ORIGINAL ARTICLE

Metabolic Energy Insufficiency in Mice Kidney following Short-term Exposure to Lead: An *In-vivo* Study

Pritha Das, Sudipta Pal*

Abstract

Background: Metabolic stress is one of the risk factors of lead-induced functional abnormalities of the tissue. The present study aims at determining the effect of lead on certain energy metabolic profiles in renal tissue of mice.

Method: Swiss albino mice were exposed to Pb by gavages at a 5mg/kg body weight dose for 30 days. After treatment, urinary glucose, glucose 6-phosphatase, lactate dehydrogenase, TCA cycle enzymes, different proteolytic enzyme activities, total, acidic, basic and neutral proteins, protein carbonyl content, glyceraldehyde 3-phosphate dehydrogenase activity, tissue Pb deposition and renal morphology were examined.

Results: Lead exposure resulted in glycosuria and decreased glucose 6-phosphatase activity in the kidney, whereas the lactate dehydrogenase activity was increased in that tissue. The malate dehydrogenase and succinate dehydrogenase activities were stimulated by Pb, whereas the glyceraldehyde 3-phosphate dehydrogenase activity was inhibited. Degradation of tissue protein was accompanied with enhanced protein carbonylation. Significant changes in trypsin, cathepsin and pronase activities were noted. Decreased amino acid nitrogen in kidney was associated with altered transaminase activity. Additionally, fatty infiltration was observed in association with accumulation of elemental lead.

Conclusion: It is suggested that sub-acute Pb exposure modulated certain enzymes and intermediates of major energy metabolism pathways in the renal tissue of mice to establish an adaptive mechanism against metabolic distress.

Keywords: Glycolysis, Glyceraldehyde 3-phosphate dehydrogenase, Lead, Proteolysis, Renal morphology, TCA cycle enzymes.

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Introduction

Lead (Pb), being one of the toxic heavy metals, participates in environmental pollution and health complication. Various chemical forms of this heavy metal are found in nature among which lead oxide is very common.[1] People generally get exposed to Pb via contaminated air, drinking water, food and dermal contact.[2] It imposes adverse effect on human health when accumulated in the body over the years. In some cases, acute toxic effects also happen due to accidental exposure, causing health hazards. The severity of the physiological problems caused by Pb depends on the concentration and duration of its exposure. Significant deposition occurs in liver and kidney after bones due to the differential capacity of the organs to accumulate lead.[3] Its excess deposition in hepatic tissue results in metabolic perturbation inducing hepato-necrotic dysfunctions such as defective DNA synthesis and hepatic hyperplasia[4], nephropathies, and renal tubule obstruction in the formation of lead inclusion body in kidney.[5] Pb-induced metabolic alteration includes changes in the activities of the acid and alkaline phosphatases, lactate dehydrogenase, transaminase and serum lipid profiles that may attribute to organ toxicity.[6] The current study aims to explore how short-term lead exposure influences renal metabolic profiles with reference to energy production and morphological alteration.

Material and Methods

The inorganic salt of lead-acetate was procured from the Pioneer Concern of India, other chemicals like, diethylether, sodium potassium tartarate, boric acid, hydrochloric acid (HCL), acetic acid, casein, ethanol, sodium cyanide, sodium carbonate ninhydrin, leucine, urea, isopropanol, methyl cellosolve, sodium citrate, copper sulfate, ethanol, H_2SO_4, magnesium chloride ($MgCl_2$), sodium carbonate, and glucose 6-phosphate were purchased from Merck (India); dichlorophenolindophenol (DCPIP), bovine serum albumin (BSA), and hemoglobin were of analytical grade and procured from Sigma-Aldrich (India); sucrose and trichloroacetic acid (TCA) were purchased from SRL (India). Biochemical kits such as SGPT and SGOT kits and cholesterol kits were purchased from Transasia Bio-Medicals Ltd, Mumbai, India Coral clinical systems, Verma Industrial Estate. To prepare the reagent ultrapure water from Millipore was used to avoid metal contamination.

Nutritional Biochemistry and Toxicology Laboratory, Department of Human Physiology, Tripura University, Suryamaninagar, West Tripura, Tripura, India

***Corresponding author:** Dr. Sudipta Pal, Nutritional Biochemistry and Toxicology Laboratory, Department of Human Physiology, Tripura University, Suryamaninagar, West Tripura, Tripura, India, Email: sudiptapal@tripurauniv.ac.in, sudiptapal841@gmail.com

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Animal Procurement

Swiss albino male mice (N=12), weighing 25–30 g were procured from the Chakraborty Enterprise, Kolkata (India), an authorized animal supplier nominated by Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Govt. of India. As per the guideline of CPCSEA, animals were housed in polypropylene cages and acclimatized in adequate pathogen-free laboratory conditions for one week before starting the experiment.

