Received: 3 October 2017 | Revised: 6 March 2018 | Accepted: 25 March 2018

DOI: 10.1002/jemt.23036

RESEARCH ARTICLE

WILEY MICROSCOPY RESEARCH & TECHNIQUE

The influence of lead nanoparticles on the morpho-functional changes of rat liver during the postexposure period

Vasyl Aleksiichuk | Sergiy Omelchuk | Liudmyla Sokurenko | Rostyslav Kaminsky | Oleksandr Kovalchuk | Yuri Chaikovsky

Bogomolets National Medical University, Kyiv, Ukraine

Correspondence
Liudmyla M. Sokurenko, Bogomolets National medical university, Kyiv, Ukraine.
Email: l-sokurenko@i.ua

Review Editor: Prof. Alberto Diaspro

Abstract

Lead as any heavy metals may be found in soil, water, air, and is used in everyday life. Once in the body, it causes toxic effect, making the liver, which is one of the main organs of detoxification, suffer. Recently, the study of the action of not only ionic forms of lead, but also its nanoparticles, has become topical. The study aims at determining changes in the liver of rats and biochemical changes in their blood both at late term of exposure to nanoparticles of lead compounds and in the post-exposure period. The study was performed on 120 male rats of Wistar line, which were divided into two series, each series containing four groups. The first and the second groups of animals were intraperitoneally injected with colloidal solution of nanoparticles of lead sulfide of 10 and 30 nm in size, and the third group were intraperitoneally injected with a solution of lead nitrate. The fourth group of animals served as control. In the first series, the investigated substances were administered 60 times within 12 weeks. In the second series, after 60-fold administration of the investigated substances, the exposure was discontibued and animals were observed for 6 weeks—overall duration of 18 weeks. Histological, morphometrical and biochemical methods were used. The body weight was reduced in the rats exposed to PbS_{nano1} at week 12 of experiment and in rats exposed to both PbS_{nano1} and $Pb(NO_3)_2$ in the second series. Absolute liver weight increased at week 12 of experiment in all experimental groups. In the second series this value almost reached that of the control level. Relative liver weight in the animals of all experimental groups was higher than that in the control at week 12 of experiment. In the second series this value remained higher in rats exposed to PbS_{nano1}. After 12 weeks of exposure dystrophic changes in the liver were found in all experimental groups. At week 6 after the exposure (the second series) destructive changes in the liver decreased. Total protein, albumin, glucose, total lipids, cholesterol, triglycerides content in blood serum corresponded with morphological data. The experiment has demonstrated that the 12 weeks long exposure to lead nanoparticles had harmful effect on the liver. Within the postexposure 6-weeks period structural changes in the liver and biochemical changes in blood serum decreased. Biochemical changes in blood serum corresponded to the morphological data. By many parameters PbS_{nano1} had more pronounced harmful effect. Toxicity of PbS_{nano2} and $Pb(NO_3)_2$ were comparable.

KEYWORDS

lead nanoparticles, lead sulfide, liver morphology, serum biochemistry

1 | INTRODUCTION

Heavy metals may be found in soil, water, air, and are used in everyday life (Tsuzuki, Sugiyama, & Haramaki, 1994; Sokurenko & Chaikovskii, 2014; Van Sprang, et al., 2016; Sokurenko & Chaikovskii, 2016; Sokurenko, Savchyna, Litus, Kaminsky & Chaikovsky, 2017). Once in the body, they excessivly accumulate in the tissues causing the whole range of symptoms of poisoning (such as abdominal discomfort, vomiting, weight loss, heart and kidney failure, alimentary syndrome, neuromuscular syndrome (more often seen in adults), cerebral

syndrome, lead encephalopathy, neurological defects, psychopathological disorders (Kamynsky, Primachenko, Sokurenko, and Chaikovsky, 2016; Rostislav, Liudmyla, and Yuri, 2016; Doudi & Setorki, 2015) at the same time making the liver, which is one of the main organs of detoxification, suffer (Hadrup & Lam, 2014).

Recently, the study of the effect of not only the ionic forms of heavy metals but also their nanoparticles has become topical. However, the available literature data regarding the toxic effects of nanoparticles of heavy metals like lead on the body (Omelchuk, Aleksijchuk, Sokurenko, Blagaia, & Prudchenko, 2016) are scare. Moreover, there are no data on their impact in case of long-term exposure and in the post-exposure period.

The study aims at determining changes in the liver of rats and biochemical changes in their blood both at late term of exposure to nanoparticles of lead compounds and in the post-exposure period.

