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

Uğur ÖZDEK[1]   Hasan TOZ[2]   Ahmet Ufuk KÖMÜROĞLU[1]   Leyla MİS[3]

Zübeyir HUYUT[4]   Yeter DEĞER[2]

[1] Van Yuzuncu Yil University, Health Services Vocational School, Van, Turkey
[2] Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Biochemistry, Van, Turkey
[3] Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Physiology, Van, Turkey
[4] Van Yuzuncu Yıl University, Medical Faculty, Department of Biochemistry, Van, Turkey

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*

ÖZ   **Rat Böbrek Dokusunda Kurşunun Neden Olduğu Oksidatif Strese Karşı Kitosanın Koruyucu etkisi**

Kitosan antioksidan ve şelatör özelliğe sahip doğal bir polimerdir. Bu çalışmada kurşun toksikosyonu oluşturulmuş ratların böbrek dokusu kurşun (Pb), molandialdehit (MDA), 8-hidroksi deoksiguanozin (8-OHdG), glutatyon (GSH), seroloplazmin konsantrasyonu ve katalaz aktivitesi üzerine kitosanın etkisi araştırıldı. Her grupta sekiz adet olacak şekilde 28 adet erkek Wistar albino rat, kontrol (C), kurşun grubu (Pb grubu), kurşun+kitosan (Pb+CS grubu) ve kitosan (CS grubu) olmak üzere dört gruba ayrıldı. Kurşun grubuna 5 gün, 50 mg/kg kurşun asetat intraperitonel (ip) ve kitosan gruplarına (CS+Pb ve CS grupları) 28 gün boyunca 200 mg/kg kitosan gavaj yoluyla uygulandı. Çalışma sonunda, kurşun, MDA, 8-OHdG, seruloplazmin, GSH konsantrasyonu ve katalaz aktivitesi böbrek dokusunda ölçüldü. Kontrol grubu ile karşılaştırıldığında, Pb uygulanan gruplarda böbrek dokusunda Pb, MDA, 8-OHdG ve seruloplazmin seviyesi arttı, GSH seviyesi ile katalaz aktivitesi ise azaldı ($p < 0.05$). Kurşun ile birlikte kitosan verilmesi böbrek dokusunda Pb, MDA ve seruloplazmin seviyelerini azalttı, CAT aktivitesini arttırdı ($p < 0.05$). GSH ve 8-OHdG seviyelerinde önemli değişiklik olmadı ($p > 0.05$). Elde edilen sonuçlar, kitosanın, kurşun uygulaması oluşan oksidatif streseten böbreği koruduğunu göstermektedir.

Anahtar Kelimeler: *Kitosan, Kurşun, Böbrek, Oksidatif stres, Antioksidan*

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; Matos 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

Corresponding author: Uğur ÖZDEK
Van Yüzüncü Yıl Üniversitesi, Sağlık Hizmetleri MYO, Van, Türkiye . e-mail: ugurozdek@yyu.edu.tr

187

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 (HO2-), hydrogen peroxide (H2O2), superoxide anion (O2-), and hydroxyl (OH-) (Bokar et al. 2008; Ercal 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 (Finkel 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 (Ercal 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 oxidation damage (Finkel 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 meals. This changing consumer lifestyle emphasizes the need for better, effective ways of controlling food hygiene (Baş et al. 2006).

It has been shown that the reactive hydroxyl and amine groups of chitosan (a natural cationic polysaccharide) inhibit the formation of reactive oxygen radicals and prevent lipid peroxidation in biological systems (Chien et al. 2007; Je and Kim 2006; Kim and Thomas 2007; Park et al. 2004; Xing et al. 2005). Chitosan is seen as a useful candidate for non-toxic substances thanks to its antioxidant property (Wang et al. 2012). Today, many studies focus on antioxidant effects of chitosan (Guo et al. 2006; Guo et al. 2005; Guo et al. 2008; Je and Kim 2006; Jurczuk et al. 2006; Liu et al. 2009; Park et al. 2004; Sun et al. 2004; Sun et al. 2008; Xing et al. 2005; Yang et al. 2010). This study investigated the effects of chitosan in MDA, 8-OHDG, GSH and ceruloplazmin levels and CAT activity in kidney of Pb-treated rats.

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.

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 HCI 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) H2PO4 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

test to compore 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) ($p<0.05$).

In the Pb-treated groups (Pb, Pb+CS) when compared with the control group, the lead concentration in the kidney tissue was increased but decreased after administration of chitosan (Table. 2) ($p<0.05$).

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 ($p<0.05$) (Table. 2). Co-administration 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 ($p>0.05$).

