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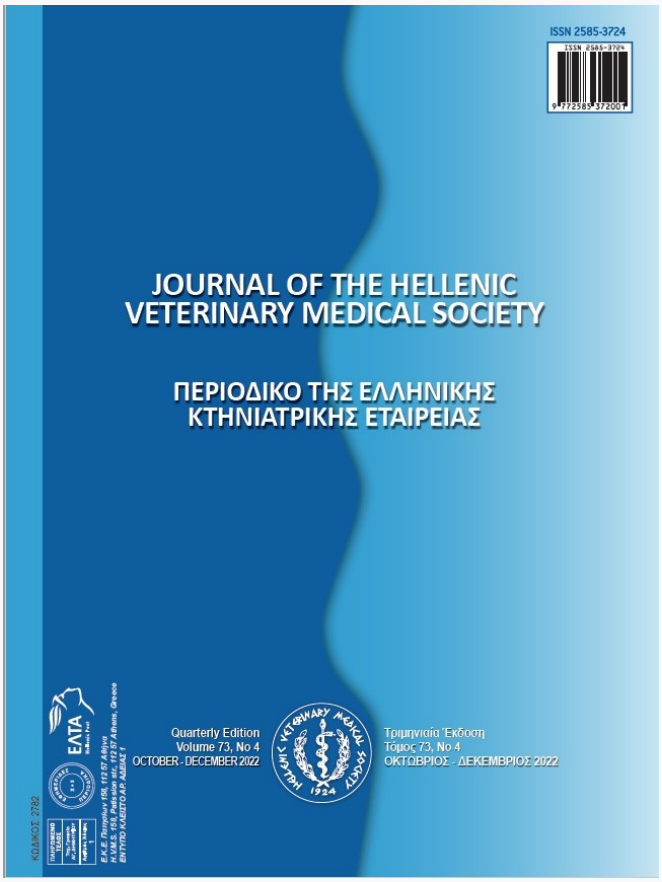

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Research article
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The Impact of Chitosan on Oxidative Stress in Liver of Rats Loaded With Lead Acetate

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ABSTRACT: This study was conducted to determine the impact of chitosan on lead (Pb)-induced hepatotoxicity. Thus, lead acetate was administered intraperitoneally (50 mg/kg for 5 days) and chitosan was given as 200 mg/kg via oral gavage for 28 days. When the trial was terminated, it was determined that aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities in serum as well as Pb, 8-hydroxy-deoxyguanosine (8-OHdG) and malondialdehyde (MDA) levels and catalase (CAT) activity in liver tissue significantly increased, however reduced glutathione (GSH) and ceruloplasmin (Cp) levels in liver and high density lipoproteins (HDL) in serum had a significant decrease in the Pb group when compared to the control group. The administration of chitosan significantly prevented Pb-induced changes in serum liver enzyme activities, 8-OHdG and MDA levels. In addition, chitosan showed a statistically insignificant effect on reduced GSH, Cp, HDL levels and CAT activity. Accordingly, administration of chitosan can strengthen the antioxidant defence system of liver tissue and may decrease oxidative stress.

Keywords: antioxidant parameter, chitosan, lead, liver, lipid peroxidation

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INTRODUCTION

Environmental contamination or occupational exposure to lead (Pb) is a risk factor in development of diseases such as nephrotic, haematologic, and hepatic disorders. Lead exposure occurs via contaminated air, water and food, thus the main target organ for lead absorbed through the portal system is liver. Having a negative effect on liver function, Pb also damages cholesterol and nucleic acid mechanisms (Abdel-Moneim et al., 2011). It has been reported that different mechanisms are effective on the development of lead-induced liver damage. This metal causes structural disorders such as necrosis and hydropic degeneration in liver cells as well as oxidative stress, and hepatotoxicity by increasing free radical (ROS) production (Abdel-Moneim et al., 2011). It was also suggested that lead may weaken the antioxidant defence system via antagonist interaction with minerals that are essential for antioxidant enzymes (Markiewicz-Górka et al., 2015).