Animal Diet

Mice were supplied with a standard protein diet prepared by 18% casein, 9% fat and 71% amylum, and vitamins and minerals used earlier.[7] Moreover, drinking water was supplied *ad-libitum* and the animals were kept in the treatment room, maintaining the temperature at 22 to 25°C and humidity at 50% with light and dark exposure for 12 hours.

Animal Treatment

Mice were divided into two groups namely control and Pb-treated group, where each group consisted of six (n=6) numbers of animals. They were kept in separate cages. The treatment was followed by gavage using orogastric feeding needle to the Pb-treated group of animals at a dose of 5 mg per kg body weight per day for a period of 30 days, and the respective control group received an oral invasion of 0.9% NaCl for the same duration. The lead acetate dose was selected based on the dose-dependent study performed earlier.[8] The treatment was given after providing food to the mice at a particular time once daily to promote better absorption.

Animal Sacrifice and Collection of Kidneys

After the treatment period was over, mice were sacrificed by cervical dislocation according to Institutional Animal Ethical Committee guidelines. Ethical approval was taken from the Institutional Animal Ethical Committee prior to animal experimentation (Approval No. TU/IAEC/2015/XI/2-2 dated 28th July, 2015). After sacrifice, kidneys were taken out from all the animals, the outer fatty layer was removed by forceps and the tissue was washed in ice-cold saline (0.9% NaCl solution), blotted dry, weighed and kept at -20˚C until biochemical analyses were performed.

Isolation of Mitochondrial Enzyme

According to the modified method of Sordahl *et al.* [9], a 10% tissue homogenate was prepared in 0.01 M Tris-HCl buffer containing 0.25 M sucrose and 1-mM EDTA for mitochondrial fraction separation. The tissue (kidney) was centrifuged at 15,000 rpm for 5 minutes to isolate the mitochondrial enzyme. Then the resultant supernatant was centrifuged at 30,000 rpm for 10 minutes. The pellet was re-suspended in isolation medium and centrifuged for 10 minutes at 30,000 rpm.

Analyses of Physio-biochemical Parameters

Body Weight and Kidney-Somatic Index (KSI)

The bodyweight of each of the animals was observed and noted down routinely throughout the whole experimental period until sacrifice. Additionally, all the animals' organ weight (kidney) was also recorded after sacrifice. The kidney-somatic index (KSI) was calculated using the following formula of Khallaf and Authman.[10]

$$\text{Organo-somatic index} = \frac{\text{weight (g) of the organ}}{\text{Day 30 total body weight (g)}} \times 100$$

Analysis of Tissue Lead Content

A definite amount of target tissue was taken and kept overnight for dehydration. Then the tissue sample was digested with acid mixture containing HNO_3, H_2SO_4, and PCA (6:1:1) over a regulated heater and carried out the digestion process for 6-8 hours to obtain a clear transparent solution at 65–70˚C. During the acid digestion of tissue ultrapure water was added occasionally to prevent overdrying. The digested sample was then made up to a definite volume with ultrapure water. The sample was then applied for estimation of lead content using the flow injection atomic spectrophotometer fitted with a graphite furnace according to the method as suggested by Ghosh *et al.*[11] The lead residues were expressed in terms of µg/g of tissue.

Trypsin Activity

To determine the trypsin activity in the 5% tissue homogenate in phosphate buffer (pH 7.4), the method of Green and Work[12] was employed. The enzyme activity was calculated as nmoles of tyrosine liberated/min/mg of tissue protein.

Cathepsin Activity

The cathepsin activity in renal tissue was measured by the method of Pokrovsky *et al.*[13] Tissue cathepsin activity was expressed in terms of nmoles of tyrosine released/min/ mg of tissue protein.

Pronase Activity

The pronase activity was estimated following the method of Barman.[14] The enzyme activity was expressed as nmoles of tyrosine produced/min/mg of tissue protein.

Tissue Protein Content

The acidic, basic, neutral and total protein contents were measured separately according to the methods of Shashi,[15] Trivedi *et al.*[16] and Lowry *et al.*[17] using bovine serum albumin as standard. The protein content was expressed as g per 100 g of tissue.

Protein Carbonyl Content

To calculate the protein carbonyl content in the kidney, the procedure of Stadtman and Levine[18] was employed. The results were expressed as nmol of DNPH-incorporated/ mg protein based on the molar extinction coefficient of 22,000/M/cm.

Free Amino Nitrogen Content

The amount of free amino nitrogen in kidney was estimated

by the method of Rosen.[19] The amino nitrogen content was expressed as mg of leucine per g of tissue.

