

Large scale toxicological evaluation of lead acetate in broiler chicken

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Abstract

Among heavy metals, lead is one of the very toxic pollutants of the environment. Its accumulating nature in the body makes it a great threat to public health particularly when humans consume lead intoxicated foods like chickens. The main purpose of the conducted research was to elucidate the bioaccumulation of lead in different organs of chickens and its toxicological effects on various organs and biochemical parameters. An experimental study on the effect of lead acetate toxicity in chicks was conducted by orally administration for consecutive thirty days. Thirty-chicks were categorized into A, B, C, D, E, and F groups with lead acetate dose rate 0, 71, 142, 213, and 284 mg/kg body weight, correspondingly. During the experiment, various biochemical parameters (uric acid, GPT, creatinine, ALP, LDH, ASAT, ALT, glutathione, superoxide dismutase) were measured employing commercially available kits. At the end of experimentation, and lead accumulation in liver, kidney and brain was estimated by absorption spectrophotometer. Some biochemical parameters like uric acid, GPT, creatinine, ALP, LDH, AST, and ALT were increased while the level of glutathione and superoxide dismutase, was found to be decreased after exposure to lead acetate. In present study, the pattern of metal accumulation in different organs directly related with concentration of metal. The order of metal accumulation in organs is; liver > kidney > brain. In the present study, supplementation of lead acetate has affected the exposed chicken. Mostly the blood profile and chemistry are perturbed. These effects might be due to the accumulation of lead in the brain, kidneys, and liver which may result in neurotoxicity, nephrotoxicity, and hepatotoxicity. To refine such outcomes, further studies in the future are recommended.

Introduction

Heavy metals resulting from anthropogenic activities are a source of pollution and accumulation in food chains because they are not metabolized by the organisms (El-Beltagi et al. 2017). Among heavy metals, lead (Pb) is one of the most noxious heavy metals that are capable to cause poisonousness at very low concentrations and damage many organs (Amadi et al. 2019). It has long been a widespread concern for public health (Kamińska et al. 2020). The non-biodegradable nature of this heavy metal in different metrics makes it affects both the environment as well as living bodies. In poultry and other domestic animals, lead acetate has been differentiated as an accidental poisoning source (Batra and Yadav, 2019).

Toxic substances ammonia gas can damage chicken liver (Li et al., 2021) and kidney (Han et al., 2020a). Common health consequences of Pb include abdominal pain, weight loss, carcinoma of various tissues, changing blood parameters, kidney disorders, anemia, disorders related to the brain and liver (Rashid et al. 2019; Kshirsagar et al. 2019). It induces the overproduction of free radicals that might be enhanced the rate of lipid peroxidation and oxidative stress (Morkunas et al. 2019). The toxic effects include morphological and biochemical changes in different tissues such as hepatic, and renal systems (Younus, 2017). Serum enzymes are an important indicator of chemical-induced toxicities in experimental animals. Some studies showed that lead acetate led to the elevation of serum enzymes such as GPT, and AST as well as reduced the antioxidant enzymes *e.g.*, catalase, glutathione peroxidase, and superoxide dismutase in mice (Khanam et al. 2016). Hematological parameters (Hb, MCHC and PCV) are mainly

significant for the analysis of anemia in most animals (Hasan et al. 2016). The observed decrease in total erythrocyte count depends on the duration and dose of lead acetate consumption. Lead acetate increases the number of leukocytes and increases leukocyte differential count which may be linked to the increased inflammatory process (Offor et al. 2017).

The poultry in Pakistan is the greatest source of protein and nutrition. Exposure to lead in poultry can take place through different routes such as water, air, and feed (Naicker et al. 2018). Organ weight and liver damage had been linked with Pb exposures in animals (Offor et al. 2017; Kim et al. 2020). The aim of the present study was to evaluate the bioaccumulation of lead in different organs of chickens and impact of lead exposure on biochemical profile of chicken.

Materials And Methods

Ethical statement

All animal trials were executed according to local and worldwide procedures. The nearby way is the Wet op de dierproeven (article 9) of Dutch law (international) and an associated rule planned via the Bureau of Animal Research Licensing, Local University as detailed in our earlier papers (Saba et al. 2021; Ali et al. 2020; Hussain et al. 2020; Ali et al. 2020; Ara et al. 2021; Ali et al. 2020; Khan et al. 2019; Ali et al. 2019; Mumtaz et al. 2019; Mughal et al. 2019; Dar et al. 2019). The rearing and use of animal were carried out using NIH Publication "Guide for the Care and Use of Laboratory Animals" (NRC 2004) and by the local bioethical committee of the University on animal experimentation.

Chemicals

Lead (II) acetate trihydrate $[(\text{CH}_3\text{COO})_2 \text{Pb} \cdot 3\text{H}_2\text{O}]$ (Merck, Germany) purity 99.5%, with CAS number 6080-56-4 was used to prepare different concentrations .

Experimental design and management of chicks

Two weeks old thirty chicks (*Gallus domesticus*) Ross 308 was purchased from the local market (Hussain et al. 2020). The birds were kept in floor pens and fed on a commercially available corn-based starter diet and drinking water at their satiation. After acclimatization of three weeks, these thirty healthy chicks were randomly divided into five groups and measured initial weight of the chicks in each group.. Group A, B, C, D, and E of experimental animals were treated with the concentrations of lead acetate 0, 71, 142, 213, and 284 mg/kg of body weight, respectively using gavage/ dropper for one month. The weight of chicks was measured every fifth day using a digital weighing balance. Each dose was prepared in distilled water just for five days after that dose was changed according to the new bodyweight of the chick.

Blood collection

The blood samples (3 ml) of each specimen were taken from the vein of the wing aseptically on days 0, 5, 15, 25, and 30 of the research. The collected blood samples were shifted to vacutainer 3 ml ImuMed[®] coated with EDTA.K3 and preserved at 4 °C till advance investigation.

Biochemical analyses (Blood biochemistry)

Serum was isolated from plasma via centrifuged at 2000 rpm for 10 minutes to analyze the biochemical parameters. For biochemical analyses, serum samples were kept at -20°C (Hussain et al. 2020; Naito, 1984). Various biochemical parameters were analyzed by commercially available respective kits or already designated methods (Suleman et al. 2016; Mumtaz et al. 2019; Ali et al. 2019; Khan et al. 2019; Hussain et al. 2020)

Dissection

After one month of treatment, chicks were retained for the other 5 days without the administration of lead acetate. All chicks were dissected on the 35th day after blood sampling and different organs (brain, liver and kidneys) were preserved for estimation of metal accumulation.

At the end of experiment, different organs (liver, Kidney and brain) were collected from each chick and oven dried till consistent weight. Each dried organ of known weight was shifted in flask, added 1 ml perchloric acid (65 %) and 5 ml of nitric acid (55%) and kept for 24 hrs in fume hood. Next day, 5 ml nitric acid (55%) and 4 ml perchloric acid (65%) was added in each flask. The flasks were placed on hot plate (250 °C) to digest the organs. Each sample mixture was evaporated until a clear solution was obtained. The solution was then evaporated up to 0.5 ml. The solution was diluted up to 10 ml with distilled water by properly rinsing the digestion flasks and filtered. Afterward, atomic absorption spectrophotometer was used to determine the concentrations of lead (Du Preez and Steyn, 1992; Shakir et al. 2005; Ali et al. 2019).