2 | MATERIALS AND METHODS

The studies were carried out on 120 male Wistar rats weighing 160–180 g. The animals were kept in vivarium conditions on a standardized diet with free access to drinking tap water. The experiment was carried out in accordance with the Council of Europe Convention on the protection of vertebrates, which are used for scientific purposes. The investigated substances were injected intraperitoneally daily 5 times a week (to simulate the working week). The animals were divided into two series, with four groups in each series (15 animals per group). In Groups 1 and 2 colloidal solution of lead sulphide nanoparticles was injected at a dose of 1.08 mg/kg (in lead equivalent to 0.94 mg/kg lead), their size being 10 nm in the Group 1 of animals (PbS_{nano1}) and 30 nm in the Group 2 (PbS_{nano2}). Group 3 was injected (Pb (NO3))—a solution of lead nitrate in ion form in a dose of 1.5 mg/kg (0.94 mg/kg lead in lead equivalent). The fourth group of animals (control) was administered 1 ml of normal saline. In the first series, the investigated substances were administered 60 times within 12 weeks. In the second series, after 60-fold administration of the investigated substances, the exposure was discontinued and animals have been observed for 6 weeks, the overal post exposure period lasting for 18 weeks.

Colloid solutions of lead sulphide nanoparticles were prepared by chemical synthesis, for them to contain concentration of the metal cation similar to that in the ionic form solutions. Same amount of lead nitrate was added to the distilled water, from which Pb nanoparticles were later prepared in the followingreaction: $Pb(NO_3)_2 + Na_2S\text{-}2NaNO_3 + PbS$. Sodium polyphosphate was used as the nanoparticles stabilizer. The size of the NP of Pb sulphide in colloid solutions depended on the changes of the temperature setting during their synthesis.

All the animals were euthanized by decapitation under mild ether anesthesia. The blood and liver of the experimental animals as well as their general status, behavior, average body weight, absolute and relative liver weights were studied.

The specimens of the experimental animals' livers were fixed in 4% formaldehyde solution and after standard histological procedures paraffin slides 7–8 μm thick were made. General morphology was studied after azure-II-eosine, Van Gieson's staining and PAS-reaction. Einarson's gallocyanin-chrome alum method was used to perform cytophotometric quantification of the nucleic acids (NA; Crissman et al., 2004; Neef, Nikula, Francke-Carroll, and Boone, 2012). The cross-sectional area of the cell nucleus (N area, μm^2), specific optical density of the cell nucleus (N DM in ODU—optical density units) were determined in 30 stained cells. Content of NA was calculated in the reconstructed volume of the cell nucleus (N Coef NA) for the extrapolation of measurement results in the cross-section area (Hrabovoy, Zarets'kyy, & Klymnjuk, 2012):

$$\text{N Coef NA} = \text{N IntDen} \cdot 3/4 \cdot \text{N area} \cdot \sqrt{(\text{N area}/\pi)} \quad (1)$$

When studying the cytoplasm of cells we have also determined the cross-sectional area of the cell cytoplasm (C area, μm^2), optical density of the cell cytoplasm (C DM in optical density units, ODU), NA content in the reconstructed volume of the cell cytoplasm (by the formula similar to 1; C Coef NA). Total protein, albumin, glucose, total lipids, cholesterol, triglycerides levels in blood serum were determined by Vitros-250 biochemical analyzer. Biochemical studies are presented according to the International System of Units recommended for use in clinical laboratory practice.

The average body and liver weights and biochemical parameters were evaluated by variational statistics using *t* Student and Fisher test, after confirming the normality of distribution. The average value (M) and the standard deviation (m) were determined.

3 | RESULTS

The post-exposure observation of the animals has not revealed any significant deterioration, with their motor and behavioral activity being in the normal range. At the same time the reduced consumption of food and water by rats exposed to $Pb(NO_3)_2$ and animals exposed to $PbS_{nano1}$was recorded.

At week 12 (the first series) a significant decrease in body weight was observed only in animals exposed to $PbS_{nano\ 1}$ (271.5 ± 3.1 g and 293.5 ± 1.34 g in the control). Absolute and relative liver weight were significantly increased ($p \leq .05$) in rats of experimental groups as compared to the control.

At week 18 (the second series) the average body weight of rats exposed to both $Pb(NO_3)_2$ (284.5 ± 14.4 g) and PbS_{nano1} (249.5 ± 5.5 g) was significantly lower, than in the control group animals (343.0 ± 4.1 g). The average body weight of rats exposed to PbS_{nano2} was significantly higher than that of those exposed to PbS_{nano1} in this series and did not differ from the control (Figure 1).

