Environmental Toxicology and Pharmacology 40 (2015) 568–574

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Protective effects of sodium selenite on lead nitrate-induced hepatotoxicity in diabetic and non-diabetic rats

Suna Kalender[a,*], Fatma Gökçe Apaydin[b], Hatice Baş[c], Yusuf Kalender[b]

[a] Gazi University, Faculty of Gazi Education, Department of Science, 06500 Ankara, Turkey
[b] Gazi University, Faculty of Science, Department of Biology, 06500 Ankara, Turkey
[c] Bozok University, Faculty of Arts and Science, Department of Biology, 66100 Yozgat, Turkey

ARTICLE INFO

Article history:
Received 22 April 2015
Received in revised form 7 August 2015
Accepted 8 August 2015
Available online 14 August 2015

Keywords:
Lead toxicity
Sodium selenite
Oxidative stress
Histopathology
Biochemical alterations
Diabetes mellitus

ABSTRACT

In the present study, the effect of sodium selenite on lead induced toxicity was studied in Wistar rats. Sodium selenite and lead nitrate were administered orally for 28 days to streptozotocin induced diabetic and non-diabetic rats. Eight groups of rats were used in the study: control, sodium selenite, lead nitrate, lead nitrate + sodium selenite, streptozotocin-induced diabetic-control, diabetic-sodium selenite, diabetic-lead nitrate, diabetic-lead nitrate + sodium selenite groups. Serum biochemical parameters, lipid peroxidation, antioxidant enzymes and histopathological changes in liver tissues were investigated in all groups. There were statistically significant changes in liver function tests, antioxidant enzyme activities and lipid peroxidation levels in lead nitrate and sodium selenite + lead nitrate treated groups, also in diabetic and non-diabetic groups. Furthermore, histopathological alterations were demonstrated in same groups. In the present study we found that sodium selenite treatment did not show completely protective effect on diabetes mellitus caused damages, but diabetic rats are more susceptible to lead toxicity than non-diabetic rats.

1. Introduction

Lead nitrate (LN) is one of the effective toxic heavy metals that have been found in environment and biological systems (Guimaraes et al., 2012). Many researchers have reported that lead exposure could induce pathological changes and also oxidative stress in experimental animals (El-Neweshy and El-Sayed, 2011; Dai et al., 2013). After absorption, LN is also distributed to soft tissues and bone. LN leads to many toxicological effects such as hematological and neurological damages (Yuan et al., 2014).

Among the soft tissues, liver shows extensively higher lead concentration (Guimaraes et al., 2012). Liver is the target organ for the toxic effects of xenobiotics (Mehana et al., 2012). It is demonstrated that heavy metals cause hepatotoxicity via changes the profile of liver marker enzymes such as ALP, AST, LDH and histopathological changes. Therefore, in toxicological studies biochemical analysis and histopathological lesions have been widely used as biomarkers. (El-Neweshy and El-Sayed, 2011; Demir et al., 2011).

The general toxic effect of heavy metals is considered to be due to oxidative damage by formation of reactive oxygen species (ROS) (Kalender et al., 2013). It is known that oxidative stress is related to heavy metal toxicity mechanisms (Apaydin et al., 2014). Lipid peroxidation (LPO) has been extensively used as a marker of oxidative stress (Uzun and Kalender, 2013).

Selenium (Se) is an essential trace element for animals and humans, and protects cells from oxidative stress. Se is an important essential element in almost all biological systems and well-established antioxidants. It has been shown to detoxification effects on various heavy metals (Liu et al., 2013). Antioxidant enzymes are important cell protectors against oxidative stress induced cell injuries (Messarah et al., 2012). SOD and CAT are the two enzymes that help to scavenge superoxide and hydroxyl ions; respectively (Sankar et al., 2012). GST is a member of enzymes involved in the detoxification of xenobiotics (Ashok et al., 2014). In addition, GPx also can convert hydrogen peroxide into H_2O_2 (Uzun and Kalender, 2013).

Diabetes mellitus (DM) is a chronic disease which characterized by high blood glucose levels. The streptozotosin (STZ) induced diabetes models utilizes the STZ toxin to damage pancreatic ß cells (Chung et al., 2014). It has been shown that under diabetic conditions, increased of lipid peroxidation and alters of antioxidant enzyme activities detected (Schmatz et al., 2012).

* Corresponding author at: Gazi University, Faculty of Gazi Education, Department of Science, Teknikokullar, 06500 Ankara, Turkey.
E-mail address: suna@gazi.edu.tr (S. Kalender).

http://dx.doi.org/10.1016/j.etap.2015.08.011
1382-6689/

STZ is used to induced rat model of type 1 diabetes in this study. The aims of the present study were: (i) to evaluate the effect of lead nitrate on lipid peroxidation and antioxidant enzyme systems, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) of liver tissue, (ii) to evaluate of biochemical changes in blood like ALT, AST, LDH. (iii) to evaluate of histopathological changes in the liver tissue and (iv) to investigate the possible protective role of sodium selenite against this heavy metal both diabetic and non-diabetic groups.