Statistical analysis

Statistics were presented as mean \pm standard error of mean then analyzed statistically via one-way ANOVA with "Dunnett's Multiple Comparison Test", to find any considerable variances amongst the means of a group. GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for the study. Values of $P < 0.05$ were deliberated substantially.

Results

Glutamate pyruvate transaminase (GPT)

Orally administration of lead acetate in chicks caused a substantial elevation in GPT level at the highest concentrations. On the 30th day, the values of GPT level in group B at the dose rate of 71 mg/kg was 2.0 ± 0.1 IU/L with respect to control group (A) 1.6 ± 0.1 IU/L. For Group C, on 30th day, the level of GPT was 2.2 ± 0.1 IU/L at the dose rate of 142 mg/kg while, 1.4 ± 0.1 IU/L on 5th day of the research. The higher substantial rise in the level of GPT (2.4 ± 0.1 IU/L) was detected in group D at a higher concentration of dose 213 mg/kg on the 30th day. The highest substantial increase in GPT level from 1.8 ± 0.1 IU/L to 3.4 ± 0.1 IU/L was found at 284 mg/kg rate of dosage on the 30th day (Figure1).

Creatinine

The level of creatinine in group B was 15.9 ± 0.6 g/l on 30th day at 71 mg/kg of dose when compared to the control group (A) 14.7 ± 0.4 g/l). In group C at 142 mg/kg rate of dose creatinine level on the 5th day was 16.5 ± 0.7 g/l, whereas, on 30th day, creatinine level was 13.6 ± 0.2 g/l. The highest creatinine level was found in group D at higher concentration of dose 213 mg/kg rate of dose on the 30th day as 18.4 ± 0.7 g/l and the lowest was as 13.6 ± 0.3 mg/dl on the 5th day. On the 30th day group E showed the highest significant increase 26.9 ± 0.5 g/l in creatinine at highest concentration of dose 213 mg/kg when compared to the 5th day reading 14.5 ± 0.4 g/l (Figure2).

Uric Acid

Administration of lead acetate to chicks caused a substantial elevation in uric acid level. In group B at 71 mg/kg rate of dose the level of uric acid on day 30th was 2.4 ± 0.2 μ g/dl to control group (A) 1.7 ± 0.1 μ g/dl. Likewise, the lowest value of uric acid for group C at 142 mg/kg rate of the dose was noted as 1.7 ± 0.1 μ g/dl on day 5th and the highest value was 2.4 ± 0.2 μ g/dl on day 30th of the analysis. On the 30th the level of uric acid in group D at 213 mg/kg rate of a dose was 2.5 ± 0.1 μ g/dl when compared to untreated group (A) 1.7 ± 0.1 μ g/dl. . At the highest concentration of dose 284 mg/kg group E, showed the elevation in the level of uric acid from 1.8 ± 0.1 μ g/dl to 3.6 ± 0.2 μ g/dl on 5th to 30th day consecutively (Figure 3).

Alkaline phosphatase (ALP)

The values of alkaline phosphatase for the control group (A) were calculated as 359.5 ± 15.0 IU/L to 390.9 ± 15.2 IU/L from 5th to 30th day respectively. In-group B, at 71 mg/kg rate of dose the level of ALP increased from 379.7 ± 14.6 IU/L to 411.9 ± 12.1 IU/L. The level of ALP elevated at 142 mg/kg rate of dose from 393.7 ± 25.3 IU/L to 411.9 ± 12.1 IU/L in group C. Group D showed an increase in ALP value at a higher concentration of dose 213 mg/kg rate of dose on day 5th 390.2 ± 21.6 IU/L to 444.1 ± 6.5 IU/L

30th day. The highest significant elevation 459.9 ± 9.1 IU/L in the level of ALP was found on 30th day as compared to um-treated group (A) 401.3 ± 16.1 IU/L (Figure 4).

Lactate dehydrogenase (LDH)

Orally administration of lead acetate caused a significant elevation in the level of LDH at highest concentration of doses (213 mg/kg and 242 mg/kg). In group B at 71 mg/kg an increase in LDH value was noticed 355.3 ± 9.8 IU/L to 396.5 ± 4.3 IU/L from 5th to 30th day. In group C at 142 mg/kg rate of dose an increase in LDH was noticed as 364.2 ± 13.9 IU/L to 394.2 ± 7.6 IU/L from 5th to 30th day. In group D at a higher concentration of dose 213 mg/kg rate of dose the recorded values were 347.5 ± 12.2 IU/L to 404.1 ± 6.1 IU/L from 5th to 30th day. At highest concentration of dose 284 mg/kg an increase in LDH was noticed as 418.7 ± 15.8 IU/L when compared to control group (A) 366.1 ± 9.9 IU/L (Figure 5).

Superoxide dismutase (SOD)

A significant fall in the level of SOD was found after exposure to lead acetate in chicks. An increase in SOD values was recorded from 173.5 ± 3.8 U/L to 177.1 ± 5.2 U/L for the control group (A). For group B, at the 71 mg/kg rate of dose a decrease in SOD level was recorded as 174.7 ± 6.2 U/L to 164.1 ± 6.3 U/L. For group C at 142 mg/kg rate of dose the level of superoxide dismutase decreased from 5th to 30th day as 174.2 ± 4.5 to 178.1 ± 5.4 U/L. In group D at 213 mg/kg of dose higher significant decline in the level of superoxide dismutase 156.5 ± 3.5 U/L was found on 30th day as compared to 5th day 159.9 ± 5.1 U/L. In group E at 284 mg/kg rate of dose the highest substantial decline in the level of SOD were 158.5 ± 6.0 U/L and 150.7 ± 5.5 U/L from 5th to 30th day. (Figure 6).

Glutathione

The values of glutathione were calculated as 2.5 ± 0.1 μ mol/ml to 2.4 ± 0.0 μ mol/ml on 5th and 30th day for the control group (A). For Group B, at 71 mg/kg the GSH level decreased from 2.4 ± 0.1 μ mol/ml on day 5th to 2.2 ± 0.1 μ mol/ml on the 30th day. The GSH value in group C at 142 mg/kg was reduced from 2.3 ± 0.1 μ mol/ml to 2.1 ± 0.0 μ mol/ml on day 30th. The glutathione level decreased in group D at a higher concentration 213 mg/kg rate of dose from 2.3 ± 0.1 on 5th day to 2.0 ± 0.1 μ mol/ml on day 30th. The highest significant decrease in glutathione level was calculated in group E (284 mg/kg) as 2.3 ± 0.1 μ mol/ml on 5th day to 1.8 ± 0.1 μ mol/ml on the 30th day (Figure 7).

Aspartate amino transferase (ASAT)

The value of aspartate aminotransferase was increasing in the control group (A) from 143.8 ± 5.8 IU/L to 167.7 ± 4.7 IU/L. The level of ASAT in group B at 71 mg/kg rate of dose increased from 173.7 ± 7.7 IU/L

to 180.4 ± 6.0 IU/L on day 30th. The value of ASAT in group C at 142 mg/kg rate of was recorded as 174.7 ± 12.6 IU/ on the 5th day and 187.1 ± 4.2 IU /L on the 30th day. The ASAT level in group D at 213 mg/kg rate of dose increased as 165.9 ± 11.5 IU/L on day 5th to 201.2 ± 4.6 IU/L on day 30th. The highest substantial elevation in the level of ASAT at highest concentration of dose 284 mg/kg in group E was 177.1 ± 7.7 IU/L on the 5th day and 216.1 ± 17.9 IU/L on the 30th day (Figure 8).