The overproduced ROS or decelerated detoxification caused by deficiency of antioxidant systems leads to the accumulation of these radicals and toxic effects on lipid and protein molecules in the cell and DNA. Oxidative damage occurs in lipid structures in cellular membranes and aldehyde-structured compounds such as malondialdehyde (MDA) form. MDA is a biochemical marker used as a tissue lipid damage marker (Özkan-Yılmaz et al., 2014). 8-OHdG formation of DNA damage marker is caused by oxidative stress(Tsai et al., 2011).

Free radical levels of in cellular and extracellular body fluids are controlled by enzymatic (superoxide dismutase, catalase etc.) and non-enzymatic (reduced glutathione, ceruloplasmin etc.) antioxidant systems (Franco et al., 2007). Exogenous antioxidants play an important role in reducing oxidative stress. After exposure to oxidative pollutants, dietary antioxidant treatment is effective on reversal or treatment of the oxidative stress (Jacob, 1995). In some studies, the researchers investigated the protective effects of antioxidants on lead-induced liver damage in order to support liver function and repair liver damage (Mutlu et al., 2011).

Being produced by alkaline N-deacetylation of chitin, Chitosan (CS) has no toxic effect on living organisms. CS has attracted attention due to its anti-bacterial, anti-ulcer, anti-tumour, immunostimulant and other important biological activities. Recent studies have revealed that one of the most remarkable features of CS is antioxidant activity. The studies have indicated the antioxidant role and free radical scavenging properties of chitosan (Chiang et al., 2000; Jeon et al., 2003; Ozcelik et al., 2014; Wang et al., 2016). This study was conducted to assess the protective effect of chitosan on lead acetate-induced oxidative stress in liver tissue.

MATERIAL AND METHODS

Animals and the application

Three month-old male Wistar albino rats obtained from Van Yuzuncu Yıl University Animal Experiments Unit were used in the study. The rats weighed 200-250 g. The rats were randomly selected and were separated into 4 groups including 7 rats in each. They were kept in cages at 22±2 °C and 12:12 h dark- light cycle. Euro type 4 standard mouse cage with the size of 60x35x20 cm was used as the cage. 7 rats were housed in each cage. Sterile corn cob (MBD Feed Mill, Kocaeli, Turkey) was used as litter and changed daily. Rats were fed ad libitum with standard rat pellet feed (Bayramoglu Feed Mill, Erzurum, Turkey) and standard drinking water via bottles. For the present study, an approval was obtained from the Local Animal Experiments Ethics Committee at Van Yüzüncü Yıl University on 31.12.2020, with the decision number 2020/12-05.

Methodology

Assignment of study groups

C (Control group): Saline solution was administered orally for days 28.

Pb (Lead group): The lead acetate was dissolved in the saline solution and administered intraperitoneally *(i.p.)* for five days at a dose of 50 mg / kg (Mutlu et al., 2011).

Pb+CS (Lead+chitosan group): The lead acetate was dissolved in the saline solution and administered intraperitoneally (*i.p.*) for five days at a dose of 50 mg / kg. 200 mg / kg chitosan was administered orally throughout the study (Jeon et al., 2003) [12].

In the lead-induced groups, the behavior and health of the rats were observed during the study. There was no abnormal situation in the rats.

CS (Chitosan group): 200 mg / kg chitosan was administered orally.

The chitosan used is pure, low molecular weight and at least 75% deacetylation molecule (Sigma-Aldrich, CAS Number: 9012-76-4).

Biochemical analysis

On the final day of the study (on 29th day), rats were anaesthetized by ketamine HCL (75 mg/kg, İ.P) and blood samples were collected by cardiac puncture and immediately submitted for biochemical analyses. Animal blood and liver tissue were obtained. The blood samples were centrifuged for 10 minutes at +4^{0}C at 3000 rpm. Serum high density lipoprotein (HDL, Ref:02R06-21), cholesterol (Ref:7D62-22), triglyceride (Ref:7D74-22), alanine aminotransferase (ALT, Ref:7D56-22), aspartate aminotransferase (AST, Ref:7D81-22) and alkaline phosphatase (ALP, Ref:7D55-22) levels were determined via a modular auto-analyser device (ARCHITECT ci16200, U.S.A) using commercial kits (Abbott, Illinois, U.S.A.). Liver samples were homogenised in cold 0.1mM phosphate buffer (pH 7.4) and centrifuged at 10.000 rpm at 4°C for 15 min. After the centrifuge, the supernatant fraction was removed. In these supernatants, MDA level was determined based on the method of Draper and Hadley (1990). GSH level was measured using the method of Beutler et al., (1963). CAT activity was measured using the method of Aebi, (1984). Cp level was determined using the altered Ravin method. The measurement of 8-OHdG level was performed using commercial ELISA kit (Shanghai LZ biotech Co, Ltd, China). Protein level was measured based on method of Lowry et al., (1951) using bovine serum albumin as the standard.

