Abstract

Lead (Pb) is a widespread toxic metal found in the environment. Lead affects multiple organs in the body and results in alteration of various serum parameters. The present study was designed to examine the effect of lead on serum markers and further examine the protective effect of nutrient metal mixture of calcium, iron and zinc. Pups of wistar rats were lactationally exposed to 0.2% lead acetate through drinking water of mother from postnatal day (PND) 1 to PND 21. Another group of pups received 0.2% Pb-acetate together with a mixture of calcium, iron and zinc (0.02%) whereas control animals received only deionized water without Pb. Various serum markers like glucose, urea, creatinine, bilirubin, total cholesterol, alkaline phosphatase (ALP), serum glutamic pyruvate transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) levels were estimated in control and experimental rats of PND 28, PND 45, 4 months and 12 months aged rats. These serum markers were significantly altered in Pb - exposed rats in all age groups when compared to control rats. The nutrient metal mixture of calcium, iron and zinc significantly reversed the Pb induced changes in serum markers indicating protection against Pb - induced toxicity.

Keywords: Lead, Nutrient metal mixture, Serum markers.

Introduction

Human exposure to heavy metal lead (Pb) is a global health problem because of its widespread use in industrial and commercial products. Pb is one of the oldest known and most widely studied occupational and environmental toxins (1, 2), usually found in Pb acid batteries, pipes, paints, gasoline additives, ceramic glazers, glass and crystals, cosmetics etc.

Unlike most chemicals for which health impacts of low level doses are still uncertain, exposure to Pb, even at very low levels (10 µg/dl), is highly toxic (3). Pb has a more severe effect on the cardiovascular and haematological systems especially in Fe deficient people. Because the combination of Fe deficiency and Pb exposure cause more severe effects on the blood forming system, women and children tend to show more severe effects (4). Lead impairs the synthesis of a substance called “heme” which is extremely important to human life because it carries oxygen to tissues of the body. In chronic exposure, Pb induces anemia by both interfering with heme biosynthesis and by diminishing red blood cell survival. Pb inhibits the body’s ability to make hemoglobin by interfering with several enzymatic steps in the heme pathway. Specifically, Pb decreases heme biosynthesis by inhibiting δ-aminolevulinic acid dehydratase (ALAD) and ferrochelatase activity (5, 6). Blood parameters are probably the more rapid and detectable variations under stress and are useful in assessing the health conditions. Besides blood indices, serum parameters are also broadly affected by Pb exposure. Pb alters the serum urea and creatinine levels (7, 8) indicating renal
failure. Pb induces hypercholesterolemia and alters the liver marker enzymes such as ALT, AST and ALP (9-11).

Nutrient metals like calcium, iron and zinc play an important role in reducing Pb toxicity. Dietary Ca deficiency increases the absorption of Pb by the gastrointestinal tract and increases Pb concentrations in critical organs of the body (12, 13). Nutritional Fe deficiency also enhances Pb absorption and promotes Pb toxicity more prominently in young children and women (14). A negative relationship between dietary Fe intake and blood Pb levels was also found in a study of preschool children (15). Studies show that as dietary Zn increases, Pb absorption and its subsequent toxicity decrease, indicating that Zn exerts its effect on Pb in the gastrointestinal tract (16, 17). Interactions between Zn and Pb are possible beyond the level of the gastrointestinal tract, as Zn involves the inhibition of heme synthesis by Pb (14, 18). Since the nutrient metals play an important role in the treatment of Pb poisoning, the present study was therefore aimed at investigating the potential of Ca, Fe and Zn nutrient metal mixture in countering age dependent Pb induced toxicity in the haematological system of rats.

Material and Methods

Maintenance of experimental animals: Adult albino rats (Wistar) were obtained from Sri Venkateswara Traders, Bangalore, and from these animals a colony of rats were developed and maintained in rat cages under normal laboratory conditions (at constant temperature 28±2°C and relative humidity 60±10% with a 12 hour light/dark cycle) throughout the course of the present study. The animals were fed on rat feed supplied by Sri Venkateswara Traders, Bangalore and water was provided ad libitum.

