Lead Nitrate Induced Testicular Toxicity in Diabetic and Non-Diabetic Rats: Protective Role of Sodium Selenite

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ABSTRACT

Among heavy metals, lead is one of the common pollutants found in the environment and biological system. In the present study, streptozotocin-induced diabetic and normal non-diabetic male Wistar rats were given sodium selenite (1.0 mg/kg bw), lead nitrate (22.5 mg/kg bw) and sodium selenite plus lead nitrate (1.0 mg/kg+22.5 mg/kg bw, respectively) through gavage. At the end of 4th week, malondialdehyde (MDA) levels, antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST)], and histopathological changes of testes were investigated compared to the control group. No significant differences were observed between the control and sodium selenite treated groups. However, lead nitrate increased the levels of MDA, SOD, CAT, GPx and GST activities compared with the control group in diabetic and non-diabetic rats. Light microscopic analyses revealed that lead nitrate induced numerous histopathological changes in testis tissues of diabetic and non-diabetic rats. In the diabetic and non-diabetic sodium selenite plus lead nitrate treated groups, there were statistically significantly decreased MDA levels and antioxidant enzymes activities and mild pathological changes. As a result, sodium selenite significantly reduced lead nitrate induced testicular toxicity for both diabetic and non-diabetic rats.

Key words: Testicular toxicity, Lead nitrate, Sodium selenite, Streptozotocin, Oxidative stress, Histopathology

INTRODUCTION

Among the toxic chemicals, metals are critical environmental toxicants because they possess bioaccumulative and non-biodegradable properties and are harmful for ecological systems (Sainath et al. 2011). Lead has been one of the most important heavy metals because of its common usage in various industrial products, and therefore, is considered as a serious occupational hazard throughout the world (Ahmed et al. 2012). It is well known that testes are very sensitive to oxidative stress due to its higher polyunsaturated fatty acid content (Acharya et al. 2003). It has been shown that lead exposure enhances intracellular ROS production and lipid peroxidation, including tissue damage in animal reproductive systems (Wang and Jia 2009). It has also been reported that lead alters the activities of antioxidant enzymes, such as glutathione peroxidase, catalase and superoxide dismutase in various experimental animals (Flora et al. 2004a; Farmand et al. 2005).

Diabetes mellitus, a metabolic disorder, is characterized by fasting hyperglycemia, deficient insulin secretion or insulin receptor insensitivity (Degirmenci et al. 2005; Chen et al. 2009). It is known that diabetes mellitus affects more than 100 million people worldwide and is considered as one of the five leading causes of death in the world.
It has been reported that under diabetic conditions, persistent hyperglycemia may cause high ROS production via glucose auto-oxidation and/or protein glycation in various tissues (Saddala et al. 2013). Also, there are reports on the altered antioxidative enzymes activities and increased lipid peroxidation in animal models and humans with diabetes (Kade et al. 2009a; Schmatz et al. 2012; Prabakaran and Ashokkumar 2013).

Selenium, an essential element, has physiological antioxidant properties and occurs in the body as selenocysteine, a structural component of several enzymes such as glutathione peroxidase (Kade et al. 2009a). It has been shown to exert insulin-like actions on the glucose homeostasis of diabetic rats and has a large number of other biological functions (Kade et al. 2009b). It is known that sodium selenite is a common dietary form of selenium (Shilo et al. 2003). This study determined the possible adverse effects of lead nitrate on the reproductive system of diabetic and non-diabetic male rats and assessed whether these effects could be ameliorate by co-administration with sodium selenite.

### MATERIALS AND METHODS

**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 20±2°C. They were quarantined for 10 days before beginning the experiment. All the 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 had approved the animal experiments (Approval number: G.U. ET-11.028).

**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).

**Animal treatment schedule**

The rats were divided into two main groups: non-diabetic (n=24) and diabetic (n=24). The non-diabetic group was subdivided in four treatment groups, each consisting of six rats: Group 1–control rats (1.0 mg/kg bw distilled water per day), Group 2–sodium selenite treated rats (1.0 mg/kg bw per day in distilled water), Group 3–lead nitrate (LN) treated rats (22.5 mg/kg bw per day in distilled water), Group 4–sodium selenite plus lead nitrate treated rats (1.0 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 the 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 >300 mg/dL and then subdivided in four treatment groups, each consisting of six rats: Group 5–diabetic control rats (1.0 mg/kg bw distilled water per day), Group 6–diabetic sodium selenite treated rats (1.0 mg/kg bw per day in distillated water), Group 7–diabetic lead nitrate treated rats (22.5 mg/kg bw per day in distillated water), Group 8–diabetic sodium selenite plus lead nitrate treated rats (1.0 mg/kg+22.5 mg/kg bw per day, respectively).