Determination of liver Pb levels

For this purpose, 2% HNO_3 and 500 µL 10% Triton x 100 were added on the previously prepared 250 µL supernatant. 1250 µL 10% $(NH_4)H_2PO_4$ was added. The total volume with deionised pure water was completed to 5 ml. Then, a 286.3-nm wavelength analysis was performed in the furnace unit in the atomic absorption device (Graphite Furnace Atomiser, GFA-7000 Shimadzu) using a Pb cathode lamp (Hollow Cathode Lamp, Hamamatsu Photonics). The results were expressed as ngPb / g tissue based on the calibration curve plotted for different standard concentrations (Kummrow et al., 2008; Subramanian, 1996).

Statistical Analysis

Duncan test was determined whether or not continuous variables were normally distributed. The groups with normally distributed variables were compared by using One-way analysis of variance (ANOVA). The value of $p<0.05$ was considered as significant. SPSS (20.0) software was used to carry out all analyses.

RESULTS

The results of the study are shown in Tables 1 and 2.

Table 1. Serum biochemical parameters of control and experimental groups

Parameters	Control	Pb	Pb+CS	CS
HDL (mg/dl)	40.20±2.11 ^{a}	24.93±5.71 ^{b}	27.99±9.20 ^{b}	33.03±3.19 $^{a, b}$
Cholesterol (mg/dl)	60.83±4.26	66.20±6.30	65.40±13.18	60.50±7.97
Triglyceride(mg/dl)	95.50 ±13.08	108.83±16.58	106.75±27.37	102.50±19.94
ALT (mg/dl)	14.40± 2.30 ^{a}	32.29±2.36 ^{b}	17.58±2.36^a	14.33±2.88 ^{a}
AST (mg/dl)	99.50±4.35 ^{a}	127.50±7.31^b	109.58±5.91^a	107.76±13.06^a
ALP (mg/dl)	208.00±37.32 ^{a}	387.50±56.73 ^{b}	222.24±57.09 ^{a}	219.50±13.87^a

C : Control group, Pb: group of lead acetate, Pb+CS: group of lead acetate and chitosan, CS: group of chitosan. Each value is expressed as mean ± SE (n=7). $p<0.05$; a, b, c: The difference between the mean values of the groups shown differently from the letters in the same row is statistically significant. High density lipoproteins (HDL), Alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP).

Table 2. The liver Pb, MDA, 8-OHdG, GSH, Cp levels and CAT activity for control and experimental groups

Parameter	Control	Pb	Pb+CS	CS
Lead (ng/g tissue)	13.51±0.55^a	261.11±61.88^b	181.12±62.18^c	13.012±1.87 ^{a}
MDA(nmol/g protein)	7.80±0.38^a	13.32±2.58^b	9.06±0.03^a	8.39±0.62 ^{a}
8-OHdG(ng/g protein)	2.10±0.92^a	3.68±0.76^b	2.53±0.31^a	2.24±0.79 ^{a}
GSH(mg/g protein)	1.21±0.05^a	0.85±0.05^b	0.97±0.07^a	1.08±0.12 ^{a}
CAT (U/g protein)	10.839±2.331^a	22.762±2.349^b	20.102±2.791^b	13.974±1.349^a
Cp (mg/ L protein)	34.41±7.62 ^{a}	13.43±6.40 ^{b}	17.12±5.13^b	41.11±15.33 ^{a}

C: Control group, Pb: group of lead acetate, Pb+CS: group of lead acetate and chitosan, CS: group of chitosan. Each value is expressed as mean ± SE (n = 7). $p<0.05$; a, b, c: The difference between the mean values of the groups shown differently from the letters in the same row is statistically significant. Lead (Pb), malondialdehyde (MDA), 8-hydroxy-desoxyguanosine (8-OHdG), reduced glutathione (GSH), catalase (CAT), and ceruloplasmin (Cp).