Glutamate-Pyruvate Transaminase (GPT) and Glutamate-Oxaloacetate Transaminase (GOT) Activities

The transaminase enzyme activities in the studied tissue were determined following the method of Reitman and Frankel.[20] For this assay, the standard kit (Coral clinical systems, Goa, India) was used to measure photometrically the color intensity of the reaction mixture. The enzyme activities were expressed in terms of units/mg of protein.

Glucose 6 Phosphatase (G6PASE) Activity

To measure the G6PASE enzyme, a tissue homogenate containing 52 mg of renal tissue was mixed with 1.8 mL of substrate buffer, (pH 6.5) containing 0.1-mmol/L tris-HCl, 0.1-mol/L EDTA, 0.05 mol/L glucose 6-phosphate. The mixture was incubated at 37°C for 10 minutes. Added 1 mL ice cold 10% TCA and centrifuged the solution at 3,000 rpm for 10 minutes. As a result, a clear supernatant was found to estimate the phosphate content, according to Plummer. [21] The data were recorded with a spectrophotometer at 880 nm. The unit of glucose 6-phosphatase was expressed as µg of phosphate liberated/min/g tissue protein.

Succinate Dehydrogenase (SDH) Activity

The activity of SDH was measured by the method of Hollywood *et al.*[22] The assay mixture consisted of 1-mL phosphate buffer (pH 7.2) containing sodium succinate (0.15 M), azide (0.2 M) and the tested sample. The activity of this enzyme was figured by adding DCPIP (6 mg/mL) as a coloring reagent and finally, a spectrometer recorded the optical density at 600 nm. The result was expressed as µm of DCPIP reduced/min/mg of tissue protein.

Malate Dehydrogenase (MDH) Activity

This enzyme activity was estimated by the method of Mehler *et al.* [23] using an assay mixture containing potassium phosphate buffer (pH 7.2), 0.0076 M oxaloacetic acid and 0.005 M NADH at pH 7.4. The enzyme activity was calculated by the regular reduction of NADH at 340 nm for 3–5 min with 10 seconds interval and expressed the obtained data by mM of NADH oxidized/min/mg protein.

Isocitrate Dehydrogenase (IDH) Activity

The IDH activity was estimated following the method of King,[24] and the enzyme activity was expressed as unit per minute per milligram of protein.

Lactate Dehydrogenase (LDH) Activity

To measure the LDH activity in the target tissue (kidney), the protocol of Bergmeyer *et al.*[25] was followed. The enzyme activity was expressed as units/min/mg of protein.

Glyceraldehyde 3-phosphate dehydrogenase (G-3PDH) activity

The activity of G3PDH was measured by the method of Heinz and Freimuller.[26] The activity was expressed as mmoles of NADH /min/ mg of protein.

Alkaline phosphatase (ALP) activity

The activity of ALP in renal tissue of mice was measured according to the Kind and King's method modified by Varley.[27] The Kit-based estimation was based on the measurement of the colour intensity of the phenolic compound derived from the chemical reaction of substrate disodium phenyl phosphate in the presence of oxidizing agent like potassium ferricyanide and expressed the obtained data as KAunits/mg of tissue.

Urinary Glucose Estimation

The glucose contents of urine samples of mice were measured by Erba glucose Kit (Transasia Bio-Medicals Ltd, Mumbai, India) following glucose oxidase-peroxidase method.[28] The glucose content was expressed as mg/dl of urine. It was estimated as pooled sample from three urine samples.

Histopathological Studies

Kidneys were collected from the experimental animals and preserved in 10% formaldehyde solution for 24 hours. After gradual tissue dehydration in graded alcohol, clearing and embedding, the tissue was sliced by rotary microtome. The obtained sections were stained using hematoxylin-eosin and examined under a compound microscope under 10x, 20x, and 40x objectives.

Statistical Analyses

To compare data between two groups, all results were expressed as means±S.E.M. The experiments were repeated three times. To record the significance level of the result, paired 'Student's t test' was carried out to compare the two groups. $P < 0.05$ was considered statistically significant.

Results

Body Weight and KSI

The final body weight of the mice was reduced by 18.4% ($p < 0.001$) after exposure to lead (Table 1). The kidney somatic index (KSI) was increased by 32.5% ($p < 0.05$) in a lead-treated group of mice as compared with the control group.

Tissue Lead Content

Lead exposure significantly elevated the elemental lead concentration in the renal tissue of mice (Table 1). The increase was observed as 136.4% ($p < 0.001$).

Urinary Glucose

Glucose content in urine samples of the Pb-treated mice was elevated by almost ten times that the control group (Table 1).