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

	C	Pb	Pb+CS	CS
Urea (mg/dl)	44.83±4.57 a	59.40±5.68 c	51.33±4.22 b	46,83±4.07 a, b
Creatinine (mg/dl)	0.54±0.08 a	0.66±0.03 b	0.57±0.06 a	0.55±0.06 a

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

	C	Lead	Lead+CS	CS
Lead (ng/g)	16.244±2.457a	62.394±17.192b	35.814±8.250c	15.364±3.367a
MDA (nmol/g protein)	21.14±2.46 a	30.48±1.41 b	26.59±2.15 c	23.24±1.64 a
8-OHdG (ng/g protein)	3.98±0.59 a	5.74±1.28 b	5.25±0.31 b	4.04±0.55 a
GSH (mg/g protein)	1.13±0.11 a	0.85±0.05 b	0.88±0.03 b	1.08±0.016 a
Catalase (U/g protein)	8.82±0.62 a	2.70±0.32 b	5.25±0.41 c	8.74±0.38 a
Ceruloplasmin (mg/L protein)	8.769±2.285 a	25.447±4.607 b	18.060±5.61 c	9.397±4.145 a

Values are presented as mean ±SD (n=7). $p < 0.05$; the difference between group averages with different superscript letters on the same row 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 nitrilotriacetate 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 exaposed 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; Onsoyen 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.

REFERENCES

Abdel Moneim AE, Dkhil MA, Al-Quraishy S (2011). The protective effect of flaxseed oil on lead acetate-induced renal toxicity in rats. J Hazard Mater, 194, 250-255.

Aykin-Burns N, Laegeler A, Kellogg G, Ercal N (2003). Oxidative effects of lead in young and adult Fisher 344 rats. Arch Environ Contam Toxicol, 44, 0417-0420.

Aziz F, Maulood I, Chawsheen M (2012). Effects of melatonin, vitamin Cand E alone or in combination on lead-induced injury in liver and kidney organs of rats. IOSR J Pharm, 2 (5), 13-18.

Bas H, Kalender Y, Pandir D, Kalender S (2015). Effects of lead nitrate and sodium selenite on DNA damage and oxidative stress in diabetic and non-diabetic rat erythrocytes and leucocytes. Environ Toxicol Pharmacol, 39, 1019-1026.

Beutler E, Duron O, Kelly BM (1963). Improved method for the determination of blood glutathione. J Lab Clin Med, 61, 882-888.

Bokara KK, Brown E, McCormick R, Yallapragada PR, Rajanna S, Bettaiya R (2008). Lead-induced increase in antioxidant enzymes and lipid peroxidation products in developing rat brain. Biometals, 21, 9-16.

Chien PJ, Sheu F, Huang WT, Su MS (2007). Effect of molecular weight of chitosans on their antioxidative activities in apple juice. Food chemistry, 102, 1192-1198.

Ercal N, Treeratphan P, Hammond TC, Matthews RH, Grannemann NH, Spitz DR (1996). In vivo indices of oxidative stress in lead-exposed C57BL/6 mice are reduced by treatment with meso-2,3-dimercaptosuccinic acid or N-acetylcysteine. Free Radic Biol Med, 21, 157-161.

Farmand F, Ehdaie A, Roberts, C. K., Sindhu, R. K. (2005). Lead-induced dysregulation of superoxide dismutases, catalase, glutathione peroxidase, and guanylate cyclase. Environ Res, 98, 33-39.

Finkel T, Holbrook NJ (2000). Oxidants, oxidative stress and the biology of ageing. Nature, 408, 239-247.

Flora SJ (2009). Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. Oxid Med Cell Longev, 2, 191-206.

Flora SJ, Bhattacharya R, Vijayaraghavan R (1995). Combined therapeutic potential of meso-2,3-dimercaptosuccinic acid and calcium disodium edetate on the mobilization and distribution of lead in experimental lead intoxication in rats. Fundam Appl Toxicol, 25, 233-240.

Flora SJ, Pachauri V (2010). Chelation in metal intoxication. Int J Environ Res Public Health, 7, 2745-2788.

Flora S, Jeevaratnam K, Kumar D (1993). Preventive effects of sodium molybdate in lead intoxication in rats. Ecotoxicol Environ Saf, 26, 133-137.

Galazyn-Sidorczuk M, Brzoska MM, Jurczuk M, Moniuszko-Jakoniuk J (2009). Oxidative damage to proteins and DNA in rats exposed to cadmium and/or ethanol. Chem Biol Interact, 180, 31-38.