Chemical exposure: Young rats from PND1 were lactationally exposed to 0.2% Pb by adding Pb-acetate to deionized drinking water of the mother. Pb-exposure dose was obtained from published work (19). All pups, twenty four hours after birth (PND1) were pooled and new litters consisting of eight males were randomly selected and placed with each dam. Pb- exposure was continued up to PND21 and stopped at weaning. Calcium (Ca2+), Zinc (Zn2+) and Iron (Fe2+) in a mixture was supplemented as 0.02% in 0.2% Pb-water given to another group of pups through mother’s milk up to PND 21 and stopped at weaning. Control animals received only deionized water without Pb.

Collection of blood and serum and estimation of biochemical parameters: The control and experimental animals at the end of PND 28, PND 45, 4 months and 12 months after the treatment were sacrificed by cervical decapitation and the blood samples were collected. Blood was allowed to stand for 30 min at room temperature and then centrifuged using refrigerated centrifuge to separate the serum. Serum was stored at -40°C, and used for the analysis of various parameters like glucose, urea, bilirubin, creatinine, total cholesterol, serum alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT). Serum parameters were estimated by using the kits purchased from Biosystems, A15 hematology Analyzer (20). Results were expressed as mg/dl for serum glucose, urea, creatinine, bilirubin, cholesterol and as IU/L for serum ALP, SGOT, SGPT.

Statistical analysis: The data obtained from six separate samples were expressed as mean ± SD. Data was analyzed by two-way Analysis of Variance (ANOVA) following Standard Statistical Software Package to compare the effects among various groups. The 0.05 level of probability was used as the criterion for significance.

Results

Serum glucose levels of rats of different age groups were shown in figure1. From the results obtained in our studies, it is clear that Pb-exposure showed a significant decrease in the glucose levels of PND 28, PND 45, 4 months and 12 months aged rats (Fig 1). The decrease in glucose content of Pb exposed rats was 13.63% in 28
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days rats, 9.34% in 45 days rats, 24.36% in 4 months rats and 11.2% in 12 months rats. Maximum decrease (24.36%) was observed at 4 months of postnatal development. The decrease in glucose content was most significant at 4 months age and not significant in remaining age groups of rats (P<0.05). Ca$^{2+}$+Fe$^{2+}$+Zn$^{2+}$ supplementation reversed the effects of Pb on glucose levels of experimental animals at different time periods (Fig. 1). Maximum effect of nutrients was observed at 4 months PND (Fig. 1).

The serum urea levels of control, Pb exposed and nutrient supplemented rats were shown in figure 2. Serum urea levels increased progressively from PND 28 days to 12 months old rats (Fig. 2). The Pb-exposure showed a significant increase in the urea level of rat’s different age groups. The increase in urea levels of Pb exposed rats decreased progressively from PND 28 days to 12 months aged rats. The increase in urea of Pb exposed rats was 45.7% in 28 days rats, 43.24% in 45 days rats, 17.9% in 4 months rats and 12.8% in 12 months aged rats. Maximum increase in urea levels was observed in 28 days young rats (45.7%) than other age groups (Fig. 2). The increase in serum urea content was significant at all the age points of rats except in 12 months age rats. The administration of nutrient mixture (Ca$^{2+}$+Fe$^{2+}$+Zn$^{2+}$ ) produced reversal effect on Pb induced increase in urea levels which was maximum at 4 months rats than other age groups (Fig. 4).

Creatinine levels were estimated in serum of control, Pb exposed and nutrient supplemented rats of PND 28, 45, 4 months and 12 months aged rats (Fig. 3). The creatinine levels in control rats decreased from 28 days to 4 months age and increased in 12 months aged rats (Fig. 3). The Pb-exposure showed a significant increase in the creatinine levels of different age groups of rats. The increase in creatinine levels of Pb exposed rats from control was 60% in PND 28, 31.25% in PND 45, 26.98% in 4 months and 20% in 12 months age. Maximum increase in creatinine levels was observed in 28 days young rats (60%) than other age groups (Fig. 3). The increase in creatinine levels was significant in 28 days and 4 months age rats. Administration of mixture of Ca$^{2+}$+Fe$^{2+}$+Zn$^{2+}$ produced recovery from Pb induced increase in creatinine levels. The maximum reversal effect was observed in PND 28 rats than other age groups (Fig. 3).