There was a significant decrease in absolute liver weight ($p < .05$) of (1) animals exposed to both PbS_{nano1} and $Pb(NO_3)_2$ (8.46 ± 0.17 and 7.78 ± 0.46 g) compared with the corresponding values in the control, of (2) experimental animals compared with the corresponding values in the 1st series, and of (3) experimental animals exposed to both PbS_{nano1} and $Pb(NO_3)_2$ as compared to those exposed to PbS_{nano2}. The relative liver weight of all experimental animals (3.40% ± 0.04%, 2.86% ± 0.04%, and 2.72% ± 0.04%) was significantly lower than the

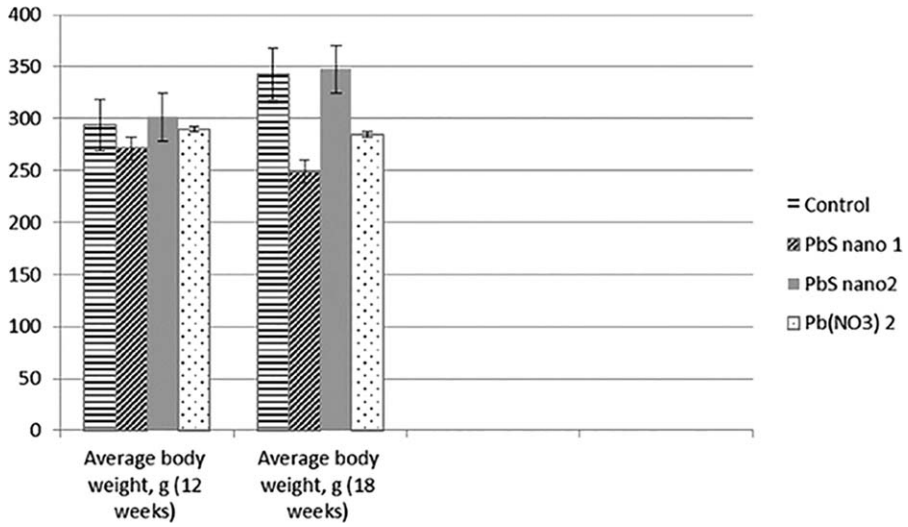

FIGURE 1 Body weight of experimental animals (M ± m), $p \leq .05$

values obtained in the first series (3.71% ± 0.08%, 3.57% ± 0.04%, and 3.84% ± 0.05%, respectively; Figure 2).

3.1 | Changes in biochemical parameters of the laboratory rats blood

At week 12 (the first series) the serum concentration of total protein in animals after exposure to PbS_{nano2} (76.9 ± 2.6 g/L) was significantly reduced as compared to the similar value in the animals exposed to Pb $(NO_3)_2$ (95.4 ± 2.97 g/L), the latter being higher than that in the control (Figure 3). Albumin levels in the animals exposed to nanoparticles was significantly lower than that in animals exposed to $Pb(NO_{3)2}$. However the albumin content (%) in the second group was significantly higher than that in the first and the third group. The glucose levels in rats of all experimental groups was significantly increased in comparison with the corresponding value of the control group. The serum levels of total lipids and cholesterol of the experimental animals tended to decrease and that of triglycerides to grow in comparison with control.

At week 18 (the second series) serum total concentration of protein in the animals exposed to $Pb(NO_3)_2$ (78.70 ± 1.6 g/L) and PbS_{nano1} (81.7 ± 2.3 g/L) was significantly reduced compared with values in the first series while in animals of the group exposed to PbS_{nano2} (88.7 ± 1.7 g/L) this value was significantly increased as compared with the control group (76.9 ± 0.9 g/L) and other experimental groups.

Albumin concentration in animals exposed to PbS_{nano1} (37.04 ± 0.7 g/L), PbS_{nano2} (38.3 ± 1.2 g/L) and $Pb(NO_3)_2$ (36.0 ± 1.0 g/L) was similar to the control values (36.8 ± 1.3 g/L). The albumin concentration in animals exposed to $Pb(NO_3)_2$ was significantly lower than the respective value in the 1st series (Figure 3).

The serum concentration of glucose in rats exposed to $Pb(NO_3)_2$ (6.09 ± 0.19 mmol/L), to PbS_{nano2} (6.17 ± 0.37 mmol/L) and PbS_{nano1} (5.96 ± 0.36 mmol/L) was significantly higher than the corresponding value of the control group (4.53 ± 0.3 mmol/L), and that at the first series.

The concentration of total lipids in the animals exposed to PbS_{nano1} (4.6 ± 0.1 g/L), PbS_{nano2} (4.8 ± 0.1 g/L), and $Pb(NO_3)_2$