Guo Z, Liu H, Chen X, Ji X, Li P (2006). Hydroxyl radicals scavenging activity of N-substituted chitosan and quaternized chitosan. Bioorg Med Chem Lett, 16, 6348-6350.

Guo Z, Xing R, Liu S, Yu H, Wang P, Li C, Li P (2005). The synthesis and antioxidant activity of the Schiff bases of chitosan and carboxymethyl chitosan. Bioorg Med Chem Lett, 15, 4600-4603.

Guo Z, Xing R, Liu S, Zhong Z, Li P (2008). Synthesis and hydroxyl radicals scavenging activity of quaternized carboxymethyl chitosan. Carbohydr Polym, 73, 173-177.

Halliwell B, Gutteridge JM, Cross CE (1992). Free radicals, antioxidants, and human disease: where are we now? J Lab Clin Med, 119, 598-62.

Han S, Qiao X, Simpson S, Ameri P, Kemp FW, Bogden JD (1996). Weight loss alters organ concentrations and contents of lead and some essential divalent metals in rats previously exposed to lead. J Nutr, 126, 317-323.

Hsu PC, Guo YL (2002). Antioxidant nutrients and lead toxicity. Toxicology, 180, 33-44.

Iqbal M, Okazaki Y, Okada S (2009). Curcumin attenuates oxidative damage in animals treated with a renal carcinogen, ferric

nitrilotriacetate (Fe-NTA): implications for cancer prevention. Mol Cell Biochem, 324, 157-164.

Je JY, Kim SK (2006). Reactive oxygen species scavenging activity of aminoderivatized chitosan with different degree of deacetylation. Bioorg Med Chem, 14, 5989-5994.

Jeon TI, Hwang SG, Park NG, Jung YR, Im Shin S, Choi SD, Park DK (2003). Antioxidative effect of chitosan on chronic carbon tetrachloride induced hepatic injury in rats. Toxicology, 187, 67-73.

Jin X, Chan HM, Lok E et al. (2008). Dietary fats modulate methylmercury-mediated systemic oxidative stress and oxidative DNA damage in rats. Food Chem Toxicol, 46, 1706-1720.

Jurczuk M, Moniuszko-Jakoniuk J, Brzoska MM (2006). Involvement of some low-molecular thiols in the peroxidative mechanisms of lead and ethanol action on rat liver and kidney. Toxicology, 219, 11-21.

Kim KW, Thomas R (2007). Antioxidative activity of chitosans with varying molecular weights. Food chemistry, 101, 308-313.

Lakshmi BV, Sudhakar M, Aparna M (2013). Protective potential of Black grapes against lead induced oxidative stress in rats. Environ Toxicol Pharmacol, 35, 361-368.

Lartillot S, Kedziora P, Athias A (1988). Purification and characterization of a new fungal catalase. Prep Biochem, 18, 241-246.

Ledwozyw A, Michalak J, Stepien A, Kadziolka A (1986). The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis. Clinica chimica acta,155, 275-283.

Lee MJ, Jung CH, Kang YM et al. (2015). Serum Ceruloplasmin Level as a Predictor for the Progression of Diabetic Nephropathy in Korean Men with Type 2 Diabetes Mellitus. Diabetes Metab J, 39, 230-239.

Li Z, Piao F, Liu S, Wang Y, Qu S (2010). Subchronic exposure to arsenic trioxide-induced oxidative DNA damage in kidney tissue of mice. Exp Toxicol Pathol, 62, 543-547.

Liu CM, Ma JQ, Sun YZ (2010). Quercetin protects the rat kidney against oxidative stress-mediated DNA damage and apoptosis induced by lead. Environ Toxicol Pharmacol, 30, 264-271.

Liu CM, Zheng YL, Lu J et al. (2010). Quercetin protects rat liver against lead-induced oxidative stress and apoptosis. Environ Toxicol Pharmacol. 29, 158-166.

Liu J, Sun H, Dong F et al. (2009). The influence of the cation of quaternized chitosans on antioxidant activity. Carbohydr Polym, 78, 439-443.

Matos RC, Vieira C, Morais S, de Lourdes Pereira M, de Jesus JP (2009). Nephrotoxicity of CCA-treated wood: A comparative study with As(2)O(5) and CrO(3) on mice. Environ Toxicol Pharmacol. 27, 259-263.

Matovic V, Buha A, Ethukic-Cosic D, Bulat Z (2015). Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys. Food Chem Toxicol, 78, 130-140.

