Summary and Conclusions
Pb is a non-essential heavy metal widely distributed in the environment. Chronic exposure to low levels of this metal is one of the problems of public health, due to its toxicity. Pb poisoning may affect numerous organ systems and is associated with a number of morphological, biochemical, and physiological changes, including kidney dysfunction, abnormal glucose metabolism, nervous system disturbances, impairment of liver function and hematological disorders (Heiskel et al., 1961; Koj 1974; Patel et al., 1989). Pb induces a broad range of physiological and biochemical alterations (Goyer, 1996; Ruff et al., 1996; Sivaprasad et al., 2004), in the central and peripheral nervous systems (Bressler et al., 1999) hematopoietic system (De Silva, 1981), cardiovascular system (Khalel-Manesh et al., 1993), kidneys (Humphreys, 1991), liver (Sharama and Street, 1980) and reproductive system (Lankranjan et al., 1975; Rom, 1980; Hsu et al., 2002) in laboratory animals and humans.

Recent studies have reported that low-level Pb exposure has a graded association with several disease outcomes such as hypertension, peripheral artery disease, kidney disease, neurodegenerative disease, and cognitive impairment (Muntener et al., 2003; Nash et al., 2003; Navas-Acien et al., 2004; Ekong et al., 2006; Menke et al., 2006). Pb potentially induces oxidative stress and evidence is accumulating to support the role of oxidative stress in the pathophysiology of Pb toxicity (Neal et al., 2000; Ehdaie et al., 2005). Pb exposure is known to induce oxidative stress leading to tissue damage (Flora et al., 2004), and lipid peroxidation by inhibiting the synthesis of some antioxidant enzymes (Kang et al., 2004).

The hematopoietic system is one of the target organs in Pb toxicity. Pb poisoning affects hematopoiesis and heme synthesis in experimental animals and humans (Doull et al., 1980). In adults, Pb exposure has been related to increased blood pressure and hypertension, conditions known to increase the risk of cardiovascular disease. Evidence suggests that Pb exposure is associated with high blood pressure, and studies have also found connections between Pb exposure and coronary heart disease, heart rate variability, and death from stroke,
but this evidence is more limited (Navasacien 2007). About 99% of the Pb present in the blood is bound to erythrocytes. Since they have a high affinity for Pb, the majority of the Pb found in the blood stream makes them more vulnerable to oxidative damage than many other cells. Moreover, erythrocytes can spread Pb to different organs of the body (Sivaprasad et al., 2003). Pb can cause damage in the erythrocytes, preventing them from carrying oxygen that increases the risk of heart attack (Vaziri et al., 1999).

Pb is known to inhibit many enzyme activities and it may interfere with the synthesis of protein or RNA or both (Nabry et al., 1972). The enzymes in the biosynthetic pathway of heme in which the effects of Pb are of the clinical interest are δ-aminolevulinic acid synthetase (δ-ALAS), δ-aminolevulinic acid dehydratase (δ-ALAD), and ferrochelatase (Jacob et al., 2000). Oxidative stress appears to be a possible mode of the molecular mechanism of Pb toxicity (Adonaylo et al., 1999; Bechara, 2004). Oxidative stress occurs when generation of free radicals exceed the capacity of antioxidant defense mechanisms. The depletion of glutathione and protein bound sulfhydryl groups and the changes in the activity of various antioxidant enzymes indicative of lipid peroxidation have been implicated in Pb induced oxidative tissue damage (Gurer et al., 1998; Neal et al., 2000). Pb seems to be quite capable of causing oxidative damage to heart, liver, kidney, reproductive organs, brain, and erythrocytes (Hsu et al., 1998; Ding et al., 2001; Patra et al., 2001; Tandon et al., 2001; Ahamed et al., 2005). Pb is known to have toxic effects on membrane structure and functions (Donaldson et al., 1993).

Albino rats (wistar) were obtained from Sri Venkateswara traders Bangalore and were acclimatized for at least 1 week to the laboratory conditions before used for experiments. Pups were lactationally exposed to 0.2% Pb by adding Pb-acetate to deionized drinking water of the mother. 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. Control animals received only
deionized water without Pb. To one batch of Pb exposed rats, Calcium (Ca$^{2+}$), Iron (Fe$^{2+}$) and Zinc (Zn$^{2+}$) was supplemented as 0.02% in 0.2% Pb-water and is given to a separate batch of mothers up to PND 21 and stopped at weaning.