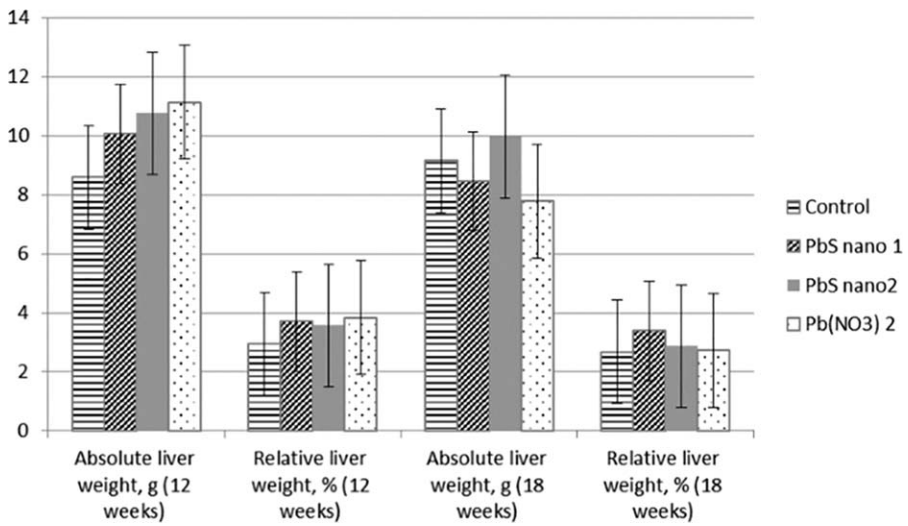

FIGURE 2 Liver weight of experimental animals (M ± m), $p \leq .05$

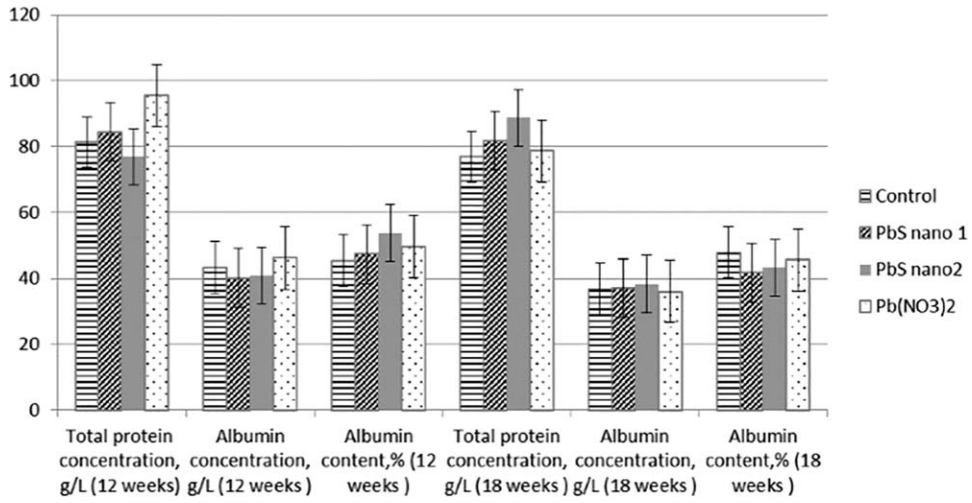

FIGURE 3 Changes in protein metabolism in the blood serum of experimental animals (M ± m), $p \leq .05$

(4.1 ± 0.3 g/L) was significantly higher than the values of the 1st series. In the group exposed to lead nanoparticles these values were significantly higher than in the control (3.9 ± 0.3 g/L).

The level of total cholesterol in animals exposed to PbS_{nano1} (1.8 ± 0.2 mmol/L), PbS_{nano2} (1.4 ± 0.2 mmol/L), $Pb(NO_3)_2$ (1.2 ± 0.1 mmol/L) was significantly lower than in the control group (2.2 ± 0.09 mmol/L) and was almost similar to the first series.

The serum concentration of triglycerides in animals exposed to $Pb(NO_3)_2$ (1.9 ± 0.2 mmol/L), PbS_{nano2} (2.0 ± 0.3 mmol/L) was significantly higher than in the control (1.3 ± 0.2 mmol/L), whereas the concentration of triglycerides in animals exposed to PbS_{nano1} (1.9 ± 0.3 mmol/L) was not different from control (Figure 4).

3.2 | Liver morphology

Microscopic examination of the liver of control animals revealed normal architecture of liver plates, moderate amount of connective tissue between lobules. Hepatocytes had clear contours and rounded nuclei. Their cytoplasm contained many glycogen granules.

At week 12 (the first series) architecture of the liver plates was slightly disorganized. Dystrophic changes of hepatocytes were seen in the liver parenchyma of all experimental groups (Figure 5a). These changes were manifested by flattening and polymorphism of hepatocytes nuclei, which were often excentrically located. Their cytoplasm was vacuolised and edematous, with significant reduction in the number of glycogen granules and unclear contours of hepatocytes. The endothelium of the capillaries was swollen. Blood vessels including the central veins were dilated. Interlobular edema and infiltration by lymphocytes and histiocytes was marked. The amount of interlobular connective tissue was higher than in the control.