The oral LD\textsubscript{50} of lead nitrate for male rats is 2250 mg/kg body weight. We used 1/100 LD\textsubscript{50} of lead nitrate. The doses used in this study were chosen on the basis of previous studies (Kara et al. 2007). The substances were administrated in the morning (between 09:00 and 10:00 h) to non-fasted rats. At the end of the 4th week (28 days) of the treatment, the rats in each group were sacrificed and dissected. The testis tissues were quickly taken to assess the histopathological changes by light microscope examination and to evaluate the antioxidative enzyme activities (SOD, CAT, GPx and GST activities), and malondialdehyde (MDA) levels.

**Biochemical Estimation**

The testis tissues were dissected and washed in sodium phosphate buffer (pH 7.2) and stored at -80°C until the analysis. The tissues were homogenized using a Teflon homogenizer (Heidolph Silent Crusher M) and then the homogenates were centrifuged at 10,000g at 4°C for 15 min. 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 the supernatant was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

**Lipid Peroxidation Assay**
MDA content was assayed using the thiobarbituric acid (TBA) test as described by Ohkawa et al. (1979). Absorbance was measured at 532 nm to determine the MDA content. The specific activity is expressed as nM/mg protein of protein.

**Measurement of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPX), Glutathione-S-Transferase (GST)**
Total SOD activity was determined according to the method described by Marklund and Marklund (1974) by assaying the auto-oxidation and illumination of pyrogallol at 440 nm for 3 min. The SOD activity is expressed as U/mg protein. CAT activity was measured according to the method described by Aebi (1984) 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 is expressed as mM/mg protein. GPx activity was measured using H₂O₂ as substrate according to the method described by Paglia and Valentine (1967). The reaction was monitored indirectly as the oxidation rate of NADPH at 240 nm for 3 min. Enzyme activity was expressed as nM/mg protein. GST activity was assayed by measuring the formation of GSH (Glutathione) and the 1-chloro-2,4-dinitrobenzene (CDNB) conjugate (Habig et al. 1974). The specific activity of GST is expressed as µM/mg protein.

**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.

**Histopathology**
For histopathological examination, testis tissues were dissected and fixed in Bouin solution. Samples were then processed using a graded ethanol series and embedded in paraffin. The paraffin sections were cut into 6-7 µm-thick slices and stained with hematoxylin and eosin for histological examination. The sections were viewed and photographed using an Olympus light microscope (Olympus BX51, Tokyo, Japan) with an attached camera (Olympus E-330, Olympus Optical Co. Ltd., Japan).

**RESULTS**

**Evaluation of biochemical parameters**
At the end of the 4th week, there were no statistically significant changes in MDA levels (Fig.1), SOD, CAT, GPx and GST activities between the sodium selenite treated group compared to the control group and the diabetic sodium selenite treated group compared with the diabetic control group (Table 1).

**Malondialdehyde (MDA) levels**
MDA levels in testis tissues was 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 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 LN group. Besides, there was statistically increased in the all diabetic groups compared to non-diabetic groups in MDA levels (P<0.05, Fig. 1).

**Antioxidant enzyme activities**
There was significant increase in the 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 testis tissues. However, all enzyme activities were decreased in the sodium selenite plus lead nitrate treated group compared to the only lead nitrate treated group. There were statistically significantly increased in the enzyme activities in the diabetic lead nitrate and diabetic sodium selenite plus lead nitrate treated groups at the end of the 4th week compared to the diabetic control group, while there was decrease in the diabetic sodium selenite plus lead nitrate treated group compared with the diabetic lead nitrate treated group in testis tissues (P<0.05, Table 1).