Acidic Protein

The acidic protein content was found to be reduced by 58.4% ($p < 0.001$) after Pb exposure (Table 2).

Basic Protein

Alteration in basic protein content showed a remarkable depressing effect of lead on this parameter in renal tissue. The decrease was found to be 39.3% ($p < 0.001$) in comparison to the control group (Table 2).

Neutral Protein

It was further revealed that the neutral protein content was decreased by 77.8% ($p < 0.001$) as compared to the control value (Table 2).

Total Protein

The total protein content was depleted in the renal tissue of mice by 66.23% ($p < 0.001$) after lead treatment (Table 2).

Protein Carbonyl Content

The protein carbonyl content in the kidney was significantly elevated in lead-intoxicated mice. The increased value was addressed by 47.4% ($p < 0.001$) as compared to the control group (Table 2).

Free Amino Acid Nitrogen

The free amino nitrogen content was reduced in renal tissue significantly. The change was represented as 33.65% ($p < 0.001$) in comparison to the control group (Table 2).

Trypsin Activity

Lead exposure caused an elevation of this enzyme activity by 60.55% ($p < 0.001$) in renal tissue of lead exposed mice (Table 3).

Cathepsin Activity

Change in cathepsin activity indicated significant increase by 115.3% ($p < 0.001$) following exposure to lead (Table 3).

Pronase Activity

The pronase activity was increased in kidney by more than four times ($p < 0.001$) in relation to the control group (Table 3).

G6PASE Activity

Lead caused a significant decrease in the G6PASE activity in the renal tissue of mice by 50.31% ($p < 0.01$) in comparison with the respective control group (Table 3).

ALP activity

The ALP activity in the renal tissue was stimulated by Pb and recorded as 34.8% increase ($p < 0.001$) as compared to control (Table 3).

SDH Activity

There was a significant increase in SDH enzyme activity in kidney of exposed mice after lead treatment. The change was recorded as 96.16% ($p < 0.001$) of the control value (Figure 1).

MDH Activity

The MDH activity in the renal tissue of mice was increased by 57.16% ($p < 0.01$) after lead exposure (Figure 2).

IDH Activity

The activity of isocitrate dehydrogenase was decreased in renal tissue of lead intoxicated mice. The change was

Table 1: Effects of Pb on the body weight, kidney somatic index (KSI), tissue lead concentration and urinary glucose content of mice

Groups of Animals of Pb exposure (g)	*Body weight after 30 Days*	*KSI*	*Lead content in kidney (µg/g of Tissue)*	*Urinary glucose content (mg/dl of urine)*
Control	42.8±0.8	1.6±0.1	1.29±0.01	0
Pb-treated	34.9±0.9***	2.12±0.04*	3.14±0.03***	9.86

Values are means±S.E.M. Figure in the parenthesis (n = 6) indicates the number of animals in each group. Significance level are indicated by $P < 0.05$*, $P < 0.001$***.

Urine glucose was estimated from three pooled samples from each group.

Table 2: Effect of Pb on differential protein contents, free amino acid nitrogen (FAAN) level and carbonylated protein (CP) content in kidney of mice

Groups of animals (n=6)	*Acidic protein* (g%)	*Basic protein* (g%)	*Neutral protein* (g%)	*Total protein* (g%)	*FAAN* (mg Leu/g tissue)	*CP* (nmol DNPH incorporated/ mg protein)
Control	2.5±0.32	7.56±0.08	13.24±1.02	20.67±0.92	14.5±1.07	28.08±0.87
Pb-treated	1.04±0.66 $p < 0.001$	4.59±0.15 $p < 0.001$	2.94±0.44 $p < 0.001$	8.33±0.56 $p < 0.001$	9.62±0.39 $p < 0.001$	39.91±1.5 $p < 0.001$

Values are means±S.E.M. (n=6) indicates the number of animals in each group. $p < 0.001$ was considered statistically high significance difference between experimental groups.

Table 3: Effect of Pb on proteolytic enzyme activities such as trypsin, cathepsin and pronase, glucose 6phosphatase (G6PASE) and alkaline phosphatase (AP) activities in kidney of mice

Groups of Animals (n=6)	*Trypsin (nmoles of tyrosine released/min/mg of tissue protein)*	*Cathepsin (nmoles of tyrosine released/min/mg of tissue protein)*	*Pronase (nmoles of tyrosine released/min/mg of tissue protein)*	*G6PASE (µg of phosphate formed/min/g tissue)*	*AP (KA Unit/mg tissue)*
Control	25.73±0.34	1.31±0.02	1.72±0.06	107.21±5.19	0.69±0.02
Pb-treated	41.31±0.36 $p < 0.001$	2.84±0.18 $p < 0.001$	7.07±0.19 $p < 0.001$	53.27±3.44 $p < 0.01$	0.09±0.02 $p < 0.001$

Values are Means±S.E.M. Significance level indicated by $P < 0.01$ and $P < 0.001$; n= number of animals in each group.

recorded as 25.7% ($p < 0.01$) in comparison to the control group (Figure 3).