It was determined that HDL levels decreased significantly in Pb and Pb+CS groups ($p<0.05$) compared to the control group. There was no statistically significant difference between CS and control groups ($p>0.05$).

While ALT, AST and ALP activities increased significantly in Pb group compared to the control group ($p<0.05$), there was no statistically significant change between the other groups and the control group ($p>0.05$).

Lead levels were significantly higher in Pb and Pb+CS groups than the control group ($p<0.05$). Also, Pb level was significantly lower in Pb+CS group than Pb group ($p<0.05$).

The MDA and 8-OHdG levels were significantly higher, whereas, reduced GSH level was significantly lower in Pb group($p<0.05$). No significant change was observed in the other groups compared to the control group ($p>0.05$).

While CAT activity was significantly higher, Cp level was significantly lower in Pb and Pb+CS groups than the control group ($p<0.05$). No statistically significant differences were found between CS and control groups ($p>0.05$).

DISCUSSION

The lead in the bloodstream is deposited rapidly into soft tissues, primarily in the liver and kidney (Demirdag et al., 2015; Jain and Singhai, 2011). Since liver is rich in oxidative substrates, it is susceptible to peroxidative agents. Lead exposure results in severe oxidative damage by inhibiting the antioxidant system in the liver and raising ROS levels (Flora et al., 2007; Liu et al., 2010). The studies have reported that lead exposure causes hepatotoxicity (Mutlu et al., 2011).

In a study, it was found that low molecular weight chitosan species administered orally in female mice were absorbed in the intestine to some extent. In addition, orally administered chitosan has been detected in liver, kidney, blood and spleen tissues. Low molecular weight (less than 60 kDa) chitosan is known to pass through the cell membrane. (Zeng et al., 2008). In the same study, it was determined that the amount of chitosan in the liver is significantly higher than that in blood.

Previous studies have revealed that chitosan has an antioxidant activity and strengthens antioxidant defence systems (Heidarian and Rafieian-Kopaei, 2013; Hossain et al., 2015; Sarkar et al., 2015). The free radicals interact with the hydroxyl or amine group to form stable macromolecule radicals (Çaylak, 2010; Flora et al., 2004; Wang et al., 2016).

Serum ALT, AST and ALP enzyme activity levels are important predictors of hepatocellular damage(-Jain and Singhai, 2011). It has been observed that ALT and AST activities change after Pb administration (Markiewicz-Górka et al., 2015; Mehana et al., 2012; Ozcelik et al., 2014; Ozkaya et al., 2018). In the present study, serum ALT, AST and ALP activities increased significantly in Pb group. In Pb-induced and CS-treated rats, increased liver enzymes significantly reduced. This result indicated that in hepatocyte membranes, CS can reduce oxidative stress and enhance endogenous antioxidant defence (Heidarian and Rafieian-Kopaei, 2013; Hossain et al., 2015; Sarkar et al., 2015).

Liver has a crucial role in lipid metabolism, several stages of lipid synthesis and transportation. For this reason, an abnormal lipid profile is rational to expect in severe liver dysfunction (Shalan et al., 2005). It has been reported that chitosan administration reduced serum total cholesterol, HDL (Antonowicz et al., 1996; A Skoczyńska and Smolik, 1994; Anna Skoczyńska et al., 1993) and LDL levels (Shalan et al., 2005). It is considered that chitosan could reduce serum lipid and plasma cholesterol levels based on its anti-lipidemic activity (Anandan et al., 2004). On the contrary, in a previous study it was stated that chitosan administration increased total cholesterol and HDL levels (Kristal-Boneh et al., 1999). In this study, a significant decrease in HDL level was detected in the Pb group. There was no significant increase in HDL level, which decreased in the Pb+CS group compared to the Pb group.