The cross-section area of hepatocytes nuclei in animals of all experimental groups was significantly higher in comparison with the corresponding value of the control group animals (Table 1). Specific optical density of the cell nuclei in animals exposed to PbS_{nano2} was

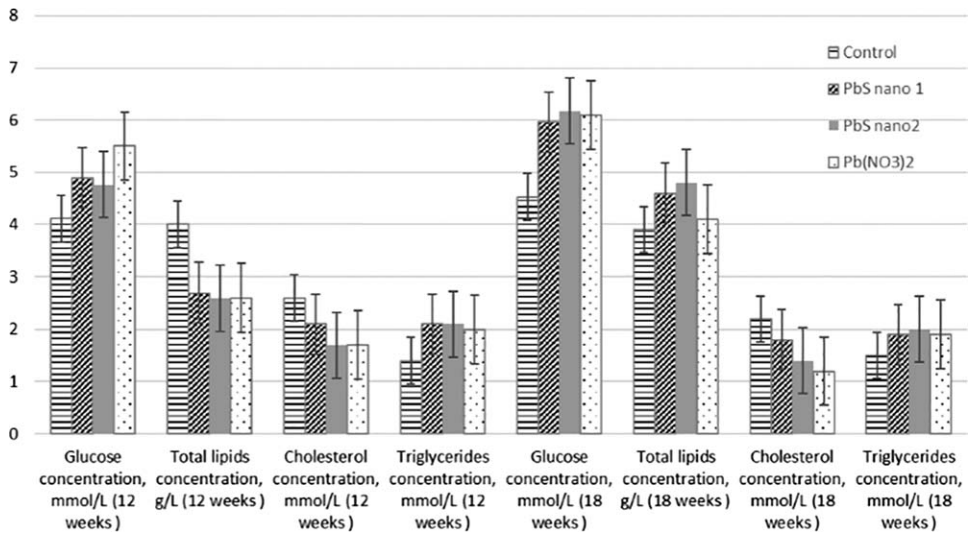

FIGURE 4 Changes in lipids and carbohydrate metabolism in the blood serum of experimental animals (M ± m), $p \leq .05$

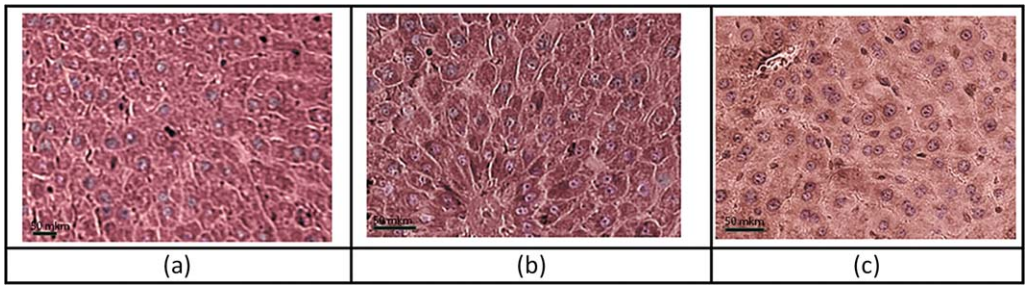

FIGURE 5 The structure of the liver in experimental groups after exposure to nanoparticles of lead sulfide. (a) First series (12 weeks), and (b) second series (18 weeks), Particles size – 30 nm. (c) Control. PAS-reaction and azur II [Color figure can be viewed at wileyonlinelibrary.com]

significantly higher than in the control group and in the animals exposed to PbS_{nano1}, this value being higher in animals exposed to $Pb(NO_3)_2$ than in the control group. Content of NA in the reconstructed cell nucleus volume in animals of all experimental groups was significantly lower than in the control group.

The cross-sectional area of hepatocytes cytoplasm in animals of all experimental groups was significantly higher compared to the corresponding value of animals of the control group (Table 2). Specific optical density of the cell cytoplasm of all experimental groups was significantly lower compared to the corresponding value of the control animals. Content of NA in the reconstructed cell cytoplasm volume in rats exposed to both PbS_{nano1} and PbS_{nano2} was significantly lower than in the control and animals exposed to $Pb(NO_3)_2$, while this value in rats exposed to PbS_{nano1} was significantly lower than in the animals exposed to PbS_{nano2}.

At week 18 (the second series) destructive changes in the liver decreased. Architecture of the liver plates did not differ from the control. Dystrophic changes of hepatocytes were not pronounced (Figure 5b). Euchromatin content in the rounded nuclei increased compared with groups of the first series. The number of glycogen granules remained lower and hepatocytes contours were clear. Interlobular edema and infiltration by lymphocytes and histiocytes were not pronounced.

The cross-sectional area of the hepatocytes nuclei in animals exposed to both PbS_{nano1} ($61.71 \pm 1.37\ \mu m^2$) and PbS_{nano2} ($66.32 \pm 2.34\ \mu m^2$) was significantly smaller as compared with the corresponding values of animals exposed to PbS_{nano1} and PbS_{nano2} in the first series of the experiment, although it was significantly larger compared with that in animals exposed to $Pb(NO_3)_2$ and the control group (Table 1).

