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Original Article

ALTERATION IN CARBOHYDRATE METABOLISM BY SUB-ACUTE LEAD EXPOSURE: A DOSE-DEPENDENT STUDY

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ABSTRACT

Objective: The study was conducted to evaluate the dose-dependent effects of sub-acute lead exposure on certain aspects of carbohydrate metabolism.

Methods: Swiss albino male mice (weighing 30-35 g) were selected for the present study and divided into five groups; one control group and others lead-treated groups i.e. Group A (5 mg/kg body weight), Group B (10 mg/kg body weight), Group C (15 mg/kg body weight) and Group D (20 mg/kg body weight). Parameters like blood and liver glucose, glycogen and pyruvic acid contents were determined in liver tissue. The enzyme activities like pyruvate dehydrogenase, malate dehydrogenase and glucose 6-phosphatase were recorded in that tissue. Additionally, free amino acid nitrogen content and transaminase enzyme activities were also evaluated in liver tissue of mice.

Results: The study reveals that lead caused a significant diminution of blood and hepatic glucose levels and fall in liver glycogen content in a dose-dependent manner, the highest effect was observed in animals treated with lead at a dose of 20 mg/kg body weight. Glucose 6-phosphatase activity was decreased significantly in all the treated groups. There was a dose-dependent increase in pyruvic acid content whereas pyruvate dehydrogenase, malate dehydrogenase and transaminase enzyme activities were significantly depressed in a dose-dependent fashion in all the treated animals. Additionally, lead treatment significantly ($p<0.001$) enhanced free amino acid nitrogen in the liver to provide a substrate for gluconeogenesis.

Conclusion: It is suggested that an adaptive mechanism is initiated by stimulating and retarding glycogenolytic and glycolytic activity and also by rising in the content of free amino acid nitrogen to recover from the lead stressed toxic manifestation.

Keywords: Lead acetate, Dose-dependent study, Glycogenolysis, Glycolysis, Tricarboxylic acid (TCA) cycle

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INTRODUCTION

Lead, being a metal is often found in paints and cosmetics, used for water pipes, in printing and in former times for health care supplies [1]. Environmental exposure of lead by widespread use of leaded gasoline in developing countries and from other sources is gaining attention due to its serious health effects [2]. Most of the environmental lead enters the body through alimentary and respiratory tracts [1]. After ingestion and absorption lead primarily accumulates in soft tissues and bones and also in teeth, liver, kidney, brain and spleen with the highest concentration [3]. Studies on the postnatal blood lead levels of women and their newborn children indicated that lead transport through the placenta is neither selective nor regulated [4].

Lead is known to be potentially harmful and therefore increasing attention to this metal has been paid during the last two decades. Moreover, lead causes hepatocellular damage [5], alters the metabolic homeostasis through denaturation of nuclear proteins and deoxy-ribonucleic acid [6], modifies the tricarboxylic acid cycle enzymes [7], causes morphological changes of liver [8] and depression of certain other metabolic enzyme functions like alanine transaminase (ALT), aspartate transaminase (AST) and acid phosphatase (ACP) [9].

The present study was aimed to evaluate the dose-dependent effects of lead exposure on glucose metabolism to see how and in which way sub-acute lead exposure altered the energy supplementation in relation to biochemical moderation during toxic condition. Thus the objective of the current study was based on profiles of carbohydrates, free amino acid nitrogen as well as transaminase enzyme activities which either supply energy or help in the survival of tissues during toxic manifestation. This study would provide new information regarding dose-dependent alteration in carbohydrate metabolism following sub-acute lead exposure.

MATERIALS AND METHODS

Materials

Lead acetate (LA) was procured from Pioneer Concern (Agartala, India); sodium citrate, diethyl ether, copper sulphate, KOH, ethanol, H_2SO_4, phenol, magnesium chloride ($MgCl_2$), sodium carbonate, glucose 6-phosphate were purchased from Merck (India); dichloro-phenolindophenol (DCPIP) and bovine serum albumin (BSA) were procured from Sigma–Aldrich (India); Trichloroacetic acid (TCA), tris-HCl, ascorbic acid, ammonium molybdate, oxaloacetic acid, dipotassium hydrogen phosphate (K_2HPO_4), sucrose, sodium potassium tartarate, 2,4-dinitrophenyl hydrazine (2,4-DNPH), toluene, ninhydrin, leucine, isopropanol, methyl cellosolve were of analytical grade and purchased from SRL (India). Glucose estimation kit was purchased from Transasia Bio-Medicals Ltd, Mumbai, India and SGPT and SGOT kit were purchased from Coral clinical systems, Verma Industrial Estate, Goa, India. Ultrapure water by Millipore was used throughout the experiment to avoid metal contamination in the preparation of reagents.

