Gazi University Journal of Science
GU J Sci
28(2):169-174 (2015)

The Effects on Antioxidant Enzyme Systems in Rat Brain Tissues of Lead Nitrate and Mercury Chloride

Hatice BAŞ[1,♠], Suna KALENDER[2], Hatice KARABODUK[3], Fatma Gökçe APAYDIN[4]

[1]*Bozok University, Faculty of Arts and Science, Department of Biology, 66100 Yozgat, Turkey*
[2]*Gazi University, Gazi Education Faculty, Department of Science, 06500 Ankara, Turkey*
[3]*Gazi University, Life Sciences Application and Research Center, 06830 Ankara, Turkey*
[4]*Gazi University, Faculty of Science, Department of Biology, 06500 Ankara, Turkey*

Received:07/11/2014 Revised: 16/12/2014 Accepted:14/01/2015

ABSTRACT

The present study was undertaken to evaluate the effects of lead nitrate and mercury chloride in brain tissues of Wistar rats. Mercury chloride (0.02 mg/kg bw) and lead nitrate (45 mg/kg bw) were administered orally for 28 days rats. The mercury chloride and lead nitrate treated animals were exhibited a significant inhibition of superoxide dismutase, catalase, glutation peroxidase and glutathione-S-transferase activities and increasing of malondialdehyde levels. In our present study mercury chloride caused more harmful effects than lead nitrate. The effect which we observed applying the lead nitrate and mercury chloride together, was more greater than when we used them alone.

Key words: *Lead nitrate, mercury chloride, oxidative stress, brain*

1. INTRODUCTION

As the amount of contaminants in environment continue to increase, the contamination of soil and water has become a real concern in recent times. Heavy metals are among those contaminants [1]. Heavy metals are commonly defined with high atomic weight such as arsenic, cadmium, chromium, copper, lead and mercury that can damage at low concentrations and that tend to accumulate in the food chain [2,3]. They enter to the human body by ingestion, inhalation or through the skin and their presence may cause serious toxicity [4].

Lead is a persistent environmental occupational toxic metal, and its poisoning remains a health threat [5]. It exposure mainly arises from contact with lead based paints in home, fertilisers, cosmetics, automobiles, disposable batteries, etc., especially in developing countries [6]. Several lines of evidence implicate that lead causes many pathological incidences including cardiac, hepatic, renal and haematological dysfunctions [7].

Mercury is a widespread industrial pollutant and it induces serious harmful effects in humans. Mercury is known to alter the intracellular redox homeostasis [8]. It is a carcinogenic heavy metal and it has been well documented that mercury may cause brain damage, dysfunction of liver, kidney, gastrointestinal tract and central nervous system [9-11].

Antioxidant enzymes are major cell protectors against oxidative stress caused damages. The main antioxidant enzymes that show effects on reactive oxygen species (ROS) are superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and catalase (CAT) [12]. The activities of these enzymes have been used to evaluate oxidative stress in cells. These antioxidant enzymes are potential targets for heavy metal toxicity [13].

In the present study, the subacute effects of lead nitrate and mercury chloride on brain tissues of Wistar rats were investigated by examination of SOD, CAT, GPx

♠Corresponding author, e-mail: htc.haticebas@gmail.com

and GST activities and also malondialdehyde (MDA) levels.

2. MATERIALS AND METHODS

2.1. Animal Care and Maintenance

24 male albino Wistar rats (300-320 g) were obtained from the Gazi University Laboratory Animals Growing and Experimental Research Center. Rats left to accommodate for 10 days before the begining of study. The animals were housed at 18-22 ^{o}C and they were supplied with standard laboratory chow and water *ad libitum*. The protocol was approved by the Gazi University Animal Experiments Local Ethics Committee (Protocol no: G.U.ET – 13.011). All animal experiments were performed accordancing to the international guidelines for care and use of laboratory animals.

2.2. Test Chemicals

Lead nitrate, mercury chloride and all the other chemicals were obtained from Sigma Aldrich. Lead nitrate and mercury chloride were dissolved in distilled water [14, 15].

2.3. Experimental Design

The rats were divided into two groups, control (n=6) and experimental group (six rats in each group). Experimental group were further divided into 3 treatment groups: Lead nitrate (LN) treatment group, mercury chloride (MC) treatment group and LN + MC treatment group.