Lead acetate concentration in tissues

Atomic absorption spectrophotometer was used to measure the accumulation of Pb in brain, kidneys as well as in the liver. The lowest concentration of Pb in the liver of group B at 71 mg/kg rate of the dose was 41.4 ± 0.6 mg/kg, whereas the highest concentration of Pb in group E at the highest concentration of dose 284 mg/kg rate of dose was 178.4 ± 3.0 mg/kg. The least accumulation of Pb in kidneys of group B was 30.3 ± 0.6 mg/kg while at the highest concentration of dose 284 mg/kg group E revealed the utmost bioaccumulation of lead as 107.5 ± 1.4 mg/kg (Figure 9).

Discussion

The current study was undertaken to evaluate the toxicological effects of lead acetate on biochemical parameters as well as its bioaccumulation in the liver, kidney and brain of broiler birds. The influence of lead acetate disclosure was equally time and concentration dependent (Hussain et al. 2020). Lead is one of the noxious heavy metals that cause toxicity in human beings and birds (Jiao et al. 2017). In the conducted research, a considerable increase in the level of serum ALP, AST, GPT, and LDH was found when broiler chicks were exposed to lead acetate while a significant reduction in GSH, and SOD was recorded at a dosage rate of 213 and 284 mg/kg B.W., respectively.

Nadia (2013) stated that when mice were exposed to 15 mg/kg B.W. of lead acetate for a consecutive one week, the level of ALP was considerably increased in serum regarding to the untreated group. An elevated level of ALP in patients who have a chronic kidney disorder affects the inflammatory responses (Bera et al. 2020). In the current research, the level of ALT, ALP, and AST increased in plasma of lead-exposed broiler chicks in comparison with control group. Our outcomes are in line with Omobowale et al. (2014) and Bera et al. (2020), who reported that exposure to lead acetate causes alterations in the cholesterol level of the liver as well as raises the various enzymes of the liver *e.g* ALT, ALP, and AST in plasma. (Omobowale et al. 2014). Elevation in the level of liver biomarkers might be occur due to disruption of metabolite transport, infiltration of inflammatory cells, necrosis, and destruction of the morphologic structures of hepatocytes via exposure of lead (Ashrafizadeh et al. 2018; Rafiei et al. 2018). It has been stated that when food polluted with lead was given to the chicks raise number of apoptotic cells in the liver (Najafi et al. 2019). Hasanein et al. (2017) reported that the reservoir of RBCs, dilation of sinusoids in the hepatocytes, vacuolization and rupture of mitochondria, disruptions in the scaffolding of the liver lobules are consequences of lead poisoning. It has been stated that lead disrupts the morphology of liver, due to cellular disruptions, apoptosis, and penetration of provocative cells in the

hepatic system (Sawada et al. 2020). Abdoua and Hassan (2014) described that when mice exposed to 15 mg /kg B.W of lead-acetate intraperitoneally for 1 week caused a decline in activity of catalase, glutathione, and superoxide dismutase. Similar results were found in the current research. When mice were exposed with Pb (1.0, 0.5, and 25 mg/mL) for 7 days, a significant elevation in the level of ALT, AST, and ALP was recorded while, decline in glutathione, catalase and superoxide dismutase was found (Abdou and Hassan, 2014). For example, excess toxic gas ammonia caused the thymuses, bursa of fabricius, and damage of kidney in broilers. Furthermore, it caused oxidative stress as well as decreased SOD in thymuses and kidneys, decreased glutathione in thymuses and kidneys of broilers ((Han et al. 2020b; Shah et al. 2020a, b). In the current research, various doses of lead acetate were given to the birds that raise the level of glutamate pyruvate transaminase. Al-Wabel *et al.* (2007) described that when lead acetate was administered to rats, the level of GPT was increased from 24.0 to 38.3 IU/L. Ashmawy *et al.* (2005) stated the similar outcomes which revealed that supplementation of lead acetate enhanced the level of glutamate pyruvate transaminase and have lethal influences on the hepatic system.

Abdoua and Hassan (2014) reported that intraperitoneal disclosure of lead acetate 15 mg /kg B.W to mice for one week, caused a major drop in the level of GPx, CAT, and SOD in plasma. In present study, when broiler chicks were treated with lead acetate for 30 days, a substantial drop ($p \leq 0.001$) in the level of SOD and GSH was perceived at a prescription rate of 284 mg/kg. The substantial reduction in the level of GPx and SOD was found in the serum of mice after treatment with lead acetate for 1 week at a dose rate of 0.25, 0.5, and 1.0 mg/mL (Abdou and Hassan, 2014). Bahnasy et al. (2020), reported that exposure of lead causes the oxidative stress which results in the reduction of antioxidant defense enzymes interfering with the disruption of cell membrane integrity, lipid peroxidation and extensive impairment in fleshy tissue. Shah et al. (2020), stated that exposure of ammonia can affect various organs of birds and humans including the bursa of fabricius, heart, liver kidney, brain and spleen (Xing et al., 2019). In past studies, it was also described that more concentration of ammonia cause oxidative stress in chicken thymus tissues via significantly alteration in the oxidative stress markers *e.g.*, CAT, SOD and GSH-Px (Chen et al. 2020).

Han et al. (2020b) reported that exposure of ammonium to chickens at higher concentrations caused a significant ($P < 0.05$) down-regulation in the level of antioxidant enzyme activities such as CAT, GSH-Px, and SOD. The supplementation lead acetate indicated a considerable ($p < 0.05$) rise in creatinine and urea with regarding the untreated group (Salim et al. 2015). Elevation in the creatinine level in conducted research is in favor of the results stated by Obi-Ezeani et al. (2020). Ahmadi and Ashrafizadeh (2019), showed that elevation in the creatinine level due to lead poisoning might be occur due to increased intracytoplasmic calcification, production of intracellular inclusions ,and focal atrophy in proximal tubules. It has also been reported that lead increased the cellular apoptosis, focal fibrosis and infiltration of lymphocytes in the lead exposed rats (Babiker et al. 2018)._ It has also been reported that exposure of NH₃ to chickens for 42 days caused a substantial rise in the level of uric acid and creatinine in serum which revealed that the high concentration of ammonia caused a decline in renal function (Sahebi-Ala et al. 2021). The microstructural alterations in renal tissues of chickens that after exposure of ammonia

were observed e.g., edema, hyperemia, hypertrophy and mononuclear cell infiltration (Craig et al., 2014; Zhang et al., 2020).

In the present study, higher accumulation of Pb in kidneys, brain and liver of birds exposed with a dosage of 284 mg/kg with respect to former groups. In present study, the pattern of metal accumulation directly related with concentration of metal. The order of metal accumulation in organs is liver>kidney>brain. The results of Suleman et al. (2016) agreed with our results, they stated that the accumulation of lead increased in the hepatic as well as a renal system when the concentration of dose increased in broiler chickens which result in nephrotoxicity and hepatotoxicity. It has been found that more concentration of Pb accumulates in the kidney of many bird species than other tissues by increasing the concentration of dose (Pineau et al. 2017). Hamidipoor et al. (2018) also found that lead is capable to accumulate in the brain of broilers. Mehrotra et al. (2008) has also reported that when quail were exposed to lead acetate highest concentration in the liver was observed than gonads after 21 days of treatment According to Winiarska-Mieczan and Kwiecień (2016) when Wistar rats were treated with 50 mg/ kg of feed or water containing lead acetate highest concentration of lead accumulated in brain than spleen, and heart. Our outcomes were also consistent with Borowska et al. 2018; Kulas et al. (2019), who found that when adult mice were treated with lead and cadmium the highest concentration of lead approximately (0.71 %) accumulated in the brain and liver when compared to the accumulation of cadmium (0.5 %) in the spleen.