Animals

For the present study, Swiss albino male mice (N=30) weighing 30-35 g were purchased from Chakraborty Enterprise, Kolkata-700011, an authorised animal supplier nominated by Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Govt. of India. The animal ethical committee of Tripura University approved the protocols for the experiments before experimentation (Approval No. TU/IAEC/ 2015/XI/2-2 dated 28th July 2015). Animals were housed in polypropylene cages, acclimatised under laboratory conditions for one week before starting the experiment. Animals were provided with 18% protein (casein) diet used in earlier occasions [10] and supplied with drinking water *ad libitum* throughout the experiment.

They were kept in the animal house by maintaining standard air conditions of temperature (22 °C to 25 °C) and humidity (50%) with 12 h alternating light and dark cycles.

Experimental design

The animals were divided into five separate groups of six animals each of equal average body weight (30-35 g), one control group and rests are lead-treated groups. An aqueous solution of lead was prepared by dissolving LA in distilled water at four different concentrations such as 50 mg%, 100 mg%, 150 mg%, and 200 mg% to carry out the dose-dependent study. The animals of the treated groups were administered with 10 µl/g body weight LA solution by orogastric feeding needle and the volume of lead solution was changed according to the alteration in body weight throughout the period of treatment.

The treatment schedule was fixed once daily for a period of thirty days. The animals were then subjected to the following treatment protocol.

Control group

Animals received distilled water orally by gavage once daily for 30 d.

Lead-treated groups

Group A:-Animals of this group were given 10 µl of 50 mg% LA per g body weight (equivalent to 5 mg/kg body weight) orally by gavage once daily for a period of 30 d.

Group B:-The animals were treated with 10 µl of 100 mg% LA per g body weight (equivalent to 10 mg/kg body weight) by gavage once daily for 30 d.

Group C:-LA was administered in a volume of 10 µl of 150 mg% per g body weight (equivalent to 15 mg/kg body weight) by gavage once daily for 30 d.

Group D:-10 µl of 200 mg% (equivalent to 20 mg/kg body weight) of the tested compound was administered per g body weight of mice by gavage once daily for the same duration.

Animal sacrifice

Animals were sacrificed after treatment by cervical dislocation by maintaining guidelines of the institutional animal ethical committee.

Collection of blood

After sacrifice, blood was collected from the hepatic vein of all experimental animals and transferred into a tube containing 3.8% sodium citrate (9:1 with blood sample) as an anticoagulant for blood glucose estimation.

Separation of tissue sample

The liver tissue was collected from all the animals and washed in ice-cold saline (0.9%), blotted dry, weighed and kept at-20 ˚C for biochemical analyses.

Preparation of tissue homogenate

A 10% liver tissue homogenate was prepared in 0.01 M Tris-HCl buffer containing 0.25 M sucrose and 1 mmol EDTA for mitochondrial separation. Additionally, 10%, as well as 5% liver tissue homogenates, were prepared in 0.1 M phosphate buffer (pH 7.4) and 0.25 M sucrose solution for different biochemical analytical methods.

Biochemical analyses

Body weight and liver somatic index (LSI)

The body weight of each animal was taken throughout the treatment period and also noted after a definite time interval until sacrifice. The liver weights of respective groups were recorded on the day of sacrifice. Then the LSI was calculated using the following formula according to Khallaf and Authman [11].

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

Blood and liver glucose

Glucose contents of blood and liver samples were measured by Erba glucose Kit (Transasia Bio-Medicals Ltd, Mumbai, India) following glucose oxidase-peroxidase method of Barham and Trinder [12]. Briefly, 1 ml of the working reagent was mixed with 10 µl of the samples (blood and 5% liver tissue homogenate) and incubated for 10-15 min at 37 °C. Similarly blank and standard were prepared by using 10 µl of distilled water and the same volume of standard solution containing 5.55 mmol/l glucose, respectively. After incubation, the absorbance was taken at 500 nm. The levels of blood and liver glucose were expressed as mg/dl and mg/g of liver tissue respectively.

Liver glycogen content

Liver glycogen content was determined according to Montgomery [13]. In brief, approximately 200-300 mg liver tissue was mixed with 1 ml 30% KOH solution, heated for 15-20 min in a boiling water bath, cooled at room temperature. 1 ml ethanol was added and the mixture was heated again for 5-10 min and cooled. The mixture was centrifuged at 3000 rpm for 10 min and the supernatant was discarded. The pellet was washed twice with 1 ml ethanol, and the resultant precipitate was taken for estimation of glycogen by mixing with 2.5 ml concentrated H_2SO_4 and 0.5 ml 80% phenol. The glycogen content was expressed in µg/g liver tissue.

Glucose 6 phosphatases (G6PASE) activity

To measure the G6PASE, 52 mg liver tissue homogenate was mixed with 1.8 ml of substrate buffer (pH 6.5) containing 0.1 mmol/l tris-HCl, 0.1 mol/l EDTA and 0.05 mol/l glucose 6-phosphate. The mixture was incubated at 37 °C for 10 min. 1 ml ice cold 10% TCA was added and the mixture was centrifuged at 3000 rpm for 10 min. The resultant supernatant was taken to estimate the phosphate content according to Plummer [14]. The optical density was measured at 880 nm. The unit of G6PASE was expressed as µg of phosphate liberated/min/g tissue protein.