During 28 days, 1ml/ kg b.w (body weight) distilled water for control group, 0,02 mg/kg b.w (1/50 LD_{50}) for mercury chloride treatment groups [14] and 45 mg/kgb.w (1/50 LD_{50}) for lead nitrate treatment groups [15, 16]. Lead nitrate and mercury chloride treatment groups were given to rats daily via gavage.
The chemicals were administered in the morning (between 09:00 and 10:00) to non-fasted rats. At the end of the 4th week, animals were sacrificed and dissected, and brain tissues were taken for examination of changes on antioxidant enzyme activities and MDA levels.

2.4. Biochemical Assays

2.4.1. Measurement of MDA levels

MDA is the individual aldehyde resulting from lipid peroxidation. MDA content was assayed using the thiobarbituric acid test as described by Ohkawa et al. [17]. MDA reacts with TBA to form a colored complex. Absorbance was measured at 532 nm. The level of MDA is defined as nmol/mgprotein.

2.4.2. Measurement of antioxidant enzyme activities

The SOD activity was estimated according to Marklund and Marklund [18] and GST according to Habig et al. [19]. CAT and GPx activities were assayed by the method of Aebi [20], Paglia and Valentine [21] respectively. The activities of SOD, GST and GPx were defined as nmol/mgprotein, CAT activity was defined as μmol/mgprotein.

2.5. Statistical analysis

Results are shown as Mean ± Standard Error of the Mean and statistical analyses were done by one-way analysis of variance (ANOVA) test followed by Tukey. The level of significance was set at $P < 0.05$.

3. RESULTS

3.1. Evaluation of malondialdehyde levels

MDA levels were measured in all groups of rats' brain tissues. After four weeks, LN treated, MC treated and LN + MC treated groups showed increasing of MDA level compared to control. We observed more increasing in MC group than LN group. Treated with combination of LN and MC caused more harmfull effects than use of them alone (Figure 1) (P<0,05).

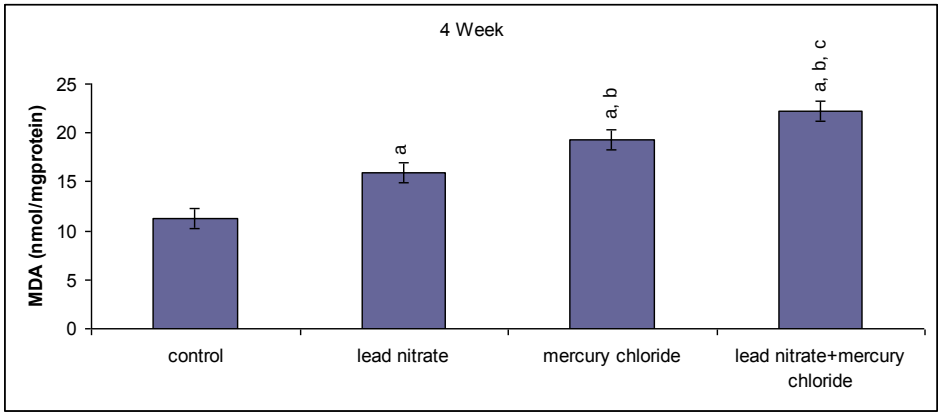

Fig. 1. Effects of subacute treatment of LN and MC on MDA levels (nmol/mg protein) in the brain tissues of rats. Each bar represents mean±SD of six animals in each group. Significance at $P < 0.05$. [a]Comparison of control and other groups. [b]Comparison of lead nitrate group and other groups. [c]Comparison of mercury chloride group and other groups

3.2. Evaluation of antioxidant enzyme activities

Treatment with LN and MC alone, decreased the activities of antioxidant enzymes (SOD, CAT, GPx and GST) in brain tissues but MC showed more toxicity than LN. In combination with LN and MC caused more damagess than use of them alone (Figures 2, 3, 4, 5) (P<0,05).