The control and experimental animals at the end of 28 days, 45 days, 4 months and 12 months of the treatment were sacrificed and the heart tissues were quickly isolated under ice cold conditions. The tissues were stored in deep freezer at -80°C and used for biochemical analysis. Blood samples (3 to 5 ml) were taken from the rats at the end of dosage period. The serum was separated using refrigerated centrifuge and stored at -40°C. The samples were thawed and mixed carefully prior to the estimation of the biochemical parameters. Haematological parameters were determined in total blood.

In the present study, haematological alterations were estimated during different time periods in control, Pb treated and Pb+nutrient supplemented rats. Pb-exposure produced a decrease in RBC count, Hb content and PCV concentrations in young and aged animals. The administration of a mixture of Ca$^{2+}$, Fe$^{2+}$ and Zn$^{2+}$ produced recovery from the Pb induced decreases in RBC, Hb and PCV levels. Haematological system is one of the target systems of Pb toxicity.

Pb exerts its adverse effect on erythropoietic tissue, suppresses bone marrow haematopoiesis, probably through its interaction with the enteric Fe absorption and by causing cytotoxic damage to bone marrow cells leading to disturbances in cell differentiation during haematopoiesis. Therefore, the RBC count was decreased following Pb-exposure. Anemia observed during Pb-exposure might result from the inhibition of heme synthesis and a decrease in the lifespan of the erythrocytes. Pb inhibits ALAD, a cytosolic sulfhydryl enzyme, and also decreases ferrochelatase activity at the last step of heme synthetic pathway. The shortened life span of RBC is probably the consequence of increased mechanical fragility of the cell membrane. Furthermore, decreased
haematocrit and haemoglobin levels might arise from reduction in serum copper as well as reduced Fe metabolism induced by Pb.

Unlike the alterations observed in RBC, Hb and PCV, the WBC of Pb-exposed rats showed an increase with postnatal age. The Pb induced increase in WBC may be due to animal’s increased defense mechanism against toxicity caused by Pb. In the present study, the administration of Fe, Ca and Zn nutrient metal mixture reduced the Pb induced alterations in RBC levels, Hb content, PCV and WBC levels.

Present study showed decrease in glucose levels in Pb exposed rats when compared to control rats. The administration of nutrient metal mixture Fe, Ca and Zn significantly reversed increase in glucose levels, and the levels reached to near control levels. The reduction in serum glucose concentration following the administration of Pb may be due to inhibition of the uptake and transport of glucose by Pb (Fowler et al., 1980). The observed decrease of glucose concentration in serum of treated rats may be due to decreasing of thyroid hormones that have antagonist relationship with serum insulin hormone. The decrease of thyroid hormone led to increase of insulin hormone that may be responsible in stimulating the glycogenesis and ultimately leading to decrease of glucose level in the serum. Supplementation of Ca$^{2+}$+Fe$^{2+}$+Zn$^{2+}$ mixture reversed the effect of Pb on glucose metabolism. These nutrient metals offered protection against the alterations caused by Pb in the supplemented group of rats.

In the present study, serum urea level was increased in Pb exposed rats. Urea is the principal end product of protein catabolism. Enhanced protein catabolism together with accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpret the elevated levels of urea. In the present study, Ca$^{2+}$+Fe$^{2+}$+Zn$^{2+}$ nutrient metal mixture reversed the alterations in urea levels caused by Pb. These nutrient metals protected the rats from the adverse effects of Pb.
In the present study, serum bilirubin level was increased in Pb exposed rats. The results showed that bilirubin levels among Pb exposed rats were high as compared to control group. The increase in bilirubin may be due to more haemolysis of red blood cells. \( \text{Ca}^{2+}\text{Fe}^{2+}\text{Zn}^{2+} \) nutrient metal mixture significantly reversed the alterations in urea levels caused by Pb.