Conclusion

It was concluded that exposure to lead acetate exerted a hepatotoxicity, neuro, and nephrotoxicity in chickens especially at the highest concentration (213 and 284 mg/kg) of dose. Accumulation of Pb was also found in liver, kidney, and brain at highest concentrations. Hence, it is found that lead acetate is a toxic bio-accumulator and affects the biochemical profile of the chicks.

Declarations

Consent to Publish

“Not applicable”

Consent to Participate

“Not applicable”

Authors Contributions

Conceptualization: Maria Akram Minhas, Shaukat Ali: **Data Curation:** Shaukat Ali, Maria Akram Minhas, Uzma Azeem Awan, Shumaila Mumtaz, Hafiz Abdullah Shakir, Hafiz Muhammad Tahir, Mazhar Ulhaq and Saiqa Andleeb: **Formal Analysis:** Shaukat Ali, Hafiz Abdullah Shakir: **Investigation:** Shaukat Ali, Hafiz

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Availability of data and materials: Most of the data generated during this study are included in this article. However, raw datasheets are available from the corresponding upon reasonable request.

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Figures

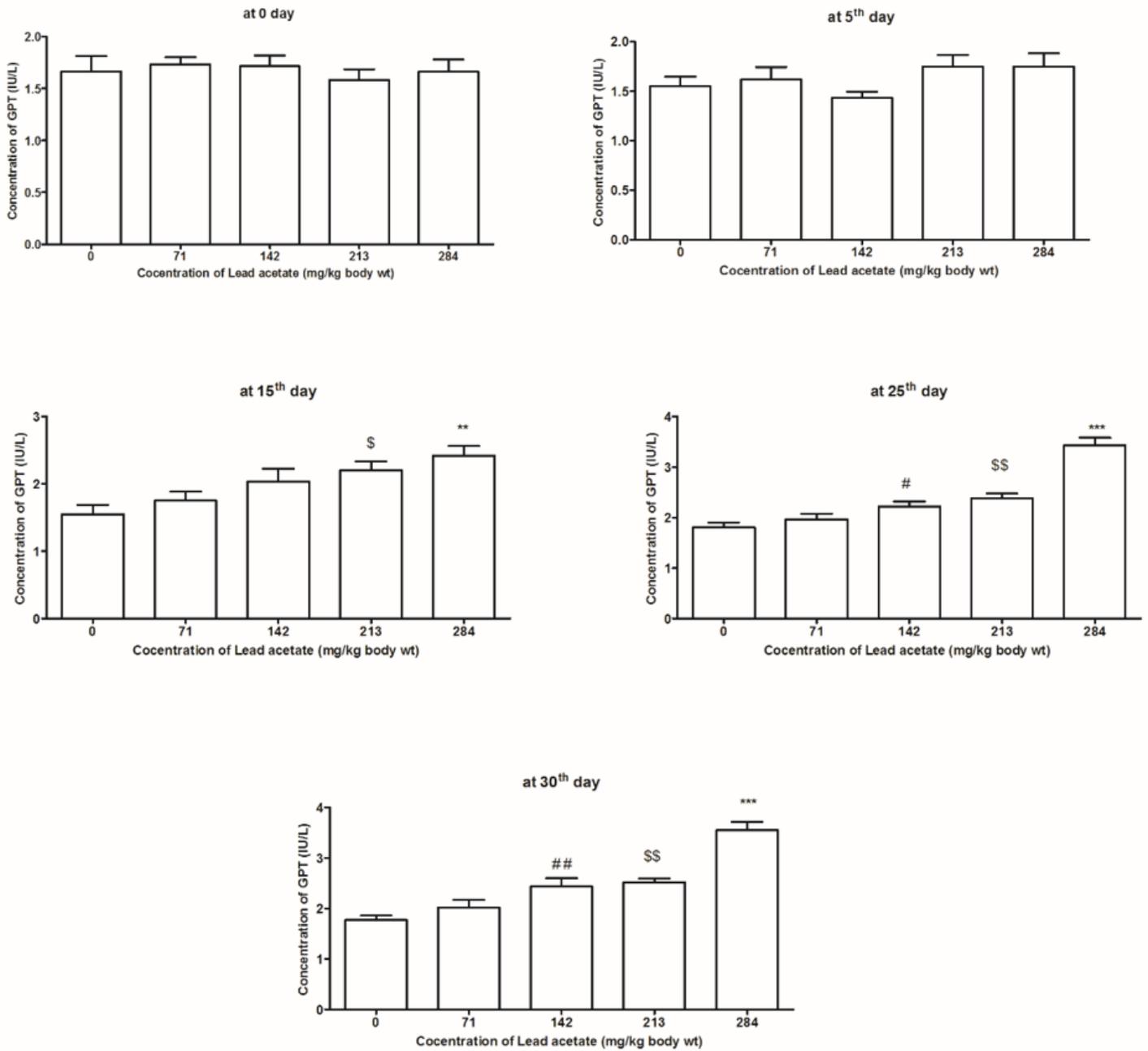


Figure 1

Measurement of Glutamate Pyruvate Transaminase (GPT) in treatment groups. Abbreviations and keys: GPT stands for Glutamate Pyruvate Transaminase. '#' designates the substantial variance among Group C and control, '\$' specifies the substantial variance among control and Group D and '*' displays the substantial variance among Group E and control. Every bar signifies the mean value of six duplicates and SEM. Arithmetic representations: #, \$= $p \leq 0.05$, ##, \$\$, **= $p \leq 0.01$, *** = $p \leq 0.001$.