Serum bilirubin levels of control, Pb exposed and nutrient supplemented rats of different age groups were shown in figure 4. Serum bilirubin levels of control rats were increased from 28 to 45 days and decreased in 4 months and 12 months aged animals (Fig. 4). The Pb-exposure showed a significant increase in the bilirubin levels of rats of different age groups. The increase in bilirubin level was 91.6%, 55.5%, 132.3% and 25% respectively in PND 28, 45, 4 months and 12 months age. The increase was greater in 4 months aged rats (132.3%) than other age groups of rats. The increase in bilirubin levels was significant in all age groups except in 12 months aged rats. Administration of nutrient mixture (Ca$^{2+}$+Fe$^{2+}$+Zn$^{2+}$) produced reversal effect on Pb induced increase in bilirubin levels which was maximum at 4 months rats than other age groups (Fig. 4).

The cholesterol level was estimated in serum of control, Pb exposed and nutrient supplemented rats of PND 28, 45, 4 months and 12 months aged rats (Fig. 5). The cholesterol level gradually decreased in the serum of control rats from 28 days to 4 months age and again increased in 12 months aged rats (Fig. 5). The cholesterol levels were significantly altered in Pb-exposed developing, young, adult and aged rats. The increase in serum total cholesterol in Pb exposed rats was 38.29%, 105%, 44.11% and 28.8% respectively in PND 28, 45, 4 months and 12 months aged rats. Maximum increase was observed at PND 45 days young rats. The increase in cholesterol levels was significant in all age groups except in 12 months age rats (Fig. 5). The administration of Ca$^{2+}$+Fe$^{2+}$+Zn$^{2+}$ supplements produced recovery from the Pb induced increase in cholesterol levels (Fig 5).
Fig. 1. Effects of Pb exposure and nutrient metals supplement to Pb on serum glucose levels. Pups were exposed to deionized water (control), or Pb acetate (0.2 %), calcium, zinc and iron combination together with Pb (0.02 % in 0.2 % Pb water) from PND 1 to PND 21. Serum glucose levels were estimated in control, Pb exposed and supplemented rats of different age groups (PND 28, 45, 4 and 12 months). All the values are mean ± SD of six individual observations. The 0.05 level of probability was used as the criterion for significance.*p<0.05, **p< 0.01, ***p< 0.001.

Fig. 2. Effects of Pb exposure and nutrient metals supplement to Pb on serum urea levels. Pups were exposed to deionized water (control), or Pbacetate (0.2 %), calcium, zinc and iron combination together with Pb (0.02 % in 0.2 % Pb water) from PND 1 to PND 21. Serum urea levels were estimated in control, Pb exposed, supplemented rats of different age groups (PND 28, 45, 4 and 12 months). All the values are mean ± SD of six individual observations. The 0.05 level of probability was used as the criterion for significance.*p<0.05, **p< 0.01, ***p< 0.001.

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Fig. 3. Effects of Pb exposure and nutrient metals supplement to Pb on serum creatinine levels. Pups were exposed to deionized water (control), or Pbacetate (0.2 %), calcium, zinc and iron combination together with Pb (0.02 % in 0.2 % Pb water) from PND 1 to PND 21. Serum creatinine levels were estimated in control, Pb exposed, supplemented rats of different age groups (PND 28, 45, 4 and 12 months). All the values are mean ± SD of six individual observations. The 0.05 level of probability was used as the criterion for significance. *p<0.05, **p< 0.01, ***p< 0.001.

Fig. 4. Effects of Pb exposure and nutrient metals supplement to Pb on serum bilirubin levels. Pups were exposed to deionized water (control), or Pb acetate (0.2 %), calcium, zinc and iron combination together with Pb (0.02 % in 0.2 % Pb water) from PND 1 to PND 21. Serum bilirubin levels were estimated in control, Pb exposed, supplemented rats of different age groups (PND 28, 45, 4 and 12 months). Values are mean ± SD of six separate experiments. The 0.05 level of probability was used as the criterion for significance. *p<0.05, **p< 0.01, ***p< 0.001.

