Protective Effect of Chitosan Against Lead-Induced Oxidative Stress in Rat Kidney

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ABSTRACT Chitosan is a natural polymer with antioxidant and chelating properties. This study investigated the effects of chitosan on lead (Pb), malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG), glutathione (GSH), ceruloplasmin concentrations and catalase (CAT) activity in the kidney tissue of the rats exposed to lead. 28 male Wistar albino rats were divided into four groups of eight each: control (C), lead group (Pb group), lead+chitosan (Pb+CS group), and chitosan (CS group). The lead group was administered 50 mg/kg lead acetate intraperitoneally (ip) for 5 days, and the chitosan groups (CS+Pb and CS groups) were administered 200 mg/kg chitosan for 28 days via gavage. At the end of the study, Pb, MDA, 8-OHdG, ceruloplasmin, GSH concentrations and catalase activity were measured in the kidney tissue. In the Pb-treated groups when compared with the control group, Pb, MDA, 8-OHdG, ceruloplasmin concentrations were significantly increased, and GSH concentration and catalase activity were significantly decreased (p<0.05). Coadministration of chitosan with lead significantly decreased Pb, MDA, and ceruloplasmin levels and significantly increased CAT activity in the kidney tissue (p<0.05). There were no significant changes in GSH and 8-OHdG levels (p>0.05). The obtained results show that chitosan protects the kidney against lead-induced oxidative stress.

Keywords: Chitosan, Lead, Kidney, Oxidative stress, Antioxidant

INTRODUCTION

Kidney is an organ that maintains the internal environment of the body. The renal tubular system is susceptible to damage caused by environmental chemicals and drugs. Heavy metals can cause kidney damage, such as nephrotoxicity (Liu et al. 2009; Matus et al. 2009). Lead induces tumor formation in different organs, but it is most commonly associated with kidney cancer in rodents. Renal adenoma and/or adenocarcinoma often occur in rats...
chronically exposed to lead ingestion (Tokar et al. 2010). Lead poisoning is thought to be caused by oxidative stress. Recent studies have shown that lead induces excessive production of reactive oxygen radicals such as hydroxy peroxide (H2O2), hydrogen peroxide (H2O2), superoxide anion (O2-), and hydroxyl (OH-) (Bokar et al. 2008; Eracle et al. 1996; Sivaprasad et al. 2004). Living organisms have antioxidant defense systems that prevent the formation of reactive oxygen radicals and protect against harmful effects caused by them. The increase of reactive oxygen radicals leads to oxidative stress that causes damage to cellular macromolecules containing proteins, lipids, and nucleic acids. Oxidative stress plays a role in the pathology of cancer, atherosclerosis, diabetes, and other diseases (Finkiel and Holbrook 2000; Halliwell et al. 1992; Pande and Flora 2002).

Chelation therapy is used as a medical treatment against lead poisoning. Chelates such as calcium disodium EDTA, dimercaprol, and meso-2,3-dimercaptosuccinic acid are useful in the elimination of lead by forming insoluble compounds with lead (Eracle et al. 1996; Florar et al. 1995; Flora and Pachauri 2010; Patrick 2006; Sivaprasad et al. 2004). However, the frequent clinical use of these synthetic chelating agents is limited due to their side effects (Flora et al. 1995; Flora and Pachauri 2010; Patrick 2006). Recent studies have suggested that antioxidant treatments prevent the formation of reactive oxygen species or capture free radicals and that cellular oxidizable substrates can relieve or delay lead-induced oxidative damage (Finkiel and Holbrook 2000; Flora 2009; Halliwell et al. 1992). In recent years, there has been an intense effort to develop and find safe and natural antioxidants. The effect of different protectors against kidney damage due to lead has been investigated (Wang et al. 2012).

Foodborne diseases are serious problem, both in developed and developing countries, causing a significant social and economic burden (Martins et al. 2012). The most frequent cause of outbreaks are mass catering and food service facilities (Todd et al. 2007). Therefore, the European Commission has recognized the importance of controlling food-poisoning outbreaks owing to the increasing number of meals consumed outside the home, in parallel with the ever-expanding range of pre-prepared increasing number of meals consumed outside the home, and was kept in cages where they were provided with ad libitum feed and water at a temperature of 22±2°C and under 12-h dark and 12-h light cycles. For the present study, an approval was obtained from the Local Animal Experiments Ethics Committee at Van Yüzüncü Yıl University on 05.09.2014, with the number 2014/10.