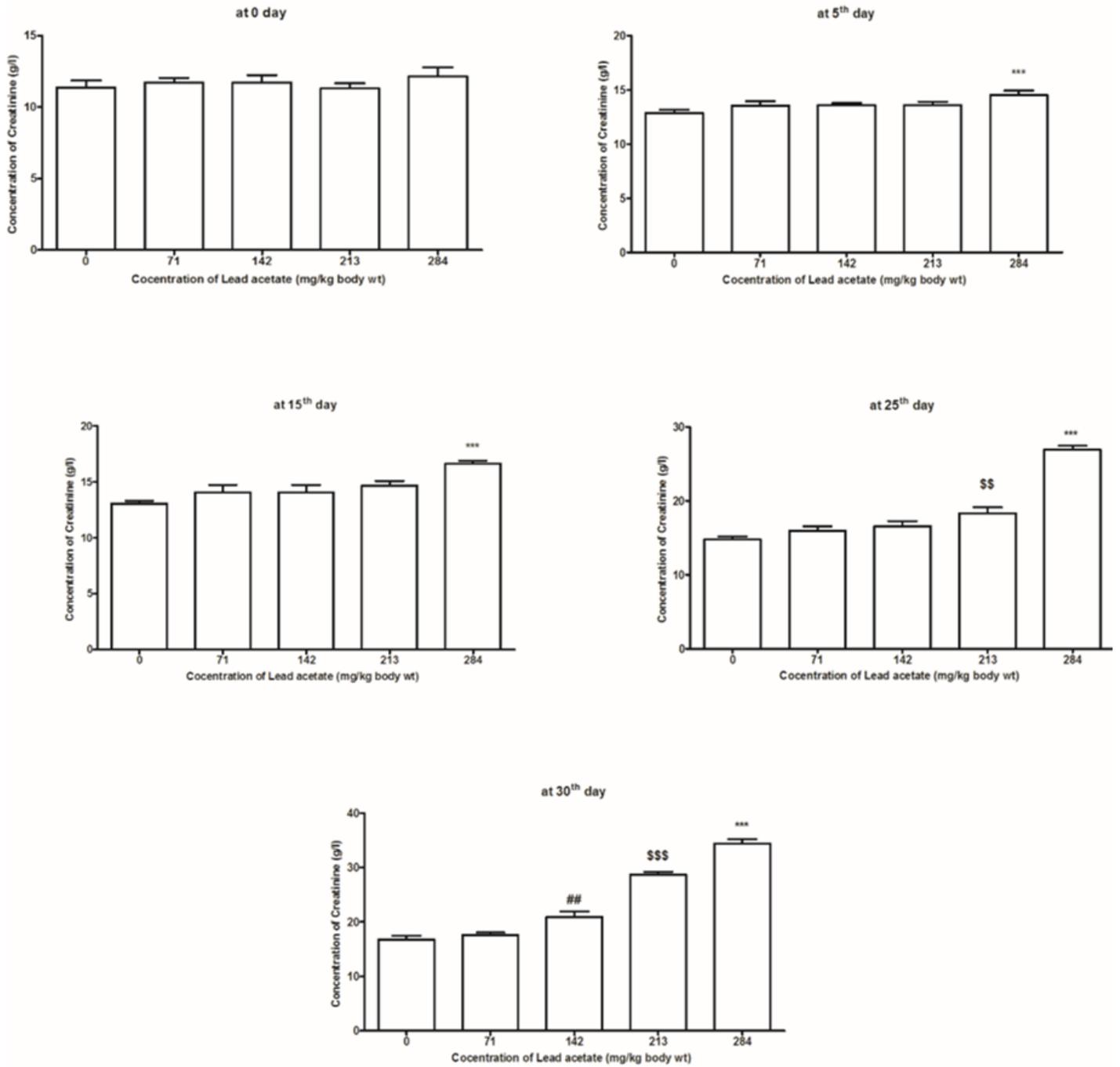


Figure 2

Measurement of creatinine level (g/l) in treatment groups: For abbreviations and keys see figure 1.

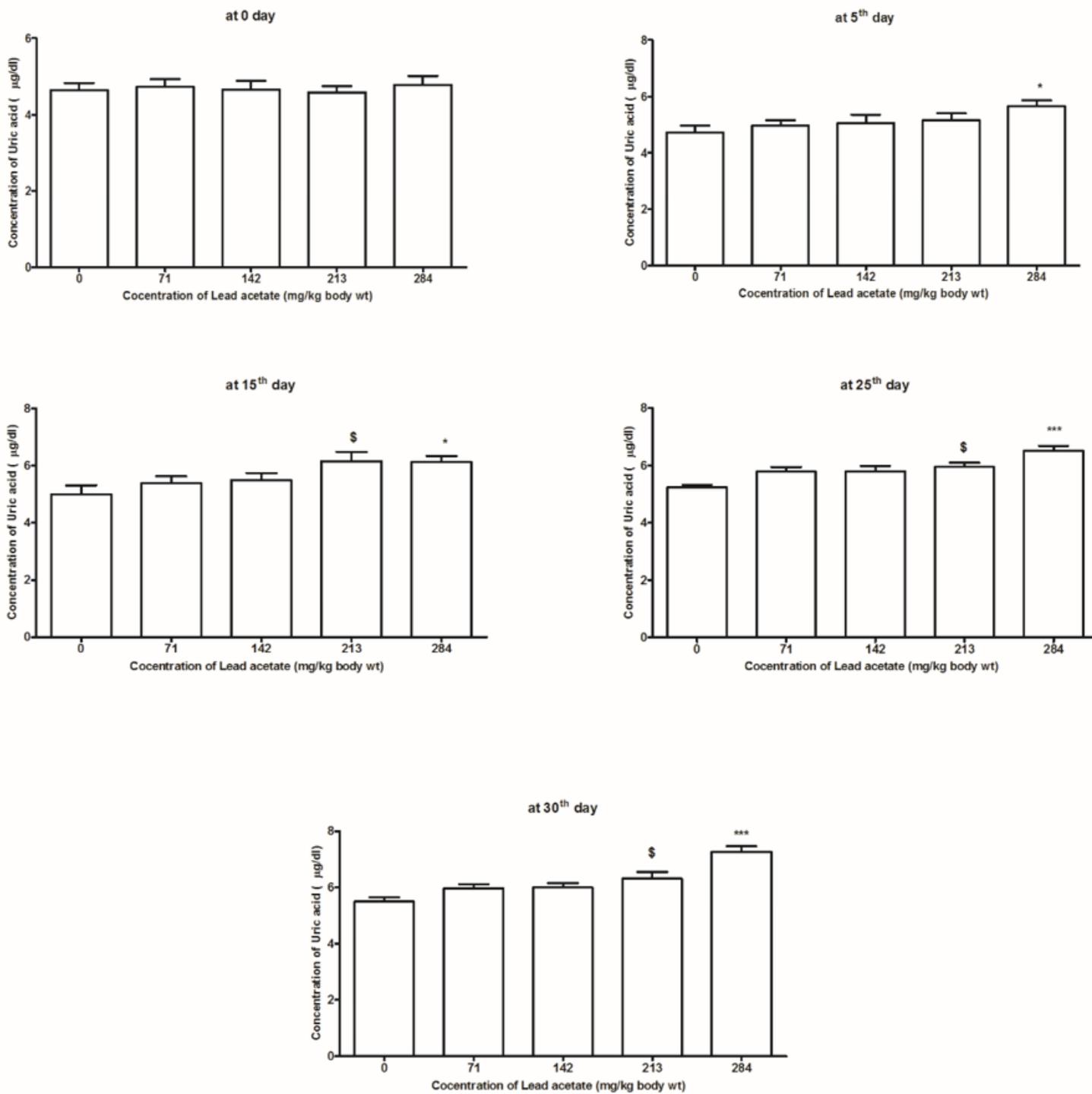


Figure 3

Measurement of the uric acid level in treatment groups. For abbreviations and keys see figure 1.