Pyruvic acid content

Pyruvic acid content in hepatic tissue was measured according to Segal *et al.* [15]. For this, 0.5 ml of a 10% tissue homogenate in 0.1 M phosphate buffer (pH 7.4) was centrifuged at 3000 rpm for 10 min with 5% TCA and the resultant supernatant was mixed with a definite volume of distilled water and 0.1% 2,4 DNPH and shake it for 3 min. Then 2.5 ml toluene was added, mixed by shaking for few min and kept the tubes in standing position for a while. The lower layer was collected and added with 10% Na_2CO_3 and 1.5 M NaOH to measure the optical density at 420 nm. The observed result was expressed as µg/g of liver tissue.

Pyruvate dehydrogenase (PDH) activity

The PDH enzyme activity was determined by the method of Liu and Bisswanger [16]. The enzymatic assay mixture was made up of triethanolamine buffer containing 0.1M $MgCl_2$, 0.1 M pyruvate, 0.01 M thiamine diphosphate and 0.01 M DCPIP at pH 7.8 and the kinetic changes at 597 nm were observed for five min at 15 s interval. The results were expressed in terms of reduction of DCPIP µmol/min/mg of protein.

Malate dehydrogenase (MDH) activity

For the isolation of this enzyme, the modified method of Sordahl *et al.* [17] was employed. The overall procedure was carried out in an ice chamber at 4 °C temperature. The liver tissue was quickly dissected from decapitated rats into ice-cold isolation medium which consisted of 0.25M sucrose, 1 mmol tris-HCl (pH 7.4) and 1 mmol EDTA (pH 7.2-7.4) and homogenized (10% w/v) using a homogenizer. The 10% tissue homogenate was first centrifuged at 700 xg for 5 min. The resultant supernatant was then centrifuged at 30,000 xg for 10 min. The pellet was re-suspended in isolation medium and centrifuged further for 10 min. The mitochondrial pellet obtained was resuspended in the isolation medium and used for the subsequent analysis. This enzyme activity was determined by the method of Mehler *et al.* [18]using an assay mixture containing potassium phosphate buffer, 0.0076 M oxaloacetic acid and 0.005 M

NADH at pH 7.4. The reduction of NADH was measured at 340 nm for 3-5 min at 10 s interval and expressed the activity as mmol of NADH oxidized/min/mg protein.

Free amino nitrogen (FAN) content

To estimate the amount of FAN content in liver 10% tissue homogenate was first dissolved in 0.67 (N) H_2SO_4 and 10% Na-tungstate to precipitate proteins and then centrifuged to get the protein-free extract. The resultant supernatant was treated with cyanide acetate buffer and 3% ninhydrin solution as per the protocol proposed by Rosen [19]. After that, the solution was heated at 100 °C in a water bath for 5 min and immediately after cooling added with isopropanol to develop a violet colour. The optical density of that coloured solution was measured in a spectrophotometer at 570 nm. The FAN 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 tissues 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 colour intensity of the reaction mixture. The colour was formed due to the chemical reaction of alanine with α-ketoglutarate to form pyruvate in the case of GPT but the aspartate was used to react with α-ketoglutarate to produce pyruvate in the case of GOT. After mixing the tissue homogenates with their specific substrates, all the tubes were allowed to incubate at 37 °C for 30 min after addition of the sample. After completion of the overall steps of the whole reaction, the optical density was read at 505 nm. The enzyme activities were expressed in terms of units/mg of protein.

Statistical analyses

For the comparison of data between different groups, all results were expressed as mean±standard error of the mean (SEM). The significance level was determined by the paired 'Student's t-test' for comparison between the groups. P<0.05 was considered statistically significant.

RESULTS

Body weight

Fig. 1 indicates time-dependent alteration in body weight of mice over the period of treatment. At the lower doses the lead-exposed animals gained the body weight as compared with their control group, but at the higher doses of exposure, the gain in body weight in lead-treated mice was found to be retarded due to toxic manifestation. In the table, 1 changes in body weight after lead exposure have been represented. It is revealed that the final body growth of the mice was reduced by 15.8% (p<0.001), 15.1% (p<0.001), 14.2% (p<0.001) and 15.5% (p<0.001) respectively in successive doses of lead treatment.

LSI

The LSI remained unchanged at the low doses of lead exposure but increased by 25.7% (p<0.05) and 31.4% (p<0.05) in the last two highest doses (table 1).