LDH Activity

The activity of LDH was enhanced by 32.64% ($p < 0.001$) in the renal tissue of lead exposed mice (Figure 4).

G-3PDH activity

The decreased activity of G-3PDH was recorded as 29.3% ($p < 0.001$) in comparison to the control value in Pb-treated kidney of mice (Figure 5).

Tissue GOT and GPT Activities

The GOT in the kidney was increased significantly by 36.9% ($p < 0.001$) following exposure to lead, whereas the GPT activity was decreased by 28.55% ($p < 0.001$) in that tissue (Figure 6).

Histological Observations

The renal structure of lead-exposed mice displayed ruptured glomerular tuft with disorganized bowmen's capsule, dilated tubular cell and aggregation of fat in the renal medulla (Figure 7a, 7b).

Discussion

The present study reveals that short-term Pb toxicity was concerned with metabolic insufficiency, especially energy metabolism within renal tissue and its structural deterioration. Decrease in body weight was observed in lead-treated mice,

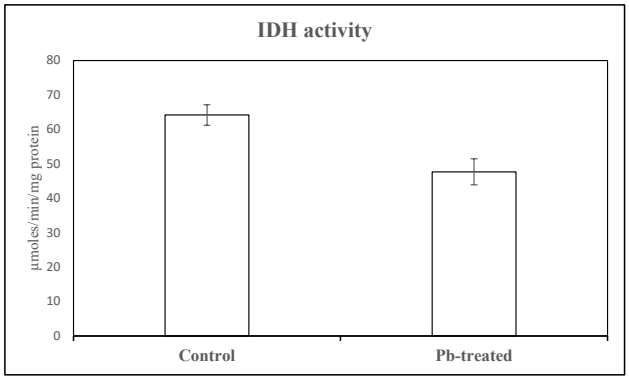

Figure 3: Inhibitory effect of Pb on isocitrate dehydrogenase (IDH) enzyme activity in kidney of mice
Values are means±SEM. Each group consisted of six number of animals. ** indicates $p < 0.01$.

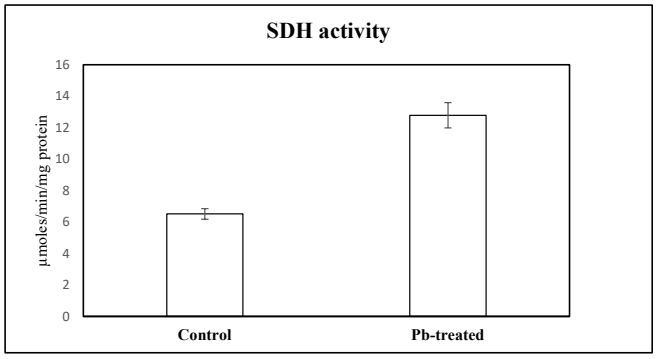

Figure 1: Stimulatory effect of Pb on succinate dehydrogenase (SDH) enzyme activity in kidney of mice
Values are means±SEM. Each group consisted of six number of animals. *** indicates $p < 0.001$.

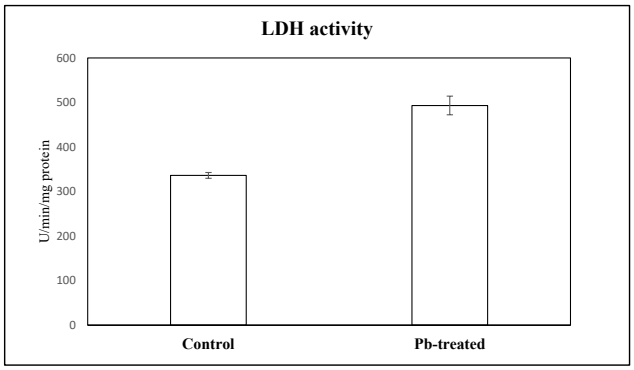

Figure 4: Stimulatory effect of Pb on renal lactate dehydrogenase (LDH) enzyme activity in mice
Values are means±SEM. Each group consisted of six number of animals. *** indicates $p < 0.001$.