Table 1 - Effects of lead nitrate (LN) and/or sodium selenite treatment on antioxidant enzymes activities in testis tissues of diabetic (STZ) and nondiabetic male Wistar rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (U/mg protein)</th>
<th>CAT (mM/mg protein)</th>
<th>GPx (nM/mg protein)</th>
<th>GST (µM/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.130±1.1095 a</td>
<td>0.719±0.0428 a</td>
<td>5.684±0.529 a</td>
<td>0.061±0.015 a</td>
</tr>
<tr>
<td>Sodium selenite</td>
<td>10.715±1.1009 a</td>
<td>0.660±0.0659 a</td>
<td>5.591±0.438 a</td>
<td>0.064±0.010 a</td>
</tr>
<tr>
<td>LN</td>
<td>19.693±1.1013 b</td>
<td>1.203±0.0448 b</td>
<td>12.318±0.731 b</td>
<td>0.147±0.011 b</td>
</tr>
<tr>
<td>LN+Sodium selenite</td>
<td>17.152±0.991 c</td>
<td>1.036±0.0614 c</td>
<td>10.092±0.563 c</td>
<td>0.120±0.014 c</td>
</tr>
<tr>
<td>STZ+Control</td>
<td>15.103±1.0353 a</td>
<td>0.856±0.0564 a</td>
<td>7.552±0.537 a</td>
<td>0.082±0.015 a</td>
</tr>
<tr>
<td>STZ+Sodium selenite</td>
<td>14.975±1.2001 a</td>
<td>0.892±0.0688 a</td>
<td>7.702±0.532 a</td>
<td>0.090±0.010 a</td>
</tr>
<tr>
<td>STZ+ LN</td>
<td>26.80±1.1116 c</td>
<td>1.530±0.0518 c</td>
<td>15.553±0.459 c</td>
<td>0.208±0.014 c</td>
</tr>
<tr>
<td>STZ+ LN+Sodium selenite</td>
<td>23.158±1.0751 f</td>
<td>1.385±0.0556 f</td>
<td>14.002±0.692 f</td>
<td>0.181±0.011 f</td>
</tr>
</tbody>
</table>

Values are mean±SD of six rats in each group. Significance at P<0.05. Within each column, means superscript with different letters are significantly different.

Histopathological changes in the testis tissues

At the end of the 4th week, the seminiferous tubules and interstitial connective tissue were structurally normal in the control, sodium selenite, diabetic control and diabetic sodium selenite treated groups. However, after 4 weeks of lead nitrate exposure, there were degenerative changes in some of the seminiferous tubules, necrosis in some seminiferous tubules and edema in interstitial tissue was observed. Besides, there were mild degenerative changes in seminiferous tubules in sodium selenite plus lead nitrate treated groups. In addition, there were degenerative changes and edema in diabetic lead nitrate treated group; similarly there were mild degenerative chances and edema in seminiferous tubules in the sodium selenite plus lead nitrate treated group (Fig. 2A-H).

Figure 2 - (A) Testicular section of control rats showing seminiferous tubules (S) and interstitial tissue (I), x100. (B-C) Testicular sections of lead nitrate-treated rats showing necrosis (●) in seminiferous tubules and degenerative changes (▲) x200 and (C) necrosis (●) in seminiferous tubules, x100. (D) Testicular sections of lead nitrate plus sodium selenite treated rats showing (D) edema in interstitial tissues (●) x200. (E) Testicular section of diabetic control rats showing seminiferous tubules (S) and interstitial tissue (I), x100 (F-G) Testicular section of diabetic lead nitrate treated rats showing degenerative changes (▲) in seminiferous tubules, and edema in interstitial tissues (●) x200. (H) Testicular sections of diabetic lead nitrate plus sodium selenite treated rats showing degenerative changes (▲) in seminiferous tubules and edema in interstitial tissues (●) x200.
DISCUSSION

Oxidative stress has been considered as a result of alteration antioxidant system (Farmand et al. 2005; Eraslan et al. 2007). It has been reported as one of the important mechanism of toxic effect of lead, like other heavy metals (Flora et al. 2004b). In the previous studies, heavy metal exposure including lead has been shown increase of ROS production in various experimental animal tissues (El-Sokkary et al. 2005; Xia et al. 2010).

It is known that lead causes biochemical and physiological dysfunctions in humans and laboratory animals (Zhang et al. 2010; Kalender et al. 2013). Malondialdehyde (MDA) is a good marker of membrane lipid peroxidation, resulting from the interaction between ROS and cellular membrane (Durak et al. 2010). Lead has been documented to increase the MDA levels in various rat tissues such as liver, brain, kidney and testis (Flora et al. 2004a; El-Sokkary et al. 2005; Sainath et al. 2011). The lipid peroxidation observed as a result of lead administration could be due to the formation of free radicals. In the present study, MDA levels increased in the lead nitrate, sodium selenite plus lead nitrate treated and all diabetic groups. This increase in MDA levels could be due to an increase in free radicals, resulting from the induction of oxidative stress.