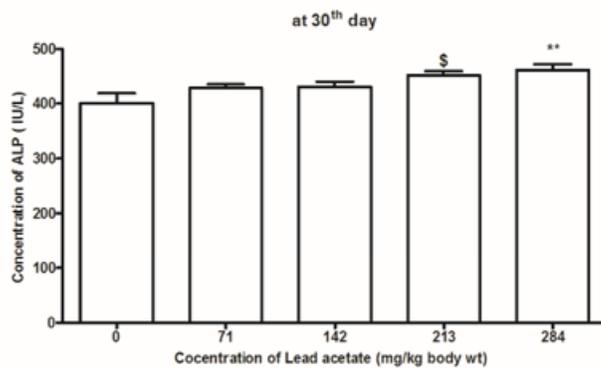
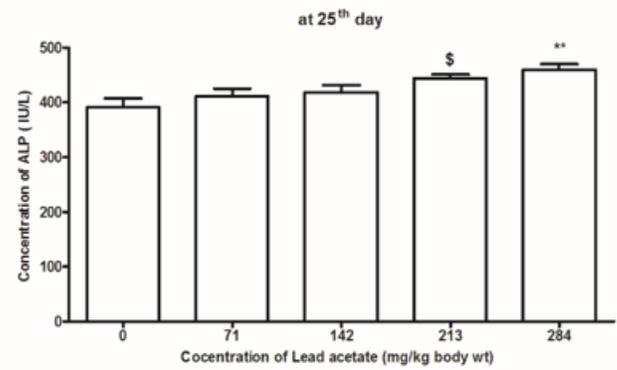
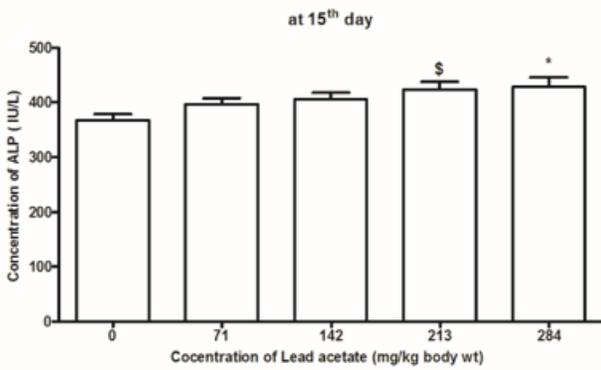
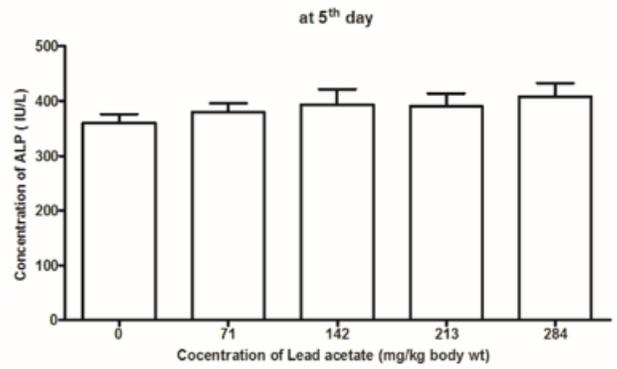
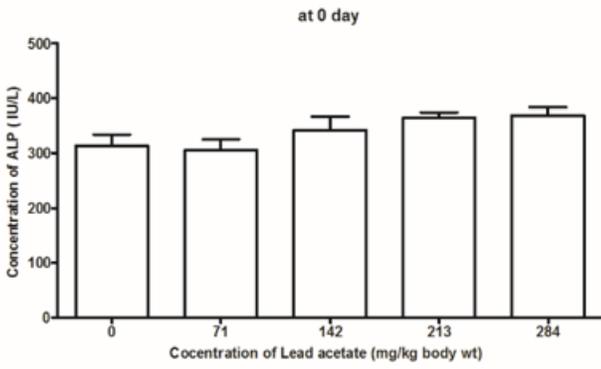


Figure 4

Measurement of ALP level in treatment groups: For abbreviations and keys see figure 1

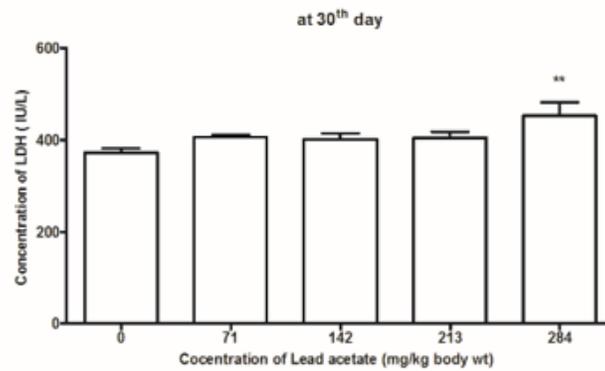
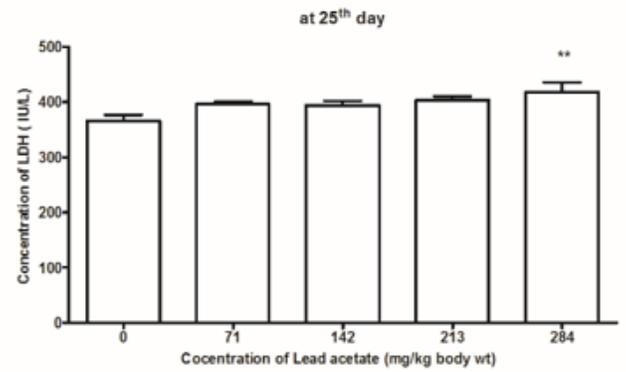
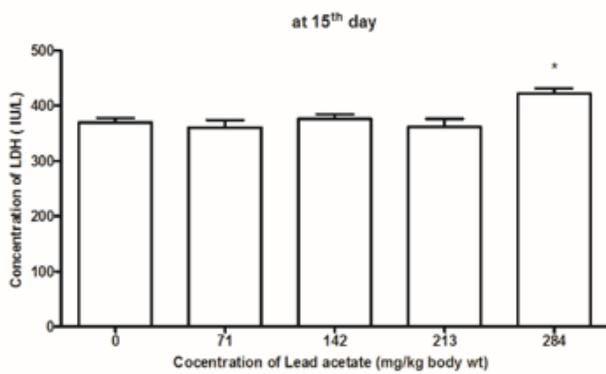
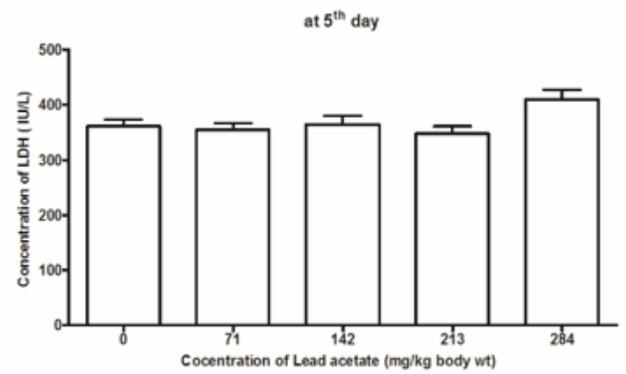


Figure 5

Measurement of Lactate Dehydrogenase (LDH) level in treatment groups: For abbreviations and keys see figure 1.