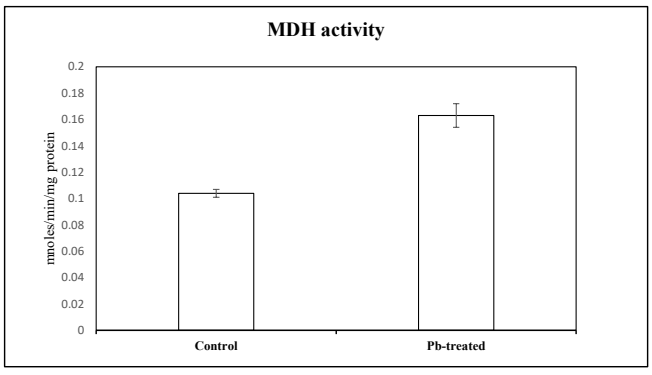

Figure 2: Stimulatory effect of Pb on malate dehydrogenase (MDH) enzyme activity in kidney of mice
Values are means±SEM. Each group consisted of six number of animals. *** indicates $p < 0.001$.

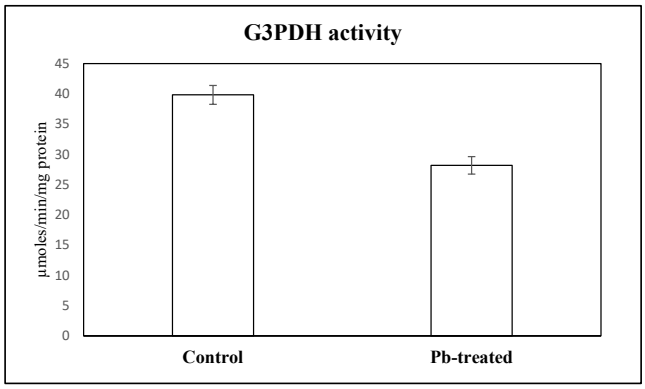

Fig. 5: Inhibitory effect of Pb on the glyceraldehyde 3-phosphate dehydrogenase (G3PDH) activity in mice kidney
Values are means±SEM. Each group consisted of six number of animals. *** indicates $p < 0.001$.

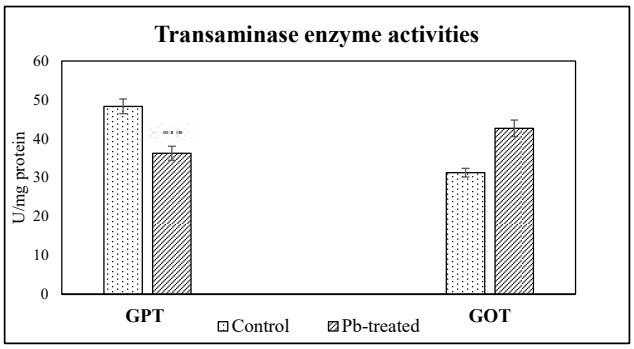

Fig. 6: Effect of Pb exposure on glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) enzyme activities in mice kidney
Values are means±SEM. Each group consisted of six number of animals. *** indicates $p < 0.001$.

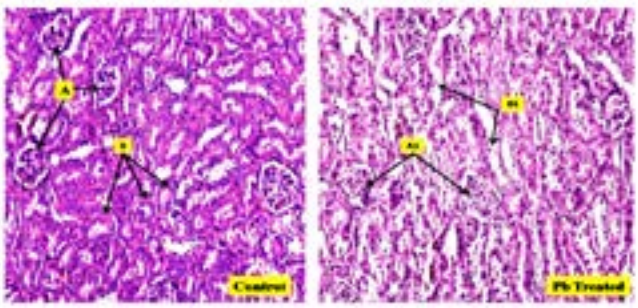

Figure 7a: kidney section of control mice (Cortical region) with glomerulus in cup shaped bowman's capsule (A) and tubular cell (B). The lead treatment destroyed glomerular tuft and ruptured the bowman's capsule area (A1), dilatation of tubular cell (B1) (Hematoxylin and Eosin staining, 20X).

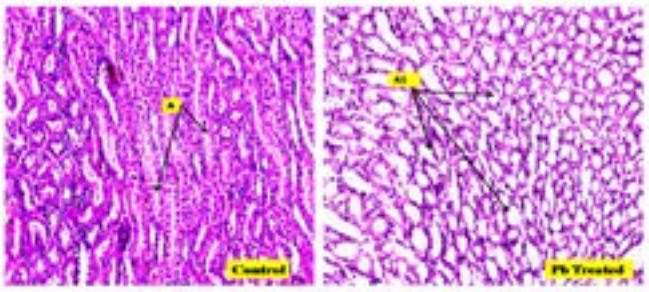

Figure 7b: Represents the histology of kidney of control mice with normal medullary tubular cell (A). Whereas, the lead treated kidney displayed fat infiltration in the medullary area (A1) (Hematoxylin and Eosin staining, 20X).