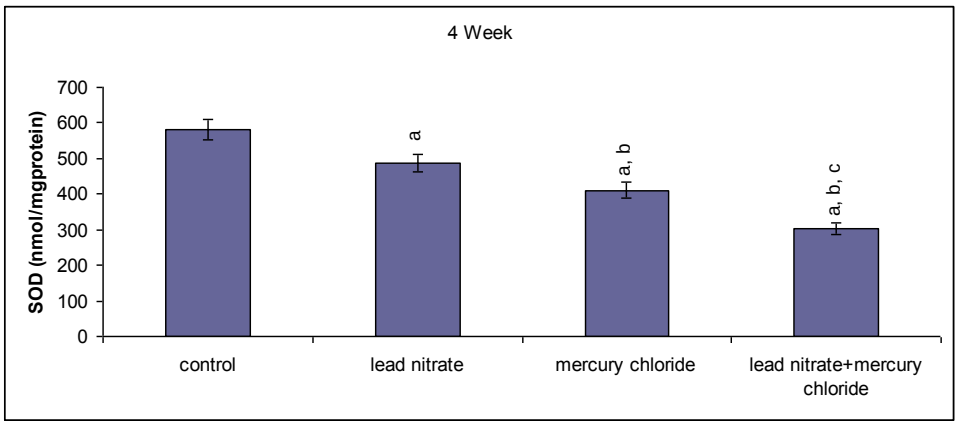

Fig. 2. Effects of subacute treatment of LN and MC on SOD levels (nmol/mg protein) in the brain tissues of rats. Each bar represents mean±SD of six animals in each group. Significance at P < 0.05. [a]Comparison of control and other groups. [b]Comparison of lead nitrate group and other groups. [c]Comparison of mercury chloride group and other groups.

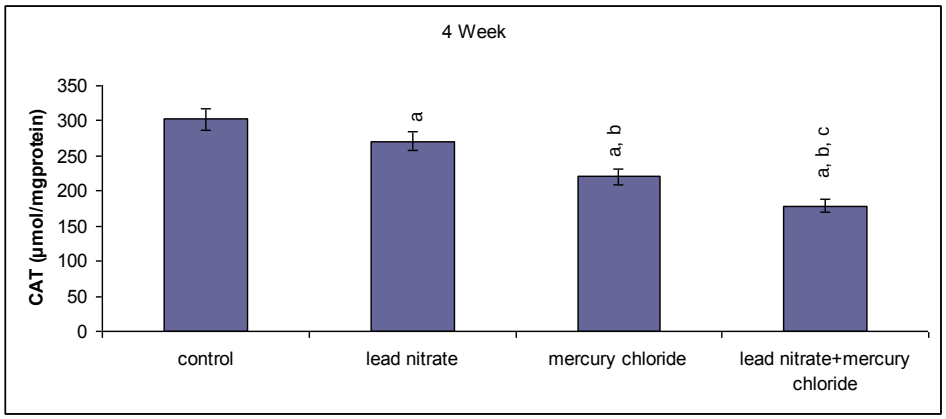

Fig. 3. Effects of subacute treatment of LN and MC on CAT levels (µmol/mg protein) in the brain tissues of rats. Each bar represents mean±SD of six animals in each group. Significance at P < 0.05. [a]Comparison of control and other groups. [b]Comparison of lead nitrate group and other groups. [c]Comparison of mercury chloride group and other groups.

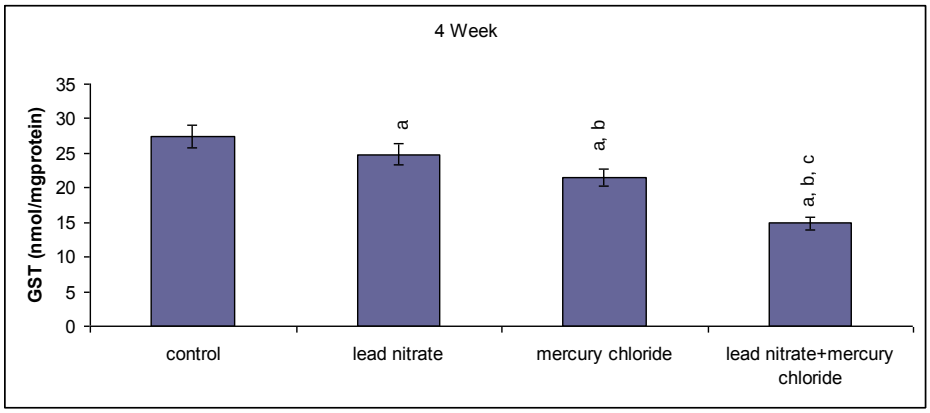

Fig. 4. Effects of subacute treatment of LN and MC on GST levels (nmol/mg protein) in the brain tissues of rats. Each bar represents mean±SD of six animals in each group. Significance at P < 0.05. [a]Comparison of control and other groups. [b]Comparison of lead nitrate group and other groups. [c]Comparison of mercury chloride group and other groups.