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Fig. 5. Effects of Pb exposure and nutrient metals supplement to Pb on serum total cholesterol levels. Pups were exposed to deionized water (control), or Pbacetate (0.2 %), calcium, zinc and iron combination together with Pb (0.02 % in 0.2 % Pb water) from PND 1 to PND 21. Serum total cholesterol levels were estimated in control, Pb exposed, supplemented rats of different age groups (PND 28, 45, 4 and 12 months). Values are mean ± SD of six separate experiments. The 0.05 level of probability was used as the criterion for significance.*p<0.05, **p< 0.01, ***p< 0.001.

Fig. 6. Effects of Pb exposure and nutrient metals supplement to Pb on serum ALP activities. Pups were exposed to deionized water (control), or Pbacetate (0.2 %), calcium, zinc and iron combination together with Pb (0.02 % in 0.2 % Pb water) from PND 1 to PND 21. Serum ALP activities were estimated in control, Pb exposed, supplemented rats of different age groups (PND 28, 45, 4 and 12 months). Values are mean ± SD of six separate experiments. The 0.05 level of probability was used as the criterion for significance.*p<0.05, **p< 0.01, ***p< 0.001.
Activity of the serum alkaline phosphatase was estimated in the serum of control, Pb exposed and Ca\(^{2+}\)+Fe\(^{2+}\)+Zn\(^{2+}\)supplemented rats at different time periods such as PND 28, PND 45, 4months and 12 months are presented in Fig 6. The activity of ALP of control rats was decreased from 28 days to 4 months and increased in 12 months aged control rats (Fig 6). Pb exposure however significantly increased the activity of ALP in all age groups of rats. The increase in ALP activity was 28.86%, 32.95%, 43.75% and 36.73% respectively in PND 28, 45, 4 months and 12 months aged rats. The increase in ALP activity due to Pb exposure was greater in 4 months rats than other age groups (Fig 6) and significant in all the age groups except in 12 months rats. The administration of Ca\(^{2+}\)+Fe\(^{2+}\)+Zn\(^{2+}\)supplement however, produced significant reversal from the Pb induced increase in ALP activity (Fig 6).

Activities of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) were estimated in serum of control, Pb exposed and Ca\(^{2+}\)+Fe\(^{2+}\)+Zn\(^{2+}\)supplemented rats at different time periods (PND 28, 45, 4 months and 12 months) (Fig 7 & 8). Exposure to Pb resulted in an increase in both the SGPT and SGOT activities in different age groups of rats. Pb exposure resulted in an increase of 68.42% in PND 28, 75% in PND 45, 69% in 4 months, 38.63% in 12 months aged animals and an increase of 81% in PND 28, 102.9% in PND 45, 66.6% in 4 months, 42.1% in 12 months aged animals for SGPT and SGOT activities respectively (Fig 7 & 8). Increases in the activities of SGPT and SGOT were significant in all age groups. The supplementation of Ca\(^{2+}\)+Fe\(^{2+}\)+Zn\(^{2+}\)reversed the effect caused by Pb exposure in SGPT and SGOT activities (Fig 7 & 8).

Discussion

In the present study, age related changes in haematological parameters were observed following exposure of rats to Pb. The haematological system is one of the important target systems in Pb induced toxicity. Pb interferes with heme synthetic pathway at various steps and cause haematological disturbances. Besides haematological parameters like blood cell count, Hb content various serum parameters were also affected due to lead exposure. In the present study, serum glucose level was decreased following Pb exposure in all age groups of rats. The reduction in serum glucose concentration following the administration of Pb observed in the present study may be due to inhibition of the uptake and transport of glucose by Pb (21). Pb appears to exert a direct or indirect effect on serum glucose of experimental rats and results in abnormal glucose metabolism. Many environmental and occupational agents including Pb have been shown to cause detrimental effects on endocrine function related to glucose metabolism (22). The observed decrease of glucose concentration in serum of treated rats may also be due to altered endocrine function and stimulating the the glycogenesis and ultimately leading to decrease of glucose level in the serum.