**Study groups**

The rats were divided into four groups as seven rats in each group, and the following items were administered to each group in the study.

The Control Group (C): Only saline solution was administered to the rats in this group for 28 days via oral gavage.

The Lead Group (Pb Group): The rats in this group were administered 50 mg/kg lead acetate dissolved in saline solution for 5 days intraperitoneally (ip) (Mutlu et al. 2011).

The Lead + Chitosan Group (Pb + CS group): The rats in this group were administered 50 mg/kg lead acetate ip for 5 days. At the same time, 200 mg/kg chitosan was dissolved in saline solution and administered for 28 days by oral gavage (Jeon et al. 2003).

The Chitosan Group (CS group): 200 mg/kg chitosan was dissolved in saline solution and administered by oral gavage for 28 days in this group.

At the end of the trial period, intraperitoneal 75 mg/kg ketamine HCl was injected, and the blood was collected by heart puncture. Then, the collected blood was centrifuged at +4°C, at 3000 rpm for 5 min to obtain serum. Urea and creatinine analyses in the extracted serum were measured on an autoanalyzer (Roche/Hitachi) using commercial kits. The rats were immediately sacrificed by bloodless method under ketamine anesthesia, and the kidney was excised. Samples of kidney tissue were homogenized in cold 0.1 mM phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm at 4°C for 15 min. The MDA (Ledwozyw et al. 1986), GSH (Beutler et al. 1963) ceruloplasmin levels (Sunderman and Nomoto 1970) and CAT activity (Lartillot et al. 1998) were measured spectrophotometrically; and 8-OHdG levels were measured in the collected supernatants by using the ELISA commercial kits (Shanghai LZ biotech Co., Ltd., China).

**Pb measurement in the kidney tissue**

500 μL of 10% Triton X-100 was added to 250 μL of supernatant. This mixture was vortexed. 1250 μL of 10% (NH4)2HPO4 and 3 mL of deionized ultra pure water were added to it (a total volume of 5ml). Then, the reading was done at the 283.3 nm wavelength in graphite furnace atomic absorption spectrometry (Graphite Furnace Atomizer, GFA-7000 Shimadzu) according to the calibration curve plotted for different standard concentrations using Pb cathode lamp (Hollow Cathode Lamp, Hamamatsu Photonics K.K Made in Japan). The obtained results were expressed as ngPb/g tissue (Reyes et al. 2000; Yahaya et al. 2011).

**Statistical analysis**

The results were expressed as the mean ± SEM of number of observation. Comporation of means carried out using one way analysis of variance (ANOVA) followed by Turkey

**MATERIALS and METHODS**

Kidney tissue of rats used in the study named "The effect of chitosan on the erythrocyte antioxidant potential of lead toxicity-induced rats" was used in the research (Toz and Deger 2017).

**Animal material**

Twenty-eight Wistar albino rats, which weighed 200-250 g, which were bred in the animal Experiment Unit of Van Yüzüncü Yıl University, were utilized in the study. The rats were divided into four groups as seven rats in each group, and were kept in cages where they were provided with ad libitum feed and water at a temperature of 22±2°C and under 12-h dark and 12-h light cycles.

**Materials and Methods**

Kidney tissue of rats used in the study named "The effect of chitosan on the erythrocyte antioxidant potential of lead toxicity-induced rats" was used in the research (Toz and Deger 2017).
test to compare means between the different groups. In the analyses, the significance level was accepted as p < 0.05. All analyses were performed using SPSS (23.0) software.

RESULTS

Serum urea and creatinine concentrations were significantly higher in rats treated with lead than in controls animals. Serum urea and creatinine concentrations were significantly lower in rats treated with lead and chitosan than in rats treated with lead (Table 1) (p<0.05).