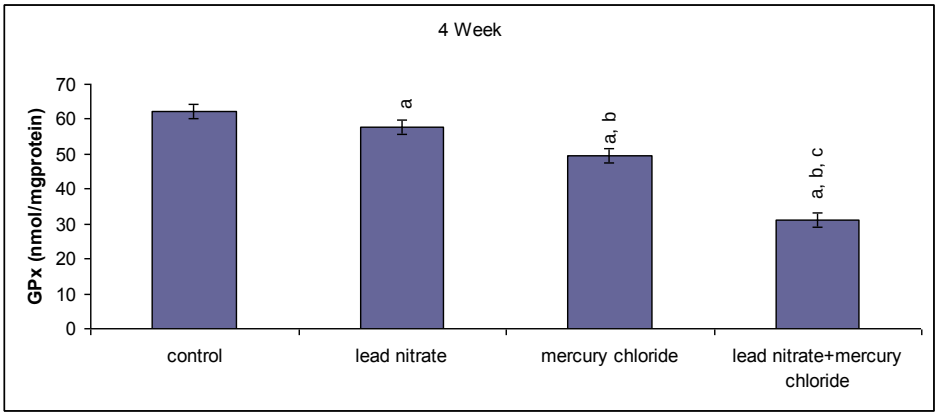

Fig. 5. Effects of subacute treatment of LN and MC on GPx levels (nmol/mg protein) in the brain tissues of rats. Each bar represents mean±SD of six animals in each group. Significance at $P<0.05$. [a]Comparison of control and other groups. [b]Comparison of lead nitrate group and other groups. [c]Comparison of mercury chloride group and other groups**.**

4. DISCUSSION

To understand the clinical syndromes of heavy metal-induced human diseases, it is important to use in vivo animal models. Heavy metal induced oxidative stress has also been the focus of toxicological research for the last decade to evaluate their possible mechanism of toxicity. Cells try to counter oxidative stress using the first line defense system such as radical-scavenging enzymes like SOD, CAT, GST and GPx [22, 23].

Even though the exact mechanism of lead toxicity is not clear, there are studies that it can cause generation of ROS and inhibits the antioxidant enzyme activities in tissues [24, 25]. It was also reported that lead increased the level of LPO [26]. The generation of ROS, stimulation of LPO and decrease of antioxidant reserves have been supposed to be main contributors to lead exposure causes diseases [7, 27]. In this study, MDA levels increased and enzyme activities decreased in LN traeted rats compared to control group. Data which were obtained from previous studies supports our findings [7, 26].

Mercury induces cellular toxicity by binding to intracellular sulfhydryl groups [9, 10, 11]. It decreases activity of metabolic enzymes, increases in LPO products [28]. Exposure to mercury compounds induces oxidative stress [29] and the formation of H_2O_2, $ROO^{\cdot}$ and $HO^{\cdot}$ that may cause cell membrane damage, inhibition of the activity of antioxidant enzymes [30]. These findings shows similarities with our study. We found that MC treatment caused changes in examining parameters. When we compared with MC traeted rats with LN treated animals we showed that these changes were greater than LN caused differences. The LD_{50} of LN for rats is 2250 mg/kg bw. For MC, the LD_{50} is 1 mg/kg bw [14, 15, 16]. As understood from the LD_{50} values, it is observed that MC is more toxic than LN.

Results from oxidative stress based in-vivo studies about hevy metals have suggested an increased free radical production and decreased antioxidant mechanisms in the brain [28]. In our study lead and mercury caused increment in MDA level and decreasing in antioxidant enzyme activities. These changes may be occured because of lead and mercury caused oxidative stress. ROS essentially generates oxidative stress and compromises antioxidant defense. The production of ROS is a normal aspect of cellular metabolism, but increased production of ROS may lead to oxidative stress consequently impairing the antioxidant defense system. LPO produces conjugated diene and MDA [23]. The level of MDA production in rat brain was found to be high till the end of exposure of lead and mercury.

Since oxidative stress is the first response to the environmental pollutants, brain cells may stimulate antioxidant and detoxification responses to counter heavy metal damages. The involvement of antioxidative enzymes such as GPx, GST, SOD and CAT play a considerable mission in protecting cells from oxidative stress [23]. So, assesment of activities of these enzymes may supply important informations about oxidative stress that cells exposed. We determined that heavy metals used in this study were decreased enzyme activities. Similar results have been also reported by Lakshmi et al., 2013 and Dewanjee et al 2013 [7, 31].

In conclusion, this study is an evident that LN and MC caused oxidative stress in brain tissues. We observed more reduction in SOD, CAT, GST and GPx activities and more increasing in MDA levels in MC group than LN group. Treated with combination of LN and MC caused more harmfull effects than use of them alone.

In conclusion, this study is an evident that LN and MC caused oxidative stress in brain tissues. We observed more reduction in SOD, CAT, GST and GPx activities and more increasing in MDA levels in MC group than LN group. Treated with combination of LN and MC caused more harmfull effects than use of them alone.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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