which might be due to malnourishment due to toxicant mediated stress that perturbed proper absorption of essential nutrients from the gastro-intestinal tract of the exposed animals.[29] The present study addressed that the kidney-somatic index (KSI) was significantly increased in the treated group compared with the control group of mice after lead exposure. The obtained result of KSI was in resemblance with the effect of lead on fish liver tissue as revealed by the studies of Khallaf and Authman,[10] which suggested that change in the organo-somatic index might be correlated with the alteration in fat accumulation in the fish tissue after lead treatment. This assumption is affirmed by the present histological study of kidney where fat deposition in the renal medulla was one of the key observations of toxic manifestation of lead. The present study also revealed a significant accumulation of elemental lead in the renal tissue that might be correlated with renal malfunctions as indicated by renal glycosuria and overaccumulation of carbonylated proteins and lipids within the affected tissue. This is supported by the earlier observation, which stated that glycosuria is one of the lead-induced nephrotoxic manifestations in human body. [30] It was further postulated that lead-induced renal glycosuria may be responsible for hypoglycaemia and associated metabolic changes in relation to lead toxicity.[8]

Alteration in carbohydrate bioenergetics in the renal tissue after short-term lead exposure was evident in the present study. Diminished activity of G6PASE, as found in the present study, might be associated with less accumulation of glucose 6-phosphate and other glycolytic metabolites in the renal tissue. It was suggested that the intermediary product, oxaloacetate formed from malate, was not sufficient to supply glucose by G6PASE enzyme after lead intoxication.[31] The present study further revealed that activity of the SDH enzyme was enhanced in the lead-treated organ that was supposed to be involved in the production of energy to maintain renal bioenergetics in a Pb-stressed environment. Normally the SDH requires basic (Arg 31, His 207) and neutral (Ser 27, Try 83) proteins to form ubiquinone binding site.[32] It is postulated that basic proteins are utilized more by the renal tissue in configuring SDH, thus enhancing its activity. The study also elucidated a significant increased activity of the MDH enzyme in Pd-treated renal tissue of mice. It conforms with Afsar (2012) reports on fish model where activation of MDH in the renal tissue by Pb was employed to produce energy during carbohydrate metabolism.[33] Elevation of blood MDH activity in occupational lead-zinc workers further provided evidence supporting the stimulatory effect of Pb on MDH activity.[34]

On the contrary, the IDH activity of the renal tissue was significantly decreased by Pb exposure in the present study. Substrate inadequacy or NAD^+ scarcity might be the reason for such enzyme suppression. The present study further acknowledged that the increased activity of lactate dehydrogenase by Pb depended on the availability of pyruvic acid, which supplies energy through the lactate cycle during insufficient supplementation of oxygen.[35] As evidenced by the present histological observation, Pb-induced damaged renal tissue might fail to utilize oxygen for its metabolic activity and thus switch over to the anaerobic process. This might also suppress the supply of pyruvate to feed the TCA cycle. On the other hand, enhanced TCA cycle enzyme activities was supposed to utilize more substrates to provide energy to the lead-stressed renal tissue. Both of these might cause serious impairment of substrate availability for the TCA cycle if the lead exposure was continued.

Moreover, enhancement of protein carbonyl content in the target tissue after Pb exposure was indicative of oxidative damage of native proteins. Significant increase in the trypsin enzyme activity in kidney might cause the breakdown of specific proteins thus serving as an adaptive mechanism to provide certain amino acids as precursors for new protein synthesis in the lead-affected tissue or the amino acids might be mobilized to the other tissues like liver and skeletal muscle to aid protein anabolism. Additionally, cathepsin activity was elevated in the kidney of Pb-treated animals. Studies of Ahmed and Zaki [36] acknowledged that a stressful environment motivated the lysosomal enzyme release from the liver to plasma, perturbing the cellular integrity along with the substrates of mitochondria and sarcolemma, which were responsible for elevation of cathepsin enzyme function. Additionally, the pronase activity was also increased after Pb exposure indicative of increased proteolysis in the renal tissue of mice.