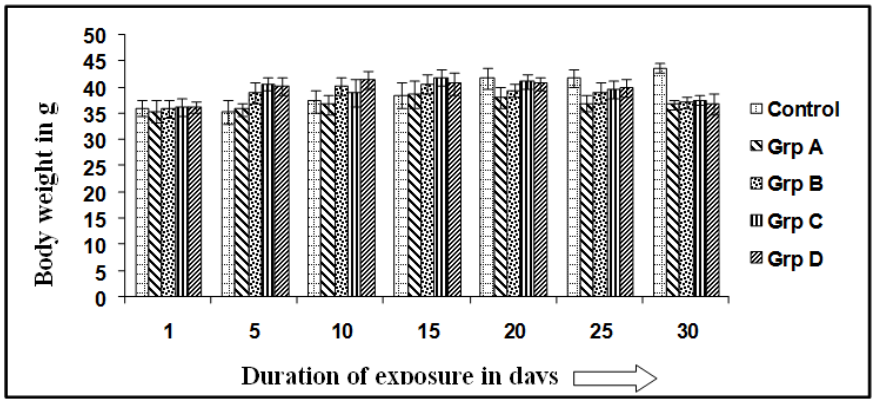

Fig. 1: Time and dose-dependent alteration in body weight of lead-exposed animals. X axis represents different lead treated groups with duration of exposure in days and Y axis represents the unit. Experiments were performed in triplicates (n=3), numbers of animal in each group were 6

Table 1: Effects of lead on body weight and LSI

Groups of animals(N=6)	Body weight(g) after 30 d	LSI Liver weight (g)× 100/body weight (g)
Control	43.8±0.8	3.5±0.4
Grp. A	36.9±0.9***	3.9±0.1#
Grp. B	37.2±1.0***	4.0±0.3#
Grp. C	37.6±1.0***	4.4±0.2*
Grp. D	37.0±2.0**	4.6±0.2*

Values are means±SEM. Significance levels are indicated by *P<0.05, **P<0.01and***P<0.001, #indicates non-significant result. Experiments were performed in triplicates (n=3), N= number of animals, LSI= liver somatic index.

Glucose content of blood

The blood glucose level was depleted following exposure to lead (table 2). The decrease was found to be 14.0% (p<0.01), 17.25% (p<0.01), 19.63% (p<0.01) and 24.6% (p<0.01) respectively in the successive doses of lead used in the present study.

Hepatic glucose level

Table 2 depicts that the lead exposure caused a significant decrease in liver glucose content in all lead-treated mice. The changes were observed as 60.9% (p<0.001), 65.9% (p<0.001), 66.3% (p<0.001) and 67.5% (p<0.001) respectively.

Glycogen content of liver tissue

As seen in table 2 lead exposure caused a significant reduction in liver glycogen content that was found to be 40.7% (p<0.001), 44.3% (p<0.001), 51.3% (p<0.001) and 61.99% (p<0.001) in different doses of lead-intoxicated groups of mice.

G6PASE activity

The data represented in table 2 indicate that LA caused a significant reduction of G6PASE activity in liver tissue of mice by 13.9% (p<0.01), 20.5% (p<0.001), 28.6% (p<0.001) and 42.1% (p<0.001) in successive doses of lead exposure.

Table 2: Effects of four different doses of lead on blood and liver glucose levels, liver glycogen content and G6PASE activity

Groups of animals (N= 6)	Blood glucose(mg/dl)	Liver glucose(mg/g of liver tissue)	Liver glycogen (µg/g tissue)	G6PASE (µg phosphate/min/g of tissue protein)
Control	121.34±3.26	18.43±1.89	103.19±5.75	45.8±1.34
Grp. A	104.29±2.45p**	7.21±0.87p***	61.20±2.67p***	39.4±2.85p**
Grp. B	100.4±5.09p**	6.29±0.43p***	57.50±2.49p***	36.4±3.04p***
Grp. C	97.52±4.64p**	6.21±0.34p***	50.28±1.15p***	32.7±3.86p***
Grp. D	91.52±4.46p**	5.99±0.51p***	39.22±3.31p***	26.5±0.76p***

Values are means±SEM. Significance levels are indicated by **P<0.01 and***P<0.001. Experiments were performed in triplicates (n=3), N= number of animals, G6PASE= glucose 6 phosphatase.

Pyruvic acid content

There was a significant elevation of pyruvic acid content in the hepatic tissue of mice following lead administration. The result depicted in fig. 2 indicates 67.5% (p<0.001), 85.8% (p<0.001), 141.8% (p<0.001) and 282.7% (p<0.001) increase in the above mentioned parameter in response to different doses of lead.

PDH activity

Changes in PDH enzyme activity (fig. 3) in mice liver reveal that lead exposure at the present doses and duration remarkably decreased the mentioned parameter in a dose-dependent manner. The decrease was found to be 10.3% (p<0.01), 16.43% (p<0.001), 30.13% (p<0.001) and 35.95% (p<0.001) in successive doses of lead exposure.