Previous studies revealed that various carcinogens such as aflatoxin B1, psychological stress, and ionizing radiation increased liver 8-OHdG content (Ichiba et al., 2003; Seki et al., 2002). It was suggested that 8-OHdG-positive hepatocyte content was correlated with AST and ALT in cirrhosis and hepatitis. The presence of a relationship between high 8-OHdG immunoreactivity and lead exposure in the liver has been indicated (Ozkaya et al., 2018). Likewise, in this study, it was observed that the 8-OHdG level of the liver increased significantly in the Pb group compared to the control group. It was found that the increasing 8-OHdG level decreased in the Pb + CS group (Ta-

ble 2). Similar to the studies mentioned above, it was found that serum ALT, AST, ALP levels decreased in parallel with the decrease in 8-OHdG level in the PB + CS group (Table 1). Lead is removed from the liver mainly through bile (Ozkaya et al., 2018). There may be a decrease in lead excretion due to liver damage. The liver tissue lead level was significantly higher in the Pb group compared to the control group. It was observed that the lead level in the Pb + CS group, depending on the chitosan application, decreased significantly compared to the Pb group. There may be a decrease in the excretion of lead due to liver damage. Chitosan is known to have chelating properties. In this study, it can be thought that the formation of chitosan-lead chelate may be effective in decreasing the increased liver Pb level. Thus, reduced Pb accumulation in CS+Pb group may be associated with a decrease in liver damage. It has been reported that chitosan application reduces the Pb levels in blood (Toz and Değer, 2018) and liver tissue (Wang et al., 2016).

Lipid peroxidation is considered as an important initiator of the toxicity mechanism of Pb, since the liver tissue has a comparatively high polyunsaturated fatty acid content. In the present study, the liver MDA level, a product of lipid peroxidation, was higher in Pb group than the control group (Ozkaya et al., 2016; Özkan-Yılmaz et al., 2014; Wang et al., 2016). The increase in the tissue MDA levels might have been induced by the possible involvement of ROS in Pb-induced toxicity (Patra et al., 2011). Recent studies have revealed that the ROS scavenging effect of CS inhibited lipid peroxidation of polyunsaturated fatty acids. In the present study, MDA concentrations decreased significantly after the administration of CS in the Pb+CS group. Furthermore, chitosan decreased ALT, AST, and ALP activities. Lipid peroxidation changes membrane integrity by impairing important metabolic functions.

Lead-induced increase in lipid peroxidation might be associated with changes in the antioxidant defence system (Ademuyiwa et al., 2009). GSH, a non-enzymatic antioxidant, contains the sulfhydryl group, which is necessary for the antioxidant functions (DeLeve and Kaplowitz, 1991). Pb is bound to the sulfhydryl group, causing a decrease in GSH levels and adversely affecting the antioxidant mechanism (Saxena et al., 2005). In the present study, liver GSH levels significantly reduced in Pb group. However, in the Pb+CS group, the decrease in GSH level caused by lead was partially prevented by chitosan application. The reason for this may be the decrease in lead level with chitosan application. It was observed in this study that liver CAT activity significantly increased in Pb and Pb+CS groups. The increase in CAT activity might be a result of the defence mechanism developed by the liver against the increased hydrogen peroxide levels. In parallel with the data of this study, it was reported that CAT activity increased in liver of lead- treated rats (Ozkaya et al., 2016). However, contrary to the abovementioned studies (Wang et al., 2016) it was found that liver CAT activity decreased after lead exposure.

Ceruloplasmin (Cp) is an acute phase protein primarily synthesised in the liver. Cp prevents the formation of free oxygen radicals and lipid peroxidation, and thus, inhibiting free radical damage in tissues and plasma (Uhlikova et al., 2008; Vassiliev et al., 2005). In their study, Mylroie et al., reported that serum Cp level significantly decreased in rats loaded with lead acetate compared to the control group (Mylroie et al., 1986). The results of the present study indicated that liver Cp level significantly decreased in Pb group when compared to the control group and CS administration after Pb exposure did not increase significantly Cp level in Pb+CS group

CONCLUSIONS

In conclusion, it is possible to assert that Pb administration leads to oxidative stress by inducing lipid peroxidation and altering the antioxidant system. Chitosan supplementation as an antioxidant and chelating agent can protect the liver tissue against lead toxicity since it reduces lipid peroxidation and improves endogenous antioxidant defence system.

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CONFLICT OF INTEREST

None declared.

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