Specific optical density of the cell nuclei in the group exposed to PbS_{nano1} (102.68 ± 1.78 ODU) was significantly lower than in the 1st series, but higher than that of the control group and animals exposed to $Pb(NO_3)_2$. Specific optical density of the cell nuclei in the group exposed to PbS_{nano2} (96.18 ± 3.07 ODU) was significantly lower than in the 1st series and did not differ significantly from that of the control group and animals exposed to $Pb(NO_3)_2$. NA content in the reconstructed volume of cell nucleus in animals exposed to PbS_{nano1} (11.12 ± 0.41 ODU) and to PbS_{nano2} (12.11 ± 2.47 ODU) was significantly higher than in the control group, in the animals exposed to $Pb(NO_3)_2$ and in the first series.

The cross-sectional area of the hepatocytes cytoplasm of rats exposed to both PbS_{nano1} ($156.17 \pm 7.17\ \mu m^2$) and PbS_{nano2} ($178.46 \pm 10.82\ \mu m^2$) was significantly smaller than that in the third group. Specific optical density of the cell cytoplasm of rats exposed to PbS_{nano1} (31.80 ± 1.95 ODU) was significantly higher than in animals exposed to PbS_{nano2} (26.02 ± 3.66 ODU), in rats of the first

TABLE 1 Morphometric and densitometric data of hepatocytes nuclei in the liver of experimental animals, $p < .05$

Groups	Cross-sectional area, μm^2 ($n = 15$)	Specific optical density, ODU ($n = 15$)	Content of NA in the reconstructed volume, ODU ($n = 15$)
12 weeks (first series)			
Control	56.45 ± 2.72	92.92 ± 1.94	7.57 ± 0.72
PbS_{nano1}	$80.66 \pm 1.79^{a,c}$	$92.08 \pm 2.04^{b,c}$	4.83 ± 1.07^{a}
PbS_{nano2}	$76.70 \pm 1.01^{a,c}$	$107.47 \pm 5.68^{a,b}$	4.55 ± 0.20^{a}
$Pb(NO_3)_2$	88.16 ± 1.96^{a}	110.44 ± 0.92^{a}	3.80 ± 0.66^{a}
18 weeks (second series)			
Control	55.97 ± 1.63	93.04 ± 1.82	7.39 ± 0.81
PbS_{nano1}	$61.71 \pm 1.37^{a,d}$	$102.68 \pm 1.78^{a,d}$	$11.12 \pm 0.41^{a,d}$
PbS_{nano2}	$66.32 \pm 2.34^{a,d}$	$96.18 \pm 3.07^{b,d}$	$12.11 \pm 2.47^{a,d}$
$Pb(NO_3)_2$	58.32 ± 1.29^{d}	$97.04 \pm 1.68^{a,d}$	8.04 ± 1.50^{d}

[a]Statistically significant differences compared with the control group ($p \leq .05$).
[b]Statistically significant differences compared with the group of animals exposed to nanoparticles of another size ($p \leq .05$).
[c]Statistically significant differences compared with the group of animals exposed to Lead Nitrate ($p \leq .05$).
[d]Statistically significant differences between groups of animals of another series ($p \leq .05$).

TABLE 2 Morphometric and densitometric data of hepatocytes cytoplasm in the liver of experimental animals, $p < .05$

Groups	Cross-sectional area μm^2 ($n = 15$)	Specific optical density, ODU ($n = 15$)	Content of NA in the reconstructed volume, ODU ($n = 15$)
12 weeks (first series)			
Control	199.20 ± 6.66	24.53 ± 3.66	23.82 ± 2.25
PbS_{nano1}	328.98 ± 2.97[a,d]	17.44 ± 2.41[a,d]	12.47 ± 3.85[a,d]
PbS_{nano2}	318.57 ± 8.10[a,d]	10.68 ± 6.05[a]	19.52 ± 1.22[a,d]
$Pb(NO_3)_2$	330.69 ± 3.07[a,d]	8.40 ± 1.42[a,d]	23.09 ± 0.64
18 weeks (second series)			
Control	200.01 ± 5.56	23.32 ± 3.54	23.25 ± 1.15
PbS_{nano1}	156.17 ± 7.17[a,d]	31.80 ± 1.95[a,d]	8.60 ± 1.00[a,d]
PbS_{nano2}	178.46 ± 10.82[a,d]	26.02 ± 3.66[b,c]	16.97 ± 4.08[a,d]
$Pb(NO_3)_2$	224.93 ± 10.71[a,d]	52.75 ± 2.27[a,d]	14,55 ± 2.38[a,d]

[a]Statistically significant differences compared with the control group ($p \leq .05$).
[b]Statistically significant differences compared with the group of animals exposed to nanoparticles of another size ($p \leq .05$).
[c]Statistically significant differences compared with the group of animals exposed to Lead Nitrate ($p \leq .05$).
[d]Statistically significant differences between groups of animals of another series ($p \leq .05$).

series, in control, but lower than in the animals exposed to Pb $(NO_3)_2$. Specific optical density of the cell cytoplasm of rats exposed to PbS_{nano2} was significantly higher than in animals of the first series, but lower than in the animals exposed to PbS_{nano1} and Pb $(NO_3)_2$ (Table 2).