Table 1. Serum urea and creatinine levels of rats treated with chitosan (200 mg/kg) for 28 days and with lead acetate (50 mg/kg) for 5 days

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>Pb</th>
<th>Pb+CS</th>
<th>CS</th>
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</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>44.83±4.57 a</td>
<td>59.40±5.68 c</td>
<td>51.33±4.22 b</td>
<td>46.83±4.07 a, b</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.54±0.08 a</td>
<td>0.66±0.03 b</td>
<td>0.57±0.06 a</td>
<td>0.55±0.06 a</td>
</tr>
</tbody>
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Values are presented as mean ±SD (n=7). p < 0.05; the difference between group averages with different superscript letters on the same rows is statistically significant.

Table 2. Kidney malondialdehyde (MDA), glutathione (GSH), ceruloplasmin, 8-hydroxydeoxyguanosine (8-OHdG) levels and CAT activity of rats treated with chitosan (200 mg/kg, vo) for 28 days and with lead acetate (50 mg/kg, ip) for 5 days

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>Lead</th>
<th>Lead+CS</th>
<th>CS</th>
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</thead>
<tbody>
<tr>
<td>Lead (ng/g)</td>
<td>16.24±2.457a</td>
<td>62.39±17.192b</td>
<td>35.81±8.250c</td>
<td>15.36±3.367a</td>
</tr>
<tr>
<td>MDA (nmol/g protein)</td>
<td>21.14±2.46 a</td>
<td>30.48±1.41 b</td>
<td>26.59±2.15 c</td>
<td>23.24±1.64 a</td>
</tr>
<tr>
<td>8-OHdG (ng/g protein)</td>
<td>3.98±0.59 a</td>
<td>5.74±1.28 b</td>
<td>5.25±0.31 b</td>
<td>4.04±0.55 a</td>
</tr>
<tr>
<td>GSH (mg/g protein)</td>
<td>1.13±0.11 a</td>
<td>0.85±0.05 b</td>
<td>0.88±0.03 b</td>
<td>1.08±0.016 a</td>
</tr>
<tr>
<td>Catalase (U/g protein)</td>
<td>8.82±0.62 a</td>
<td>2.70±0.32 b</td>
<td>5.25±0.41 c</td>
<td>8.74±0.38 a</td>
</tr>
<tr>
<td>Ceruloplasmin (mg/L protein)</td>
<td>8.769±2.285 a</td>
<td>25.44±4.607 b</td>
<td>18.060±5.61 c</td>
<td>9.397±4.145 a</td>
</tr>
</tbody>
</table>

Values are presented as mean ±SD (n=7). p < 0.05; the difference between group averages with different superscript letters on the same rows is statistically significant.

DISCUSSION

Lead-induced renal dysfunction is classified as acute and chronic nephropathy (Matovic et al. 2015). Lead-induced acute nephropathy is usually characterized by an impaired tubular transport mechanism (Fanconi syndrome), morphological changes of tubular epithelial cells, and lead-induced nuclear inclusion bodies (a lead-protein complex) (Matovic et al. 2015). Consistent with other studies, the present study found that the lead concentration in the kidney tissue was found to be significantly higher in the lead group than in the control group. It has been reported that this elevation occurred because lead binds strongly to macromolecules in the intracellular compartments (Abdel Moneim et al. 2011; Han et al. 1996). In this study, lead exposure was found to cause an increase in serum urea and creatinine levels indicating glomerular damage and renal dysfunction. The presence of lead causes the degradation of the brush border of renal epithelial cells impermeable to creatinine and urea. For these reasons, the renal function deteriorates, and blood urea and creatinine levels rise. Although the exact mechanism of renal lead toxicity is not fully understood, it has been indicated that oxidative stress plays an important role (Matovic et al. 2015).

There is a limited number of studies on lead-induced oxidative stress in animal kidney tissue. The results of this study are consistent with other studies in the literature showing that there was an increase in MDA levels in rat kidney using lead 10-150 mg/kg for 24 hours (Sharma et al. 2014), 20 mg/kg for 5 days (Abdel Moneim et al. 2011), 5 mg/kg for 30 days (Lakshmi et al. 2013), 50 mg/kg for 40 days (Sharma et al. 2010), and 500 mg/L for 8 weeks (Wang et al. 2012). It has been reported that lead exposure stimulates free radical production in the kidney by leading to the inactivation of tubular cell structure and the loss of membrane integrity, and thus causes lipid peroxidation (Aziz et al. 2012; Sharma and Singh 2014).