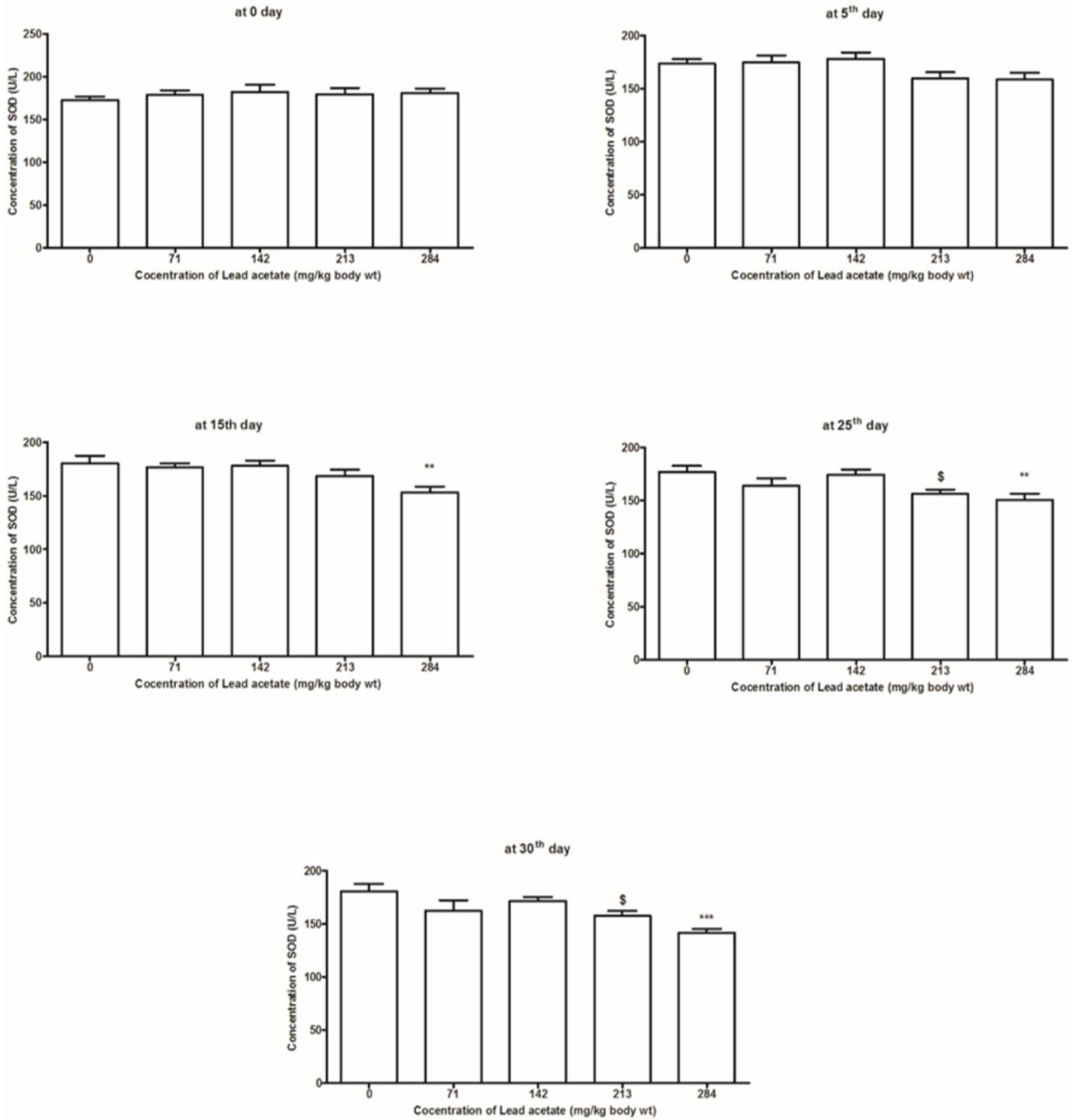


Figure 6

Measurement of SOD level treatment groups: For abbreviations and keys see figure 1.

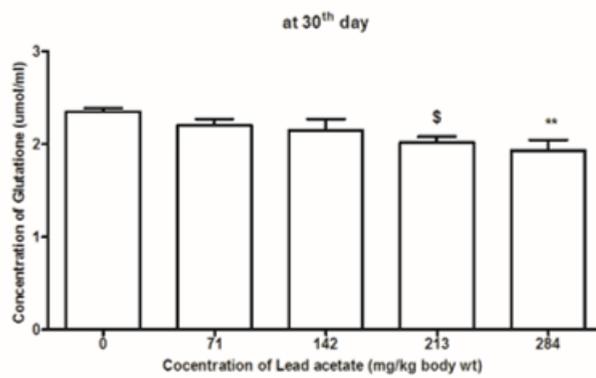
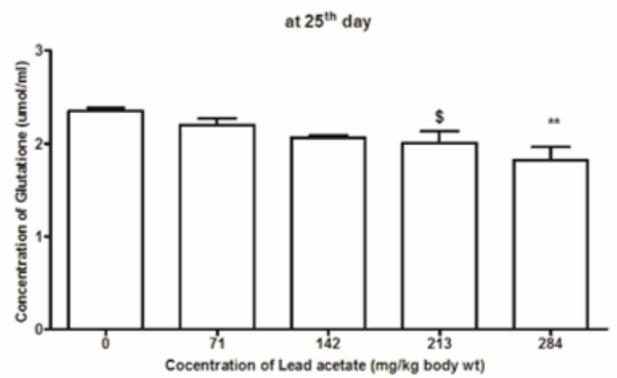
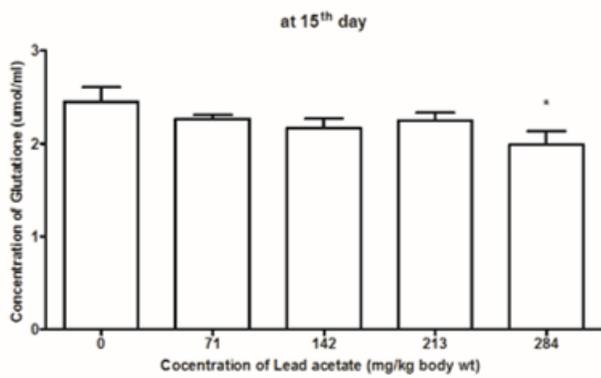
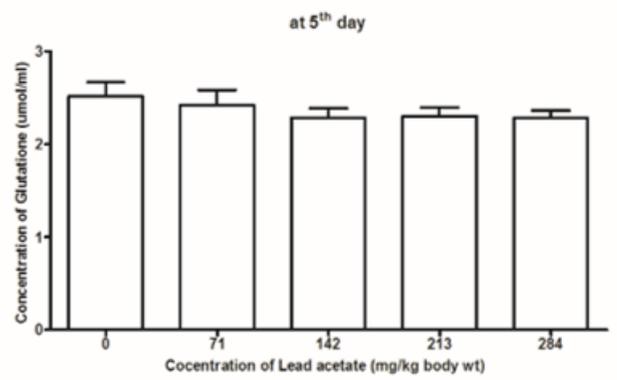
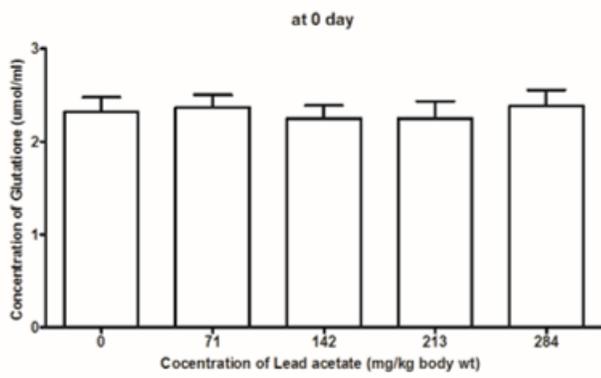


Figure 7

Measurement of glutathione level in treatment groups: For abbreviations and keys see figure 1.