In the present study, serum urea levels increased in Pb exposed animals when compared to the control animals of different age groups. Urea serves an important role in the metabolism of nitrogen-containing compounds by animals and is the main nitrogen-containing substance in the urine of mammals. Serum urea has been reported to increase in acute and chronic Pb exposure (7, 23-25). Urea is the principal end product of protein catabolism. Enhanced protein catabolism together with accelerated amino acid deamination for gluconeogenesis is probably a contributing factor for the elevated levels of urea. On the other hand, the elevated serum urea levels may also be due to the destruction of RBC’s.

The result of the present study also showed significant increase in serum creatinine levels in Pb exposed rats indicating renal deficiency. Creatinine is formed in muscle and is chiefly filtered out of the blood by the kidneys. Increased serum creatinine levels following Pb exposure was also reported earlier (7, 26) which supports
**Fig. 7.** Effects of Pb exposure and nutrient metals supplement to Pb on SGPT activity. Pups were exposed to deionized water (control), or Pb acetate (0.2 %), calcium, zinc and iron combination together with Pb (0.02 % in 0.2 % Pb water) from PND 1 to PND 21. SGPT activities were estimated in control, Pb exposed, supplemented rats of different age groups (PND 28, 45, 4 and 12 months). Values are mean ± SD of six separate experiments. The 0.05 level of probability was used as the criterion for significance.*p<0.05, **p< 0.01, ***p< 0.001.

**Fig. 8.** Effects of Pb exposure and nutrient metals supplement to Pb on SGOT activity. Pups were exposed to deionized water (control), or Pb acetate (0.2 %), calcium, zinc and iron combination together with Pb (0.02 % in 0.2 % Pb water) from PND 1 to PND 21. SGOT activities were estimated in control, Pb exposed, supplemented rats of different age groups (PND 28, 45, 4 and 12 months). Values are mean ± SD of six separate experiments. The 0.05 level of probability was used as the criterion for significance.*p<0.05, **p< 0.01, ***p< 0.001.
the findings of our present study. The observed elevation in the serum creatinine levels in Pb exposed rats suggests renal function impairment which might result from intrinsic renal lesions, decreased perfusion of the kidney obstruction of lower urinary tract or due to deranged metabolic process caused by this metal. In the present study, serum bilirubin levels increased in Pb exposed animals when compared to the control animals of different age groups. Increased bilirubin level was also observed in automobile workers (27) and Pb exposed rats (28) supporting the findings of our study. Bilirubin is the yellow breakdown product of normal heme catabolism. Increase in serum total bilirubin level in the Pb exposed rats may be due to greater haemolysis of red blood cells.

Although cholesterol is important and necessary for mammals, high levels of cholesterol in the blood can damage arteries and are potentially linked to diseases such as those associated with the cardiovascular system. Pb induced hypercholesterolemia, observed in this study could be due to increased hepatic cholesterogenesis accompanied with a decrease in triglyceride and phospholipid contents in the liver and consequently causing an increase in the blood (29). This relationship between Pb exposure and plasma cholesterol suggests an altered lipid metabolism. Increase in serum cholesterol levels was also reported earlier in Pb exposed rats (30-33). The increase in the total cholesterol of Pb exposed rats indicate that the rats were under stress and that cholesterol may be utilized for the repair of the damaged cell membrane due to the toxic impact.

In the present study, administering Pb acetate to rats resulted in a significant increase of blood alkaline phosphatase in different age groups. Alkaline phosphatase is responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. Liver enzyme ALP is a marker enzyme for liver function and integrity (34, 11). Serum ALP level usually increase in acute hepatotoxicity or mild hepatocellular injury (34, 35). In this study, administration of Pb showed significant increase in plasma ALP activity. Similar increase in serum ALP activity was also observed earlier in Pb exposed rats (28, 31, 32, 36-39) Increase in the level of serum ALP due to Pb exposure may be due to the loss of this enzyme and diffusion through cell membrane. The present data suggest that Pb exerts possible hepatotoxic effect as the increase in ALP is indicative of liver damage.