Due to its stability, 8-OHdG is one of the most reliable markers of oxidative DNA damage (Li et al. 2010). Studies on cadmium, arsenic, methyl mercury, and ferric nitrotriacetate reported that there was an increase in 8-OHdG levels in the kidney (Galazyn et al. 2009; Iqbal et al. 2009; Jin et al. 2008). Liu et al. (2010) determined that the
8-OHdG level was significantly increased in the kidneys tissue of the rats exposed to 500 mg Pb/L in drinking water for 10 weeks and suggested that DNA is a common target of lead-induced ROS in the kidney. In our study, we found that the 8-OHdG level in the kidney tissue was significantly higher in the rats treated with lead compared to the controls. The sulfhydryl groups of glutathione can act as a non-enzymatic antioxidant by directly interacting with ROS, or ROS is involved in enzymatic detoxification reactions as a cofactor or coenzyme. Because GSH is a tripeptide containing cysteine with reactive sulfhydryl groups that have a reduction potential (Hsu and Guo 2002; Sivaprasad et al. 2004). Studies showed that lead caused a significant reduction in GSH level and GSH/GSSH ratio in the kidney tissue (Aykin-Burns et al. 2003; Jurczuk et al. 2006; Lui et al. 2010; Sivaprasad et al. 2004). In the present study, the GSH level in the kidney tissue was significantly decreased in the rats treated with lead when compared with the control group. This reduction may have occurred because lead binds to the SH group of GSH (Lui et al. 2010).

Ceruloplasmin (a copper metalloenzyme) acts as an antioxidant due to its ferroxidase activity (Lee et al. 2015). However, when oxidative stress rises, it acts as a pro-oxidant by releasing free copper ions that induce the formation of reactive oxygen species and the oxidation of low-density lipoproteins (Shukla et al. 2006). It has been emphasized that elevated ceruloplasmin levels may be used as a strong marker of diabetic nephropathy (Lee et al. 2015). Increased levels of ceruloplasmin were found to be associated with oxidative stress (Mongiat et al. 1992). It was observed that there was an increase in ceruloplasmin levels in rats treated with 1,000 mg/L lead acetate for 4 weeks (Flora et al. 1993). In this study, the ceruloplasmin concentration was significantly higher in the kidney tissue of the rats treated with lead. The increase in ROS levels induced by lead causes the antioxidant defense system to weaken. Animal and human studies showed that lead changed SOD, CAT, GPx, GST and G6PD activities and GSH level (Matovic et al. 2015). Experimental studies revealed that long-term i.p. (Abdel Moneim et al. 2011; Lakshmi et al. 2013) and oral administration (Farmand et al. 2005; Bas et al. 2015; Sharma et al. 2010; Wang et al. 2012) of Pb significantly reduced the activities of antioxidant enzymes such as SOD, CAT, and GPx in the kidney tissue. The molecular structures and activities of these enzymes depend on a variety of essential elements, so antioxidant enzymes are potential targets for lead (Lui et al. 2010). In the present study, the catalase activity was found to be significantly lower in the lead-treated groups compared to the control group. This reduction may be due to increased reactive oxygen species production. After recent studies have shown that lead induces oxidative stress by deteriorating the antioxidant defense system, antioxidant therapy has gained importance. Wang et al. (2016). Chitosan exhibits antioxidant activity as a natural cationic polysaccharide. Mechanisms related to the antioxidant activity of chitosan include free-radical-scavenging action, metal-ion-chelating ability, and reducing activity (Chien et al. 2007; Kim and Thomas 2007; Onsuyen and Skaugrud 1990; Park et al. 2004; Sun et al. 2004). In the present study, co-administration of chitosan with lead significantly decreased Pb, MDA, and ceruloplasmin levels and significantly increased CAT activity in the kidney tissue. The decreased level of lead in the kidney tissue may be due mainly to the chelating property of chitosan with heavy metals. In this context, the antioxidant levels may have increased in relation to the reduction in ROS production.

CONCLUSION
Current study revealed that chitosan protects the kidney against lead-induced oxidative stress.

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