Serum creatinine level was increased in Pb exposed rats. Increase in creatinine concentration in Pb induced rats indicates impaired kidney function and considered as functional evidence of Pb induced nephrotoxicity (Qu et al., 2002; Goswami et al., 2005). \( \text{Ca}^{2+}\text{Fe}^{2+}\text{Zn}^{2+} \) nutrient metal mixture partially reversed the alterations in creatinine levels caused by Pb.

Present study showed increase in cholesterol levels in Pb-exposed rats. The increase in the total cholesterol of Pb exposed rats indicates 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. It may also be due to the decrease in cholesterol catabolism due to liver dysfunction or inhibition of lipoprotein lipase activity which would decrease the removal of lipoprotein from plasma (Hariprasad Reddy, 2001). Administration of \( \text{Ca}^{2+}\text{Fe}^{2+}\text{Zn}^{2+} \) nutrient metal mixture greatly reversed the alterations in cholesterol levels caused by Pb.

Present study showed increase in SGOT and SGPT levels in Pb-exposed rats. Increased levels of serum and liver GOT are an indication of mitochondrial damage and decreased state of respiration (Goyer and Krall, 1969) and also an indication of injury of hepatic tissue (Zayed et al., 2003). Increase in levels of SGPT and SGOT in Pb-exposed rats may be due to leakage of these enzymes from damaged liver cells suggesting the toxic effects of Pb on liver. Administration of \( \text{Ca}^{2+}\text{Fe}^{2+}\text{Zn}^{2+} \) nutrient metal mixture reversed the alterations in the activity levels of serum transaminases caused by Pb. These nutrient metals offered protection against the Pb by competing with the Pb absorption from gastrointestinal tract.
ALP is an ecto-enzyme of hepatocyte plasma membrane. The observed increase in serum ALP of Pb exposed rats reflects the pathological alteration in biliary flow and damage to the liver cell membrane (Giuliani et al., 1983, Dunsford et al., 1989). Addition of Ca\(^2+\)+Fe\(^2+\)+Zn\(^2+\) nutrient metal mixture to Pb partially reversed the alterations in ALP levels caused by Pb suggesting protective effects of these metals.

A gradual decrease was observed in the activity of serum AChE and BuChE following Pb administration. Pb accumulates in the body and found in considerable concentrations in blood. Pb in blood could interact with AChE or BuChE and cause decreased activity of these enzymes. Pb has high affinity for the sulphydryl groups in enzymes and proteins and its binding can alter their function (Chetty et al., 1990; Bagchi et al., 1997). This may also be one of the reasons for decreased AChE activity observed in Pb-exposed rats. Supplementation with Ca\(^2+\)+Fe\(^2+\)+Zn\(^2+\) reversed the Pb-induced inhibition in AChE and BuChE activities in blood. The reversal of inhibition in the activity of AChE by supplementation with Ca\(^2+\)+Fe\(^2+\)+Zn\(^2+\) may be due to competition of these metals for similar binding sites and reducing the availability of binding sites for Pb.