The present study further revealed that lead exposure caused a significant decrease in acidic protein content in renal tissue of mice. This might be due to interference of Pb with that protein synthesis or with the sulfhydryl groups of some metal-binding proteins, and their subsequent removal from the tissue through the detoxification process.[37] On the other hand, the obtained result of basic proteins might be explained by the function of trypsin as because this enzyme mainly targets the basic proteins' carboxyl sites containing amino acids such as arginine, lysine and leucine[38] and releases the end products that accumulate in the affected tissues. Relating to the inversely proportional association between the enzyme and its substrate,[39] it is postulated that the increased activity of trypsin might be the cause of decreased basic protein content in the renal tissue of mice exposed to Pb. This assumption is supported by the heavy metal toxicity in aquatic fish model.[40] Moreover debasement of basic proteins is consistent with the surge of malate dehydrogenase function as the basic proteins like arginine and histidine are needed to form the structural components of the MDH.[41] Decrease in neutral protein content after lead intoxication might be due to degeneration of amino acid like cysteine in account of the metal's ability to lure the sulfhydryl groups of protein.[42] It was further accompanied by enhanced cathepsin activity, which might utilize more neutral proteins as substrate and subsequently reduce its content in the examined tissue. Additionally, as found in the present study, the reduction of renal total protein content was supposed to be an indicator of disturbed renal metabolism.[43] The present study confirmed that the carbonylation of proteins due to oxidative stress might also be responsible for tissue protein degradation. This observation might indicate imbalanced oxidation–reduction complex formation and disintegration of the cell membrane by the reactive oxygen species produced by Pb.[44] The products of oxidative stress finally attack the functional groups of proteins like histidine, arginine and lysine to form carbonyls and ensue protein depletion.[45]

The current experiment further established a significant reduction in the level of free amino nitrogen in the target tissue, which might be due to the mobilization of free amino nitrogen to the extrarenal tissues like liver and muscle to supply the precursors for gluconeogenesis or other integrated metabolic pathways to replenish the loss of blood glucose in consequence of renal glycosuria following Pb intoxication. Increased transaminase enzyme activity such as GOT and oxidative deamination were responsible for the depletion of free amino nitrogen as these were involved in supplying intermediates of the TCA cycle or gluconeogenic pathway.[46] Possible mechanism of GOT acceleration in renal tissue might also be due to renal dysfunctions, including tubulopathy, β-2- microglobinuria, and enzymuria caused by Pb toxicity.[43] On the contrary, the activity of GPT enzyme was significantly decreased in the Pb-treated organ. The obtained result might be clarified with the destruction of acidic protein in the renal tissue as the GPT enzyme is involved in protein and amino acid metabolism;[47] so, the scared supplementation of substrates like α-ketoglutarate due to Pb-induced decreased IDH activity, as found in the current study, might be the reason of suppressed GPT function.

Moreover, increased KSI and lipid deposition in kidney might result from adipogenesis as confirmed by the histological analyses of the affected tissue. It was reported by Brömme *et al*.[48] that cathepsin L in mouse, which is homologous to cathepsin V in human, played an important role in adipogenesis and glucose intolerance via degrading fibronectin insulin receptor and insulin-like growth factor 1 receptor.[49] It was further revealed that glyceraldehyde 3 phosphate dehydrogenase activity was suppressed in the renal tissue of lead-induced mice. This observation conformed with the earlier report of Calderón *et al*., [50] suggesting that free Pb could inhibit the activity of G3PDH in erythrocyte of children. This enzyme has several metal binding domains like cysteine (Cys) 149 and histidine (His) 176 in its catalytic site, and certain metals like Co^{2+}, Cu^{2+}, Fe^{2+}, Ni^{2+} and Pb^{2+} tend to bind with this catalytic site to inhibit the enzyme activity.[51] As this enzyme utilizes glycerol as substrate to synthesize glucose via the gluconeogenic pathway, the suppressed activity of G3PDH might cause glucose inadequacy in the renal tissue. Moreover, renal glycosuria was evident in the present study indicating a disturbance in renal tubular absorption of glucose by lead toxicity, which was suggested to impose a hypoglycaemic situation in earlier occasion.[8] Alteration in renal energy metabolism due to lead was also supported by the increased alkaline phosphatase activity in that tissue which served as an adaptive mechanism to yield energy from the breakdown of ATP to support tissue survival in a metabolically stressed condition.

Conclusion

Sub-acute lead exposure significantly altered the metabolic orientation in the kidney, one of the vital detoxifying organs in mice model. An increase in organo-somatic index was

associated with fatty infiltration and significant deposition of elemental lead. Retardation of glycolytic activity after Pb elation in the target tissue motivated certain changes in protein metabolic pathways, especially degradation of tissue proteins by enhancing the proteolytic enzyme activities in response to Pb toxicity. Additionally, the decreased accumulation of free amino acid nitrogen in kidney may provide the substrate for gluconeogenic pathway in liver to compensate glucose loss due to glycosuria. The inability to supply enough energy and nutrition due to loss of blood glucose altered the relay network of the TCA cycle enzymes in the renal tissue due to Pb toxicity. Due to such imbalanced energy metabolism the renal tissue architecture was remarkably affected, which might be responsible for compromised renal function after Pb intoxication.

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Declaration

The authors declare that the present article has not been published or sent for publication in any other journal earlier, and the copyright of the article is given to The Physiological Society of India.

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