Measuring the concentrations of transaminases in the blood is important in the diagnosing and tracking many diseases. An increased serum marker (SGPT and SGOT) following Pb exposure to rats was observed previously (28, 31, 36, 38) supporting the present findings of our study. Pb may accumulate in liver and exert its toxic effect via pro oxidative damage to hepatic cell membranes causing transaminases to liberate into the serum. The increases in activities of SGOT and SGPT observed in Pb exposed rats might be due to increased cell membrane permeability or cell membrane damage of hepatocytes caused by Pb acetate. The elevation of serum SGOT and SGPT may be also due to the possible release of these enzymes from the cytoplasm, into the blood circulation rapidly after rupture of the plasma membrane and cellular damage.

Supplementation of rats with nutrient metals calcium, iron and zinc reversed the effect of Pb on serum parameters. Ca is one of the most important elements in the diet because it is a structural component of bones, teeth, and soft tissues and is essential in many of the body’s metabolic processes. Fe is one of the important nutrients as it is necessary for oxygen transport in the blood and is important for the normal functioning of all the cells in the body. Zn is a biological trace element and component of many enzymes and in many cells and is bound to a storage protein, metallothionein. Supplementation with calcium, iron and zinc decreases Pb gastrointestinal absorption and decreases tissue accumulation (40). Our earlier studies indicated reversal of the Pb induced neurochemical alterations with calcium or zinc supplements (17).
Extensive clinical and experimental evidence supports the significance of Pb-Ca\(^{2+}\) interactions (41). Ca\(^{2+}\) is a divalent cation just like Pb. The same transport mechanism is operative for absorption of Pb and Ca\(^{2+}\) from the gastrointestinal tract resulting competitive interaction between Pb and Ca\(^{2+}\) (42). Absorption of Pb by gastrointestinal tract is inversely related to the amount of Ca\(^{2+}\) present (43, 44). Ca\(^{2+}\) and Pb compete for similar binding sites on intestinal mucosal proteins, which are important in the absorptive process (45). The supplemented Ca, Fe and Zn may compete for similar binding sites as that of Pb on gastrointestinal tract. These nutrient metals offered protection against the alterations caused by Pb in the different age groups of rats. Increased Fe intake has also been suggested as one part of a multi-tiered approach to lessening Pb- toxicity (46). The mechanism by which Fe deficiency potentiates Pb toxicity is not clear, but there are several metabolic pathways in which Fe/Pb interaction is occur. The effects of Pb and Fe on the heme biosynthetic pathways have been extensively investigated and characterized. Pb inhibits two major enzymes of the heme biosynthetic pathway: δ-aminolevulinic acid dehydratase (ALAD) and ferrochelatase. It has also been suggested that these two metals might compete directly for specific erythrocyte binding sites (47). Interactions between Zn\(^{2+}\) and Pb have been investigated at absorptive and enzymatic sites (48). Zn\(^{2+}\) and Pb compete for similar binding sites on the metallothionein like transport protein in the gastrointestinal tract (49). In addition to forming strong bonds with SH and other groups like OH, NH\(_2\) and Cl in amino acids which interfere with basic enzymatic processes, toxic metals exert part of their effects by replacing essential metals such as Zn\(^{2+}\) at their sites in enzymes (50). Potential mechanisms of interaction between Pb and Zn\(^{2+}\) include an inhibition of Pb gastrointestinal absorption by Zn\(^{2+}\), which has been demonstrated in rats (51). Thus supplementation with Ca\(^{2+}\)+Fe\(^{2+}\)+Zn\(^{2+}\) reduced the inhibitory effect of Pb on the activity of blood parameters and reversed the enzyme activity nearer to control level.

The effect of Pb in haematological system is influenced directly by the dose, duration of Pb exposure and by age. The results of the present study showed that the exposure of Pb in early life can continue to influence the serum parameters even in later life suggesting the storage of Pb in critical organs of the body like bones for a long time and released oftenly into the blood stream. The supplementation of nutrient metal mixture offered protection against the Pb induced alterations in serum parameters by reducing the absorption of Pb and by interacting with Pb at various sites of the body. Thus the present data suggest that the early life exposure to Pb can continue to affect the serum markers and nutrient metal supplements provide protection against Pb induced toxicity.

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References