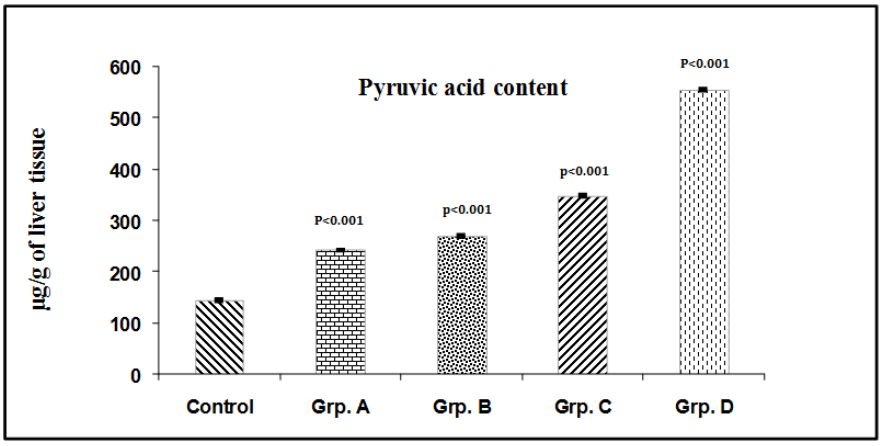

Fig. 2: Dose-dependent effect of lead on the pyruvic acid content of mice liver. Values are means±SEM. *** p<0.001 is considered as highly significant. X axis represents different lead treated groups whereas Y axis represents the unit. Experiments were performed in triplicates (n=3), numbers of animals in each group were 6

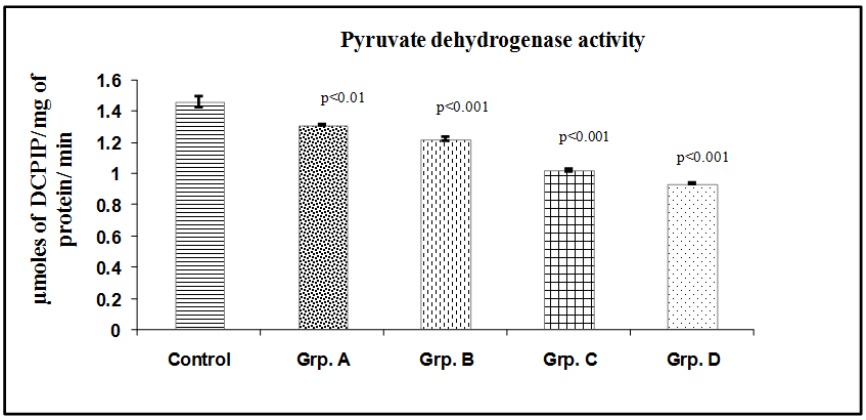

Fig. 3: Change in Pyruvate dehydrogenase activity of mice liver after different doses of lead intoxication. Values are means±SEM. ** p<0.01 is considered statistically significant and *** p<0.001 indicates the highly significant result. X axis represents different lead treated groups whereas Y axis represents the unit. Experiments were performed in triplicates (n=3), numbers of animals in each group were 6

MDH activity

Lead treatment significantly reduced the activity of MDH in hepatic tissue of mice in a dose-dependent manner (fig. 4). The decrease was found to be 88.64% (p<0.001), 89.6% (p<0.001), 90.9% (p<0.001) and 91.8% (p<0.001) respectively in four different doses of lead treatment as compared to the control group.

FAN activity

It is revealed from fig. **5** that the FAN content was increased in liver of lead-exposed mice significantly. The percentage change in observed result has been represented as 64.5% (p<0.001), 111.65% (p<0.001), 145.63% (p<0.001) and 178.25% (p<0.001) respectively in consecutive doses of lead treatment.

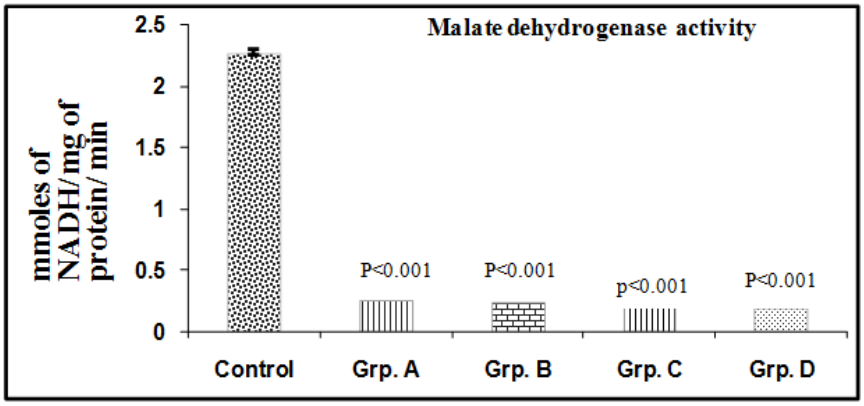

Fig. 4: Effect of lead on the activity of malate dehydrogenase of mice liver. Values are means±SEM. *** p<0.001 is considered as highly significant. X axis represents different lead treated groups while Y axis represents the unit. Experiments were performed in triplicates (n=3), numbers of animals in each group were 6