NA content in the reconstructed volume of cell cytoplasm of the animals exposed to PbS_{nano1} (8.60 ± 1.00 ODU) was significantly lower than relative values of all other groups. Content of NA in the reconstructed volume of cell cytoplasm in the animals exposed to PbS_{nano2} (16.97 ± 4.08) was significantly lower than relative values in the animals of the first series, the control, but higher than that in the animals exposed to PbS_{nano1}.

4 | DISCUSSION

Lead is known to be a toxicant that rapidly accumulates in the liver, kidneys, spleen, brain, and bones (Novikova, 2013; Omel'chuk, Aleksiichuk, & Sokurenko, 2014). It is important that in the course of Pb intake it is deposited mainly in the liver, causing toxic effect, enzymopathies, oxidative stress (Assi, Hezmee, Haron, Sabr, & Rajion, 2016) and despite of activating mechanisms of detoxification, can cause toxic hepatitis (Lyubchenko, 1990).

During the last decade toxic effects of various nanoparticles were studied (Pani, Mishra, More, Singh, & Singh, 2015; Doudi & Setorki 2015; Yamagishi et al., 2013; Thapa et al., 2017). Although hepatotoxicity of lead is well established (Chou & Bushel, 2009; Flora, Gupta, & Tiwari, 2012; Patrick, 2006), data on the impact of lead nanoparticles on liver remain sparse (Amiri, Mohammadi, & Shabani, 2016). In our study, we have compared the effect of lead nanoparticles of different sizes and its ion form. Moreover, we have obtained results not only after 12-week exposure to toxicants, but also 6 weeks into the post-exposure period.

We have found the reduction of body weight of the animals exposed to PbS_{nano1} after at week 12 of experiment and of rats exposed to PbS_{nano1} and $Pb(NO_3)_2$ in the second series. These changes indicated the overall response to toxic effect and coincided with data of (Allouche, Hamadouche, Touabti, & Khennouf, 2011; Ibrahim, Eweis, El-Beltagi, & Abdel-Mobdy, 2012). Allouche and Ibrahim on lead acetate hepatotoxicity. Our results demonstrated the long-term effect of exposure to PbS_{nano1} and $Pb(NO_3)_2$, with these toxicants preventing body weight gain 6 weeks after the end of exposure and body weight decrease being evidently more severe in the animals exposed to PbS_{nano1}.

We observed that absolute liver weight increased at week 12 of experiment in all groups. In the second series this value was almost similar to that of the control level. Relative liver weight in the animals of all experimental groups was higher than that in the control after 12 weeks of experiment. In the second series this value remained higher in rats exposed to PbS_{nano1}. These changes may be associated with reduction in the body weight of the rats.

At week 12 of exposure dystrophic changes in the liver were found in all experimental groups. The amount of glycogen granules was lower than in the control. Hepatocytes and portal tracts were edematous, blood vessels dilated, and interlobular connective tissue infiltrated by lymphocytes and macrophages. These changes were indicative of liver damage and inflammation and were also observed in rats and mice exposed to lead acetate in studies of (Liu, Ma, Ma, & Sun, 2011; Sharma, Singh, Pandey, & Dhawan, 2012). Liver edema explained increase of absolute body weight at this term.

The sectional areas of hepatocytes nuclei and cytoplasm in animals of all experimental groups were significantly higher compared with the corresponding index of animals in the control group, indicating larger amount of heterochromatine in toxic effect. Optical density of the cell cytoplasm of all experimental groups was significantly lower compared with the corresponding index of animals of the control group. These changes were the evidence of edema which was pronounced mainly in the cytoplasm.

Since heterochromatin in contrast to euchromatin is not active, its increase demonstrates the reduction of the functional activity of the cells, and the increased volume of cytoplasm may be explained by the swelling of hepatocytes, which was observed at week 12 of the

experiment. While at week 18 hepatocyte volumes reduce due to the significant reduction of swelling which also manifests itself in the decreased weight of the organ and since the ODU of the cytoplasm is calculated by the cell area, it increases accordingly. Thus, the reduction of the nuclear ODU and the sectional area of hepatocytes in the presence of cytoplasm ODU increase is a compensatory intercellular mechanism ensuring the partial recovery of the function.