Mammalian cells possess non-enzymatic and enzymatic antioxidant defense systems to prevent the oxidative stress that interact with and inactivate ROS (Farmand et al. 2005; Demir et al. 2011). SOD plays an important role in protecting the toxic effects of superoxide radical (Xia et al. 2010; Uzunhisarcıklı and Kalender 2011) and catalyzes the conversion of superoxide radicals to hydrogen peroxide (Uzun et al. 2010). CAT is a major antioxidant enzyme having heme as the prosthetic group (Xia et al. 2010) and converts hydrogen peroxide into water (Uzun et al. 2010). Therefore, increase in the SOD and CAT activities has been reported to be due to the first line of defense against free radicals and oxidative stress (Kalender et al. 2013). GPx, a selenoenzyme, plays a major role in the reduction of hydrogen peroxide and hydroperoxide to non-toxic products (Renugadevi and Prabu 2010). GST catalyses the reaction of glutathione conjugation with many xenobiotics and their reactive metabolites; therefore, it produces less toxic forms (Uzun et al. 2010).

The effect on the antioxidant defense systems of cells is defined as the second mechanism for lead-induced oxidative stress (Patra et al. 2011). Oxidative stress and a failure of antioxidant defense system cause several sperm abnormalities and result in infertility. Thus, an imbalance between the ROS generation and scavenging system might be one of the reasons for lead-induced male reproductive toxicity (Sainath et al. 2011). Similarly, in this study SOD, CAT, GPx and GST activities increased in testis tissues of rats treated with lead nitrate. This increased antioxidant activity due to lead nitrate exposure could be a counteracting mechanism adopted to eliminate lead nitrate.

The incidence of diabetes mellitus is increasing worldwide. Although, diabetes-related complications, which cause death, are vascular complications (Afridi et al. 2008). It has been shown that in the various tissues of diabetic rats there were increased lipid peroxidation and altered antioxidant enzymes (Sekeroglu et al. 2000; Duzgoner and Kaya 2007; Jin et al. 2008). In this study, results showed that diabetic rats showed improved oxidative stress by enhanced lipid peroxidation and antioxidant enzymes activities. Also, diabetic rats were more susceptible to lead nitrate-induced lipid peroxidation than non-diabetic rats in testis tissues.

It is evident that lead passes through the blood-testis barrier and induces testicular damage and then they induce oxidative stress, lipid peroxidation and ROS production that damages the biological membranes in the testes, including degeneration of the spermatogenic and Leydig cells (Wang et al. 2006; Sainath et al. 2011), like other environmental toxicants (Dirican and Kalender 2012). Lead can accumulate in the reproductive system, which may lead to a reduced number of spermatogenic cells and has been implicated in the oxidative damage in the laboratory animals (Fahim et al. 2013; Xiang et al. 2013). In the present study, lead nitrate caused several histopathological changes in testis tissues. It demonstrated the presence of diabetes-dependent effects on male reproductive system (Navarro-Casado et al. 2010). Similarly to this data, some changes in testis tissues of diabetic rats were also observed. These results showed that the lead and/or diabetes could affect both directly and by increasing the ROS production in testis tissues. It has been shown that selenium has protective effects against diabetes-induced oxidative stress in...
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CONCLUSION

In the present study, even though lead nitrate was given orally at low dose to diabetic and non-diabetic rats, histopathological and enzymatic changes were observed in the rat testis tissues, but none of the rats died during the experimental period. In conclusion, from the results of present investigations, it was evident that lead nitrate caused testicular toxicity, including histopathological changes, LPO and disturbances in antioxidant enzyme activities both diabetic and non-diabetic rats. However, sodium selenite manifested beneficial effects against the testicular toxicity. These results were probably due to generation of ROS, causing damage to cell membranes. Therefore, changes in SOD, CAT, GPx and GST activities in the testis tissue could be a response of the organ to the oxidative stress. Results showed important toxic changes in the diabetic groups, which could be due to oxidative damage in diabetes because oxidative stress has an important role play in the pathogenesis of diabetes. Results showed that Se could, therefore, play a protective role on the toxicity caused by lead nitrate and/or diabetes.

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