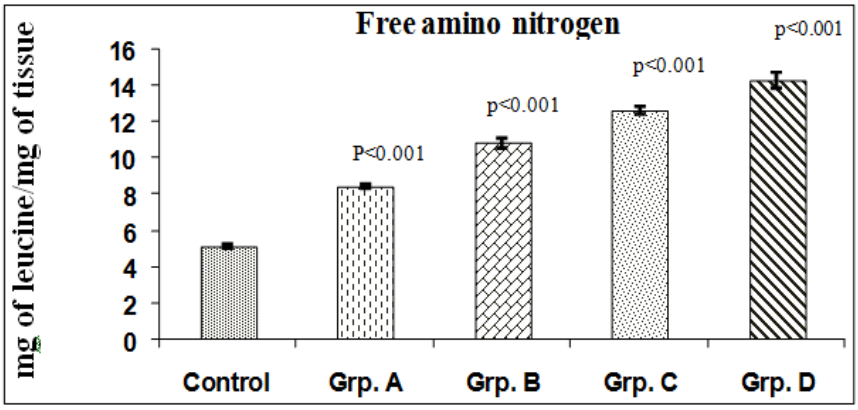

Fig. 5: Dose-dependent changes of free amino nitrogen content in the lead-exposed mice liver. Values are means±SEM. *** p<0.001 is considered as highly significant. X axis represents different lead treated groups while Y axis represents the unit. Experiments were performed in triplicates (n=3), numbers of animals in each group were 6

GOT and GPT activities in liver tissue

The activity of GPT (fig. 6) in the liver was depressed significantly by 40.2% (p<0.001), 43.13% (p<0.001), 46.8% (p<0.001) and 54.84% (p<0.001) respectively following exposure to lead at given doses. The GOT activity (fig. 7) was also decreased by 59.55% (p<0.001), 61.7% (p<0.001), 62.84% (p<0.001) and 68.74% (p<0.001) in liver in a dose-dependent manner.

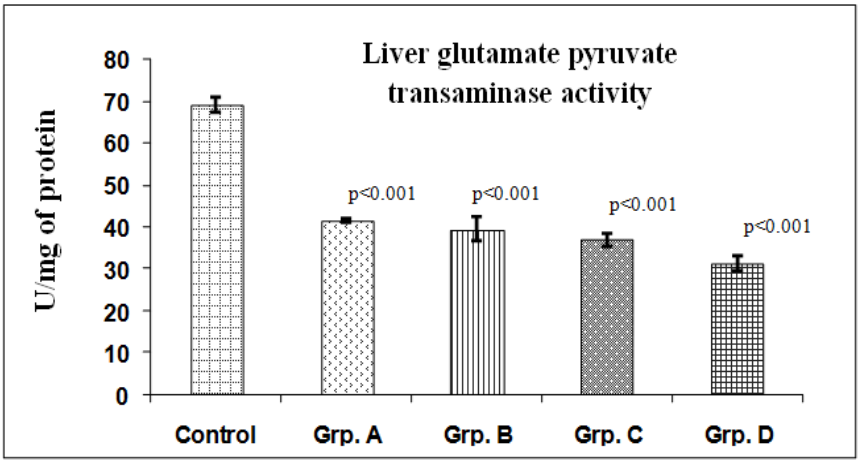

Fig. 6: Activity of liver glutamate pyruvate transaminase at different doses of lead in mice. Values are means±SEM. *** p<0.001 is considered as highly significant. X-axis represents the different lead treated groups while Y axis represents the unit. Experiments were performed in triplicates (n=3), numbers of animals in each group were 6

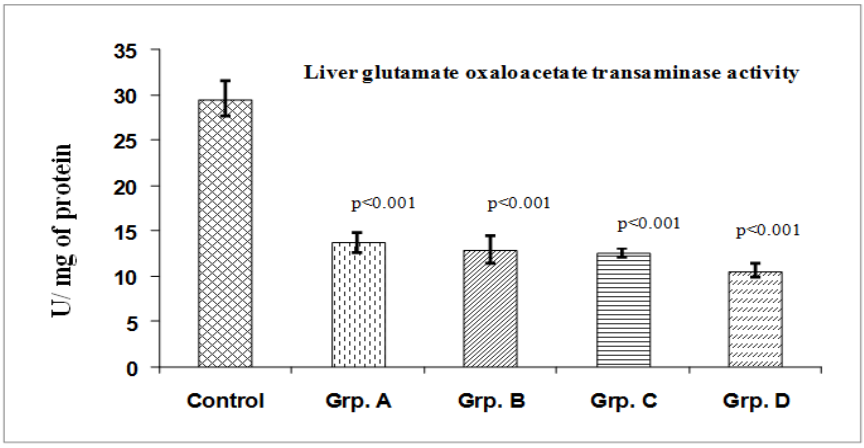

Fig. 7: Activity of Glutamate oxaloacetate transaminase in the liver of lead-induced mice. Values are means±SEM. *** p<0.001 is considered as highly significant. X-axis represents the different lead treated groups while Y axis represents the unit. Experiments were performed in triplicates (n=3), numbers of animals in each group were 6