Content of NA in the reconstructed cell nucleus volume in animals of all experimental groups was significantly lower than in the control group. NA content in the reconstructed cell cytoplasm volume in animals exposed to PbS_{nano1} and PbS_{nano2} was significantly lower than in the animals exposed to $Pb(NO_3)_2$, this value in rats exposed to PbS_{nano1} being significantly lower than in the animals exposed to PbS_{nano2}. These changes demonstrated the impairment of the transcription and translation processes which led to changed metabolism discussed further. NA content in the cytoplasm at week 18 in all groups is higher than that in the control and the previous term, which means that transcription and translation are more efficient than the control, and also provide evidence of compensatory activation of the synthetic processes.

At week 18 (the second series) destructive changes in the liver decreased which was accompanied by partial normalization of morphometrical data.

Biochemical changes in blood serum corresponded to morphological data. At week 12 of experiment albumin concentration in animals exposed to nanoparticles was significantly lower than that in animals exposed to $Pb(NO_{3)2}$. However, albumin content (%) in animals after exposure to PbS_{nano2} group was significantly higher than that in the first and the third group. Thus, the reduction in total protein concentration in the second group was not at the expence of albumin.

The concentration of glucose in rats of all experimental groups was significantly increased compared with the corresponding value of the control group. It corresponded with morphological data on the decrease of glycogen granules in cytoplasm of hepatocytes and indicated glucose mobilization in experimental setting.

The concentration of total lipids and cholesterol in serum of experimental animals tended to decrease and triglycerides—to grow compared with control. These changes coincided with morphological data on vacuolization of hepatocytes' cytoplasm and indicated lipid metabolism impairement that were associated with cytotoxic effect of metal compounds on hepatocytes. The same conclusion was made by (Haouas et al., 2014) who studied lead acetate poisoning in rats.

At week 18 concentration of total lipids and glucose in the experimental animals of all experimental groups relative to the previous study period increased. All biochemical parameters in experimental groups tended to normalize, while remaining different from control.

We believe that increasing of lipids and glucose in the experimental animals of all experimental groups at week 12 shows degranulation of hepatocytes and at week 18 in our opinion in combination with predominance of euchromatin gives some evidence of the required energy provision of the synthetic processes. Increasing of lipids in the experimental animals is likely to be conditioned by the needed recovery of biological membranes of hepatocytes and their membrane organelles.

According to the literature (Lazarenko, 2012) toxic effects of lead on cellular metabolism in the body are caused by the enzymotoxic action and membrane affinity, resulting in blocking of protein groups (sulfhydryl, carboxyl, and amine). Once lead intake by the body is discontinued, its redistribution starts. In hepatocytes Pb is partially metabolized under the influence of intracellular enzymes to form complex compounds with bile acids. They can be absorbed in the gastrointestinal tract into the bloodstream, ensuring the process of hepatointestinal recirculation. Pb accumulation exhibited the following pattern: bone > liver > kidney > gut > blood cells > muscle > brain > ovary (Nascimento, Risso, & Martinez, 2016). Further on Pb is almost completely (80%–90%) accumulated in the bones (Barry, 1975) in the form of insoluble phosphate tribasic lead which for a long time creates a depot of the metal in the body and can maintain the clinical picture of chronic toxic effect.

Comparing the action of nanoparticles of different sizes and ion form of lead, we can assume that by many parameters PbS_{nano1} had more pronounced harmful effect. Toxicity of PbS_{nano2} and $Pb(NO_3)_2$ were comparable. We believe that it is the particles size which may determine this higher toxicity; smaller nanoparticles cross the cell membrane quicker.

5 | CONCLUSION

The experiment has demonstrated that the 12-week long exposure to lead nanoparticles had harmful effect and led to significant changes in body and liver weight, total protein, albumin, glucose, total lipids, cholesterol, triglycerides content in blood serum and dystrophic changes in the liver. Within the postexposure 6-week period structural changes in the liver and biochemical changes in blood serum decreased. Biochemical changes in blood serum corresponded with morphological data. In many parameters PbS_{nano1} had more pronounced harmful effect. Toxicity of PbS_{nano2} and $Pb(NO_3)_2$ were comparable.

The research done allows to conclude that recommendations on how to prevent the potential negative impact of lead nanoparticles on human health need to be developed and implemented.

AUTHOR CONTRIBUTION

S. O. and L. S. – research concept and design; V. A. – collection and/or assembly of data; V. A. and L. S. – data analysis and interpretation; V. A. – writing the article; L. S., R. K., O. K., and Y. C. – critical revision of the article; S. O. and Y. C. – final approval of article.

ORCID

Liudmyla Sokurenko http://orcid.org/0000-0002-6870-2290

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How to cite this article: Aleksiichuk V, Omelchuk S, Sokurenko L, Kaminsky R, Kovalchuk O, Chaikovsky Y. The influence of lead nanoparticles on the morpho-functional changes of rat liver during the postexposure period. *Microsc Res Tech*. 2018;00:1–8. https://doi.org/10.1002/jemt.23036