 4. Effects of subacute treatment of LN and sodium selenite on GST levels (nmol/mg protein) in the brain tissues of rats. Each bar represents mean±SD of six animals in each group. Bars superscripts with different letters are significantly different. Significance at $P < 0.05$.

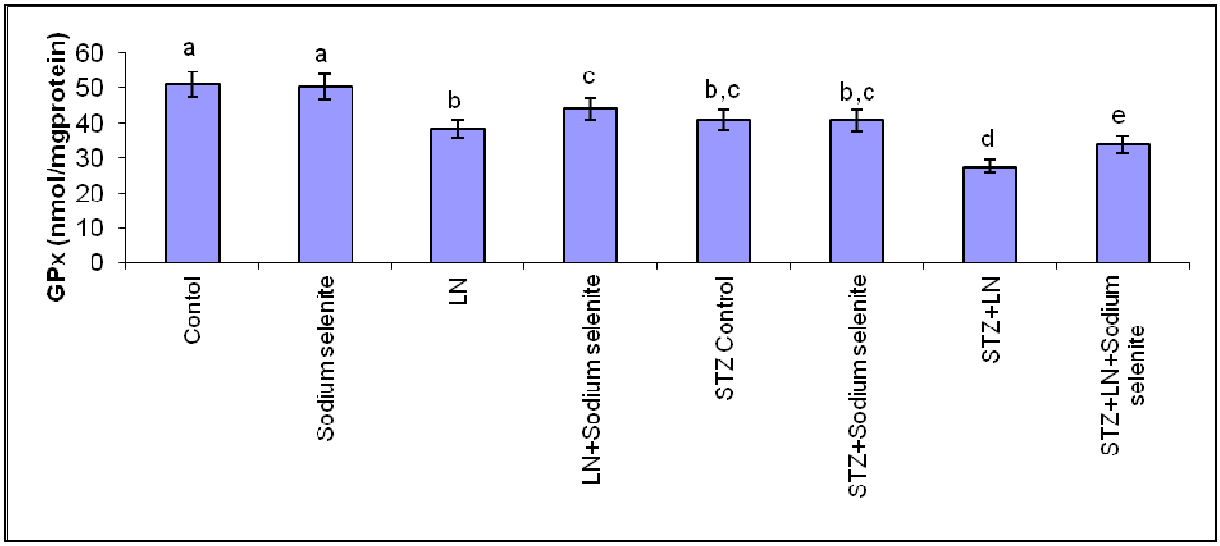

Fig. 5. Effects of subacute treatment of LN and sodium selenite on GPx levels (nmol/mg protein) in the brain tissues of rats. Each bar represents mean±SD of six animals in each group. Bars superscripts with different letters are significantly different. Significance at $P < 0.05$.

4. DISCUSSION

Lead is known cross the blood–brain barrier [18] and cause lots of toxicological problems like as inhibiting several enzyme activities [19, 20]. In addition, it is reproted that lead has toxic effects on brain, liver, bone, hematopoietic system and immune system [21]. In the present study, even though lead nitrate was given orally at low dose to diabetic and non-diabetic rats, lipid peroxidation and antioxidant enzymatic changes were observed in rat brain tissues, but none of the rats died during the experimental period.

MDA is an essential oxidation product of peroxidation of polyunsaturated fatty acids. Consequently, escalated MDA level is a substantial lipid peroxidation indicator [22, 23]. It is reproted that nervous system cells of both human and animals are specially vulnerable to oxidative damage caused by free radicals due to high concentration of membrane lipid polyunsaturated fatty acid, low level of protective antioxidant enzymes and extended axonal morphology prove to peripheral injury [24]. In the previous studies, lead has been increased MDA level in brain tissues of experimental animals [25, 26]. In the present study, MDA levels increased in the all LN treated groups. This increase might have resulted from an increment of free radicals as a result of oxidative stress in rats with LN treatment.

It is recorded that one of the major mechanisms behind heavy metal neurotoxicity has been attributed to oxidative stress [24]. Antioxidant enzymes limit the effects of oxidant molecules and they are active in the defense counter to oxidative cell damage via their free radical scavenger property [27]. SOD is an antioxidant enzyme which acts like the first line of defense against harmful effects of free radicals. It catalyzes the dismutation of superoxide radicals to molecular oxygen and hydrogen peroxide (H_2O_2) in cells. For detoxification of hydroperoxides, GPx is the most substantial peroxidase. CAT protects the SOD against H_2O_2 caused inactivation. Because of this, CAT and GPx have been thought of the principal H_2O_2 scavengers. GST is one of the antioxidant enymes that transform toxic materials into materials which have less toxicity. Therefore, the balance of these enzymes is necessary to overcome the superoxide anion and generating of peroxides [28]. Lead has high affinity for sulfhydryl groups of enzymes and molecules. Therefore, it binds to the –SH groups of various enzymes such as SOD and CAT and inhibits these enzyme activities. The inhibitory effect result in impaired antioxidant defenses and cause of oxidative stress is cells [29]. Many researchs have beeen demostrated that lead decrease antioxidant enzymes activities in brain tissues of experimental animals [26, 29]. In this study, SOD, CAT, GPx and GST activities decreased in LN treated groups. The reduction in the activities of antioxidant enzymes could indicate the adverse effects of LN on balanced antioxidant system. Similarly, in previous studies many environmental pollutants like pesticides may cause changes in antioxidant enzymes and MDA levels in different tissues of experimental animals [30, 31].

It has been indicated that oxidative stress participited in the pathogenesis and progression of many degenerative disorders including naturally occurring and chemically-induced diabetes mellitus. Also, in addition to increased production of free radicals, an antioxidant defence system has been known disturbed in diabetes mellitus [32]. Therefore, some investigations about diabetes are based on enhancing oxidant/antioxidant balance [33]. Experimental studies have been shown that in the various tissues of diabetic rats increased lipid peroxidation and altered antioxidant enzymes such as SOD, CAT and GPx [34, 35, 36]. In this study, we observed that diabetic rats have been increased lipid peroxidation and decreased antioxidant enzymes activities. Also, when compared with diabetic rats non-diabetic rats, we were determined increasing in MDA levels and reducing in activities of SOD, CAT, GST and GPx in diabetic groups. Thus, we can say diabetic rats

are more susceptible to lead nitrate-induced oxidative stress than non-diabetic rats in brain tissues.

There were several studies about selenium, in most of these; it was given to experimental animals in sodium selenite form [37]. It is known that selenium roles detoxification of toxic heavy metals [38] and has a strong tendency to form complexes with heavy metals [39]. In the present study we observed sodium selenite has protective effects against oxidative stress and lipid peroxidation in brain tissues of diabetic and non-diabetic rats. Sodium selenite may be indirectly scavenger of ROS or increase antioxidant enzyme activities; therefore it may prevent the neurotoxicity produce by lead nitrate and/or diabetes.

Biochemical evaluations showed that LN has toxic effects in brain tissues of rats. Also, in the view of the present results, we demonstrated that sodium selenite treatment may have ameliorative effects against LN treatment in diabetic and non-diabetic rats.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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