DISCUSSION

Lead, being a metallic environmental toxicant, is an issue of the global problem. The pollutant is continuously emitted from industrial and environmental sources which promote different health hazards on the human population. People are exposed to lead *via* industrial exposure as well as contamination in the drinking water. After penetration in the body of the host, it is mainly stored in the liver and impairs the metabolic function of it [3]. In the present study sub-acute exposure to a lead-induced moderate decrease in body weight of mice after treatment. The decrease was noted in all of the lead-exposed groups after exposure, but the changes did not follow any dose-dependent pattern. The loss of body growth might be due to malabsorption and difficulties in the metabolism of essential nutrients for health after toxicant exposure [21]. As like other toxic metalloids lead may induce loss of appetite and gastrointestinal disturbances leading to less intake of food [22]. Survey on established results reveals that inorganic lead selectively binds tissue proteins and disturbs the functions of those proteins (containing-SH group) resulting in growth retardation and body weight loss [23].

In the present study, there was no significant change in LSI in the first two doses of lead exposure (5 and 10 mg/kg body weight), but the considerable significant increase in LSI was observed in mice liver treated with 15 and 20 mg/kg body weight of lead. This indicates that at higher doses of lead exposure the organ weight in relation to body weight was significantly affected. This might be due to more accumulation of lead in body tissue at higher doses. The observed result of this experiment is in resemblance with the findings of Authman [24] which revealed that increased hepatic index due to lead exposure to fish might be correlated with the change in fat accumulation in the liver after lead treatment. It is further postulated that increased LSI may be due to a gathering of inflammatory cells in rat liver [25].

The present findings also reveal that the blood glucose level of lead-treated mice was decreased significantly in all the groups of animals in a dose-dependent manner indicating the marked hypoglycemic effect of lead. It may be ascribed to poor absorption of glucose in the gastrointestinal tract due to less intake of food by the lead stressed animals. Moreover, it is suggested that the decreased blood glucose level in lead-treated animals may be due to oxidative stress mediated depression of the mitochondria as a result of shifting from aerobic to anaerobic metabolism [7]. The observed result may also be correlated with the nephrotoxic manifestation of lead that could perturb the renal reabsorption of glucose leading to renal glycosuria [26]. This finding is supported by the earlier observation of Rastogi [27] who stated that glycosuria is one of the lead-induced nephrotoxic indicators in the human body.

It is further revealed that the reduced level of hepatic glucose, as found in the present experiment, is a dose-dependent effect of lead. It is suggested from the earlier report of Afsar [28] on fish model (*Anabas testudineus*) that the tissue glucose became diminished as it was utilized for the metabolic energy sources during toxic condition. Moreover, their experiments revealed that accumulation of both lead nitrate and acetate could damage the liver glucose associated with depletion of glycogen content. In the present study, the decreased hepatic glucose level following lead administration was also found to be accompanied by decreased liver glycogen content. It is postulated that toxic metals may be responsible for irregular secretion of pancreatic enzymes which may result in interruption of glucose absorption and consequently retardation of tissue glucose level [29].

Moreover, the present study thus informs that carbohydrates are the vital source of nutrients which split off to minimise the metabolic stress condition. Although the protein is the major source of energy in animals, stress or lack of oxygen causes depletion of stored carbohydrates [30]. It is well-known that glycogen is the reserve fuel that is used in different metabolic processes. In the present experiment, it has also been found that the hepatic glycogen content was decreased gradually from low dose to higher doses of lead exposure. Moreover, depletion of liver glycogen content is possibly due to lack of hormones which are involved in glycogen synthesis [31], or to an enhanced breakdown to replenish the glucose level in hepatic tissue. This may serve as an adaptive mechanism to maintain energy balance within the liver. The depletion of the glycogen content may be correlated with the lower supplementation of oxaloacetate as a substrate because it is utilized in gluconeogenic pathway.