2. Materials and methods

2.1. Animal models and chemicals

200–250 g weights adult Wistar albino rats were purchased from Gazi University Laboratory Animals Growing and Experimental Research Center. All procedures were approved by the Gazi University Committee on the Ethics of Animal Experimentation (G.Ü.ET-11.028). Lead nitrate (LN), sodium selenite, STZ and all the other chemicals were obtained from Sigma–Aldrich.

Rats were divided into eight groups (six rats in each group) for this study. These are:

Group 1: Control rats, (treated with 1 ml/kg body weight (bw) per day distilled water)
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; 1/100LD_{50} dose orally) (Sharma et al., 2010).
Group 4: Lead nitrate (22.5 mg/kg bw per day in distilled water) and sodium selenite (1 mg/kg bw per day) treated rats,
Group 5: Diabetic control rats, (1 ml/kg body weight (bw) per day distilled water)
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: Lead nitrate (22.5 mg/kg bw per day in distilled water) and sodium selenite (1 mg/kg bw per day) treated diabetic rats.

Diabetes was induced using an intraperitoneal injection of STZ at a single dose of 55 mg/kg. STZ was prepared in sodium citrate buffer at 4.5 pH. Two days after injection, the blood glucose levels were measured from the tail with a glucometer. Rats whose blood glucose levels were 300 mg/dl or higher, they were approved to be diabetic and selected for diabetic groups (Schmatz et al., 2012; Apaydin et al., 2015).

All rats were treated for 28 days. The substances were administrated in the morning (between 09.00 and 10.00 h) to non-fasted rats, and given to rats daily via gavage.

At the end of the study, rats were dissected using combination of ketamin + xylazin, than blood and liver tissues were taken for investigations of MDA and antioxidant enzymes, liver function test (Total protein, total cholesterol, triglyceride, ALP (alkaline phosphatase), ALT (alanine aminotransferase), AST (aspartate aminotransferase), and LDH (lactate dehydrogenase) and histopathological examinations.

2.2. Measurement of lipid peroxidation and antioxidant enzyme activities

Liver tissues were washed with sodium phosphate buffer (pH 7.2) then they were stored at −80 °C until the biochemical analysis. When it comes time to work, the livers were homogenized with Heidolph Silent Crusher M. The homogenates were centrifuged for 15 min. All procedures were actualized at 4 °C. Antioxidant enzyme activities and MDA levels were detected using with spectrophotometer (Shimadzu UV 1700, Kyoto, Japan). The concentration of protein was specified according to Lowry et al. (1951).

LPO in liver was analyzed at 532 nm by thiobarbituric acid method described by Ohkawa et al., 1979. MDA level was defined as nmol/mg protein. SOD activity was measured by the method of Marklund and Marklund, 1974. Activity of enzyme was monitored at 440 nm then the data was expressed U/mg protein. CAT assay was adopted from the method of Aebi (1984) at 240 nm and the activity was defined as mmol/mg protein. Activity of GPx was measured by Paglia and Valentine's study (1967). At 340 nm, the reaction was monitored and enzyme activity was stated as μmol/mg protein. According to method of Habig et al. (1974) GST activity was obtained at 340 nm. Value was given as μmol/mg protein.

2.3. Liver function tests

At the end of the 4th week, blood samples of the rats were taken from the heart and collected into sterile tubes. Blood samples were centrifugated at 3500 rpm for 20 min, and serum was separated. Total protein, total cholesterol, triglyceride, ALP (alkaline phosphatase), ALT (alanine aminotransferase), AST (aspartate aminotransferase), and LDH (lactate dehydrogenase) were assessed in serum using a commercially spectrophotometer-enzymatic kit.

2.4. Light microscopic examination

For histopathological examination, liver tissues were dissected and tissue samples were fixed in Bouin solution. Samples were then processed using graded ethanol series and embedded in paraffin. Paraffin sections were cut into 4–6 μm-thick slices and stained with hematoxylin and eosin for light microscopic 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). Ten slides were prepared from each liver tissue. Each liver tissues were examined and assigned for severity of changes using scores on scale of none (−), mild (+), moderate (++), and severe (+++) damage (Table 2).

2.5. Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) and Tukey test for comparison of groups using SPSS program for Windows (11.5 version). $P < 0.05$ was considered statistically significant.

3. Results

During 28 days, death was not observed in any of the experimental groups. The blood glucose levels of rats in diabetic groups were 300 mg/dl or higher during the experimental period.

3.1. Evaluation of lipid peroxidation and antioxidant activities

At the end of the 4th week, there were no statistically significant differences in MDA levels, 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 ($P < 0.05$, Figs. 1–5).

3.1.1. Malondialdehyde (MDA) levels

MDA levels in liver tissues were significantly increased in the LN and sodium selenite plus LN treated groups compared to the control group, while there were decreased in the sodium selenite plus LN treated group compared to the only LN treated group. When