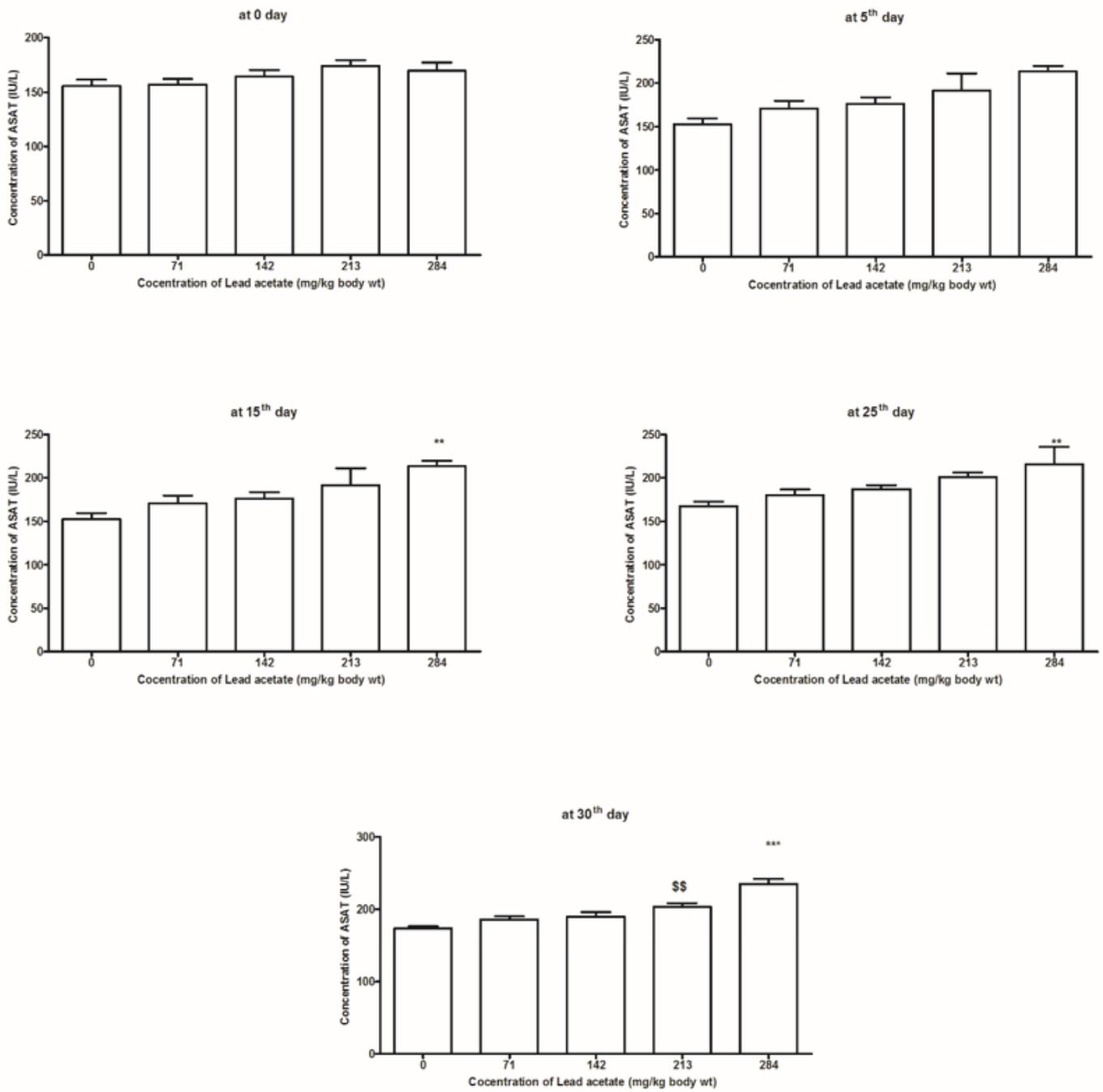


Figure 8

Measurement of ASAT level in treatment groups: For abbreviations and keys see figure 1.

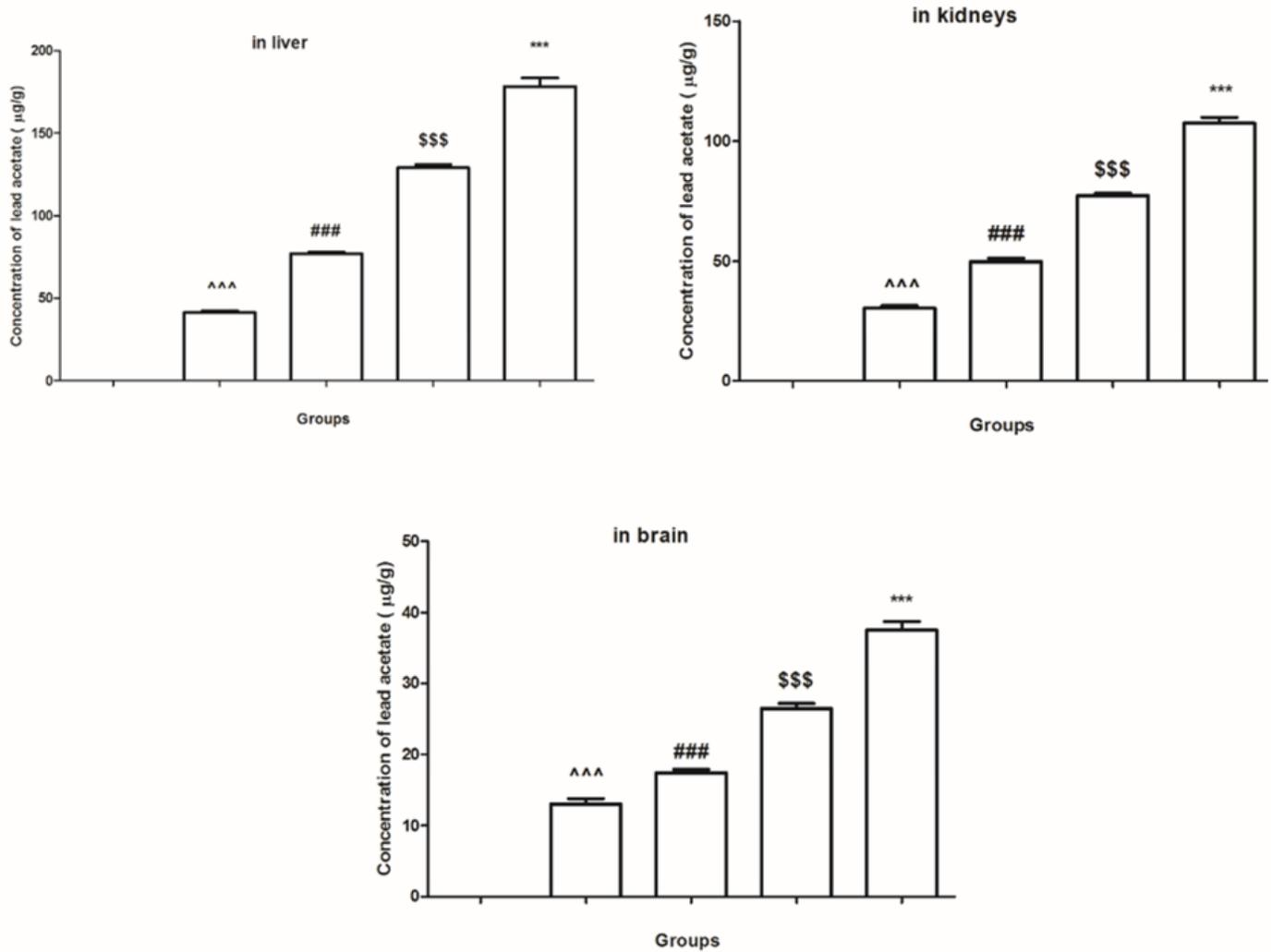


Figure 9

Measurement of lead acetate in brain, liver, and kidneys Abbreviations and keys: '^' displays the substantial variance among Group B, and control group '#' displays the substantial variance among Group A and Group C, '\$' indicates the substantial variance among Group D and control and '*' indicates the substantial alteration among control and Group E. Arithmetical signs: '^', ###, \$\$\$, *** = $p \leq 0.001$.