Mongiat R, Gerli GC, Locatelli GF, Fortuna R, Petazzi A (1992). Erythrocyte antioxidant system and serum ceruloplasmin levels in welders. Int J Occup Environ Health, 64, 339-342.

Mutlu N, Ersan Y, Nur G, Koç E (2011). Protective effect of caffeic acid phenethyl ester against lead acetate-induced hepatotoxicity in mice. Kafkas Univ Vet Fak Derg, 17, 1-5.

Onsoyen E, Skaugrud O (1990). Metal recovery using chitosan. J Chem Technol Biotechnol, 49, 395-404.

Pande M, Flora SJ (2002). Lead induced oxidative damage and its response to combined administration of alpha-lipoic acid and succimers in rats. Toxicology, 177, 187-196.

Park PJ, Je JY, Kim SK (2004). Free radical scavenging activities of differently deacetylated chitosans using an ESR spectrometer. Carbohydr Polym, 55, 17-22.

Patrick L (2006). Lead toxicity, a review of the literature. Part 1: Exposure, evaluation, and treatment. Altern Med Rev, 11, 2-22.

Reyes H, Baez ME, Gonzalez MC et al. (2000). Selenium, zinc and copper plasma levels in intrahepatic cholestasis of pregnancy, in normal pregnancies and in healthy individuals, in Chile. J Hepatol, 32, 542-549.

Saxena G, Flora SJ (2004). Lead-induced oxidative stress and hematological alterations and their response to combined administration of calcium disodium EDTA with a thiol chelator in rats. J Biochem Mol Toxicol, 18, 221-233.

Sharma S, Singh B (2014). Effects of acute and chronic lead exposure on kidney lipid peroxidation and antioxidant enzyme activities in BALB-C mice (Mus musculus). Int J Sci Res, 3, 1564-1566.

Sharma V, Sharma A, Kansal L (2010). The effect of oral administration of Allium sativum extracts on lead nitrate induced toxicity in male mice. Food Chem Toxicol, 48, 928-936.

Shukla N, Maher J, Masters J, Angelini GD, Jeremy JY (2006). Does oxidative stress change ceruloplasmin from a protective to a vasculopathic factor? Atherosclerosis, 187, 238-250.

Sivaprasad R, Nagaraj M, Varalakshmi P (2004). Combined efficacies of lipoic acid and 2,3-dimercaptosuccinic acid against lead-induced lipid peroxidation in rat liver. J Nutr Biochem, 15, 18-23.

Sun T, Xie W, Xu P (2004). Superoxide anion scavenging activity of graft chitosan derivatives. Carbohydr Polym, 58, 379-382.

Sun T, Yao Q, Zhou D, Mao F (2008). Antioxidant activity of N-carboxymethyl chitosan oligosaccharides. Bioorg Med Chem Lett, 18, 5774-5776.

Sunderman, FWJr, Nomoto S (1970). Measurement of human serum ceruloplasmin by its p-phenylenediamine oxidase activity. Clin chem, 16, 903-910.

Tokar EJ, Diwan BA, Waalkes MP (2010). Early life inorganic lead exposure induces testicular teratoma and renal and urinary bladder preneoplasia in adult metallothionein-knockout mice but not in wild type mice. Toxicology 276, 5-10.

Toz H, Deger Y (2017). The Effect of Chitosan on the Erythrocyte Antioxidant Potential of Lead Toxicity-Induced Rats. Biol Trace Elem Res, 184 (1), 114-118.

Wang J, Yang Z, Lin L, Zhao Z, Liu Z, Liu X (2012). Protective effect of naringenin against lead-induced oxidative stress in rats. Biol Trace Elem Res, 146, 354-359.

Wang Z. Yan Y, Yu X, Li W, Li B, Qin C (2016). Protective effects of chitosan and its water-soluble derivatives against lead-induced oxidative stress in mice. Int J Biol Macromol, 83, 442-449.

Xie W, Xu P, Liu Q (2001). Antioxidant activity of water-soluble chitosan derivatives. Bioorg. Med. Chem. Lett, 11, 1699-1701.

Xing R, Yu H, Liu S, et al. (2005). Antioxidant activity of differently regioselective chitosan sulfates in vitro. Bioorg Med Chem, 13, 1387-1392.

Yahaya MI, Ogunfowokan AO, Orji EO (2011). Elemental profile in amniotic fluid of some Nigerian pregnant women. East Afr J Public Health, 8, 92-97.

Yang S, Guo Z, Miao F, Xue Q, Qin S (2010). The hydroxyl radical scavenging activity of chitosan, hyaluronan, starch and their O-carboxymethylated derivatives. Carbohydr Polym, 82, 1043-1045.