Present study showed increase in blood LDH and G-6-PDH activities in Pb-exposed rats. Elevation in the activity of LDH indicates diminished TCA cycle activities. In addition, the LDH activity increases during conditions favoring anaerobic respiration to meet energy demands, when aerobic condition is decreased (Murray et al., 1995). Thus there appears to be a shift in the carbohydrate metabolism from aerobic to anaerobic oxidative condition during Pb induced stress in albino rats. The increase in the activity of G-6-PDH due to Pb intoxication might indicate an increased demand to generate reducing power in the form of NADPH under the oxidative stress induced by Pb. Supplementation with Ca\(^2+\)+Fe\(^2+\)+Zn\(^2+\) reversed the Pb-induced inhibition in LDH and G-6-PDH activities in blood. The reversal of inhibition in the activity of these enzymes by supplementation with Ca\(^2+\)+Fe\(^2+\)+Zn\(^2+\) may be due to competition of these metals for similar binding sites and reducing the availability of binding sites for Pb.
In the present study, activity of heart mitochondrial SDH, ICDH and aconitase enzymes decreased in Pb exposed rats when compared to the control rats. SDH possesses SH groups on which the enzymatic activity depends and Pb is known to interfere with these groups (Allen 1995). SDH participates in the aerobic oxidation of carbohydrates in the citric acid cycle and is bound to the inner mitochondrial membrane. The reduction of SDH activity due to Pb intoxication indicates impaired mitochondrial functions and reduction in carbohydrate oxidation. The decrease in heart ICDH in Pb exposed rats denotes the decrement in NAD+. The reduction in the heart ICDH activity might also be due to the direct interaction of Pb with mitochondria. Aconitase requires Fe as a co-factor in its active center to catalyze reactions involved in mitochondrial energy. The proposed interaction between Pb and Fe in biological systems is presumably due to similarities of both metals in their coordination chemistry. As a complete [4Fe-4S] cluster in aconitase is necessary for the enzyme to bind citrate, the replacement of Pb for the fourth labile Fe in the cubane structure would inhibit the enzyme’s catalytic function (Beinert et al., 1993; Klausner et al., 1993). Supplementation of Ca^{2+}+Fe^{2+}+Zn^{2+} nutrient metal mixture reversed the Pb-induced inhibition of heart mitochondrial aconitase, SDH and ICDH activities. The reversal of inhibition in the activity of these enzymes activity may be due to competition of these metals for similar binding sites and reducing the availability of binding sites for Pb.

The results of the present study show a significant increase in the levels of MDA on Pb administration. Malondialdehyde is a major product of lipid peroxidation. During oxidative stress, MDA and/or other aldehydes are formed in biological systems. Higher level of MDA suggests a higher degree of lipid peroxidation. The increased level of MDA in the heart of rats administered with Pb is suggestive of oxidative stress. This may be ascribed to alteration in the cellular redox status of the animals as a result of increased lipid peroxidation. The Fe^{2+}+Ca^{2+}+Zn^{2+} administration to Pb-exposed rats greatly prevented Pb induced oxidative stress and mitochondrial damage thus confirming the protective role of Fe^{2+}+Ca^{2+}+Zn^{2+} from Pb-induced toxicity.
From the results of the present study, it is clear that Pb-exposure decreased SOD activity significantly in heart tissue of young and aged rats. The decrease in SOD activity may be due to exhaustion and over utilization of these enzymes under the oxidative products. Since this is a sulfhydryl containing enzyme, decrease of its tissue level can also reflect oxidative denaturation (Kodavanti, 1999). Pb is shown to alter antioxidant activities by inhibiting functional SH groups in several enzymes such as ALAD, SOD, CAT, and GPx (Hsu, 1981; Ito et al., 1985; McGowan and Donaldson, 1986; Chiba et al., 1996).

CAT activity significantly decreased in the Pb-exposed rats. Catalase contains heme as the prosthetic group, the biosynthesis of which is inhibited by Pb and resulted in decrease catalase activation (Saga et al., 1984). This was further supported by the decrease in haemoglobin levels in Pb-exposed rats observed in the study. Supplementation with Fe$^{2+}$+Ca$^{2+}$+Zn$^{2+}$ enhanced the SOD and catalase activities, suggesting a significant role in protecting cells (Vitoria et al., 2001; Patra et al., 2001). Therefore, it could be suggested that the enhancement of the antioxidant defense enzymes in Fe$^{2+}$+Ca$^{2+}$+Zn$^{2+}$ supplemented rats may increase the detoxification process (Antonio et al., 2003).

The supplemented Ca$^{2+}$, Fe$^{2+}$ and Zn$^{2+}$ basically compete for similar binding sites as that of Pb, decreases Pb gastrointestinal absorption and tissue accumulation. Thus Ca$^{2+}$, Fe$^{2+}$ and Zn$^{2+}$ replace Pb in the body and reduce the Pb-burden. The results of the present study thus provide evidence for the suggestion that adequate Ca$^{2+}$, Fe$^{2+}$ and Zn$^{2+}$ intake greatly helps in reducing Pb-induced cardiac and haematological alterations. Therefore, these results suggest that Ca$^{2+}$, Fe$^{2+}$ and Zn$^{2+}$ nutrient mixture may be considered as therapeutic agent for the removal of Pb burden on the body.