It is further observed that the activity of G6PASE decreased significantly in lead-exposed mice liver in a dose-dependent manner. The suppression of this gluconeogenic enzyme in the lead exposed mice liver may be due to impairment of MDH enzyme activity reflecting a disturbance in between TCA cycle and gluconeogenic pathway. It is also assumed that the diminished activity of G6PASE may result from the less accumulation of glucose 6-phosphate and other glycolytic metabolites in hepatic tissue. Another possible mechanism may be like that insulin secreted during hypoglycaemia induced by lead exposure may have some inhibitory effect on G6PASE activity resulting in less release of glucose from the tissue to blood [32]. It is thus postulated that as the intermediary product oxaloacetate formed from malate is not sufficient and unable to supply glucose by G6PASE [33], the resulting hypoglycaemia occurs. It is further noted that the gradual alterations in blood glucose level following different doses of lead exposure are consistent with the dose-dependent reduction in G6PASE activity indicating a significant correlation between these parameters.

The pyruvic acid content in liver tissue was increased significantly after lead treatment. The acceleration of pyruvic acid content may be due to depletion of glucose level that further supplies energy through TCA cycle [34]. Pyruvate is the central metabolite to supply

acetyl-CoA or lactic acid by the pyruvate and lactate dehydrogenase in presence or absence of oxygen for TCA cycle [35]. The increase in pyruvic acid level may be ascribed to the conversion of hepatic glucose to pyruvic acid via glycolysis. On the other hand, the deposition of pyruvic acid in liver tissue may be due to less activity of the PDH which helps in conversion of pyruvic acid to acetyl CoA within liver mitochondria. This is supported by the present study where lead exposure at the present dose and duration causes suppressed the activity of PDH in the liver. LA induced inhibition of PDH activity was also observed in a rat model [36]. Inhibition of PDH activity may be due to the selective affinity of divalent cation as like lead towards the sulphydryl groups of the enzyme [23].

The present study further shows the suppressed activity of the MDH after lead exposure that fails to produce oxaloacetate. The obtained result is consistent with the report of Rizwana *et al.* [33] on observations made in a rat model. The suppressed activity of MDH may be due to the toxic effect of lead acetate at its synthetic level. Decreased activity of MDH may be associated with the lower supplementation of the substrate to proceed TCA cycle or may due to structural deformation [37]. As the proteins containing the thiols and other functional groups are more susceptible to metal action, lead toxicity may influence the structural modification of the enzymatic proteins. It is further noted that those metal ions are further preventing the formation of chaperone complex that is used to reform structures of denatured proteins [38].

The results further indicate that the FAN was increased in the lead-exposed mice liver in a dose-dependent manner. This acceleration of FAN may be due to supplementation of energy to the stressed tissue *via* aminotransferase enzyme that may contribute ketogenic amino acid to produce energy in the TCA cycle [39]. However, the supposedly increased transaminase activities are not observed in the present study, on the contrary, those enzyme activities were inhibited by lead exposure. This directs to postulate another mechanism that FAN may be mobilized from the peripheral tissues like kidney and muscle to the liver to provide more substrates for gluconeogenesis to replenish the loss of liver as well as blood glucose following lead exposure. The observed result may also be defined as induced FAN is simultaneously correlated with the degradation of liver glycogen to meet the energy demand by the stressed tissue and the present finding is also in resemblance with the observation of Naveed *et al.* [40].

It is also noted from the present study that suppression of tissue GPT and GOT activities follows dose-dependent alterations after lead exposure. This may be due to leakage of those transaminase enzymes from liver tissue to blood after the damage of the hepatocytes caused by lead. Pathogenic infection may also involve rushing of cytosolic or membrane-bound enzymes of mitochondria [41] and the cellular breakage or membrane disintegration may be the factor of enzyme suppression [42]. The obtained data is also furnished by Parmita and Mahanta [43]. Moreover, the decreased transaminase activities might also be guided on the basis of enzyme substrate inversely proportional correlation [44] because the current study also measured the high amount of FAN and pyruvate those may serve as substrate overload for transaminase enzymes to convert into oxaloacetate and alanine as the products.

CONCLUSION

From the overall observations, it is thus suggested that subacute exposure to lead significantly influences the glucose metabolism along with transamination reaction in mice model in a dose-dependent manner. Though the changes in body weight and LSI were significantly altered only at higher doses of lead exposure, a dose-dependent alteration in blood and liver glucose contents were found in the present study. To recover from this hypoglycemic effect, glycogenolytic activity was motivated and glycolytic activity was retarded to release the liver glucose to blood following enhanced energy demand by the body. Alteration in TCA cycle by lead was clearly indicated by suppressed activity of PDH and MDH, owing to less accumulation of liver glucose. To induce gluconeogenesis as an adaptive mechanism of hypoglycaemia and energy deprivation in the liver, FAN was accumulated in the liver. However, suppressed transaminase activities might be correlated with leakage of those enzymes from the hepatocyte as a consequence of toxic manifestation. This metabolic perturbation in liver was supposed to be involved in the alteration of energy supply to the different tissues of the body in response to lead exposure.

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CONFLICT OF INTERESTS

All the authors of this manuscript declare that they have gone through the details of the manuscript in the present form and there is no conflict of interest to publish this research paper in this journal.

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