# Biochemical effects of occupational lead exposure to workers in small scale automobile workshops of North Karnataka (India)

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## Abstract

In developing countries like India, the occupational exposure to lead still persists in the unorganised small-scale workshops. The workers are unaware of the toxic effects of lead exposure. We studied 30 automobile workers exposed to lead for periods from 2-15 years and normal healthy control subjects (N=30) who were non-occupationally exposed to lead from Bijapur, North Karnataka, India. Venous blood and urine samples were collected from both groups. The blood lead (PbB) 364%, p<0.001 and urinary lead (PbU) 176%, P<0.001 levels were significantly increased in these workers as compared to the control. The biochemical parameters related to heme biosynthesis like non-activated erythrocytes δ-ALAD (P<0.01, -18.51%), activated δ-ALAD (P<0.05, -13.29%), levels were significantly decreased and the ratio of activated/non activated δ-ALAD was significantly increased (P<0.001, 43.83%) in automobile workers as compared to the control subjects. Urinary δ-aminolevulinic acid (83.78%, p<0.001) and urinary porphobilinogen (37%, P<0.001) were significantly increased in the study group as compared to controls. The lipid peroxide, i.e. serum MDA concentration (19.31%, P<0.001) was significantly increased and the antioxidant enzymes like RBC-SOD (-16.69%, P<0.05), RBC-CAT (-11.48%, P<0.05) plasma ceruloplasmin (-29.81%, P<0.001) and plasma total antioxidant capacity (TAC) (-30.52%, P<0.001) were significantly decreased. These results indicate that the lead exposure is greater in small-scale industries.

Increased blood lead levels affect heme biosynthesis and cause imbalance between oxidative stress and antioxidant status. Therefore, early screening and regular monitoring of these workers will help to prevent the long-term deleterious effects of lead.

**Key words:** Automobile workers; Biochemical effects; Blood Lead (PbB); Car workers; Environmental health; Occupational health; Small-scale workshops; Urinary lead (PbU).

## Introduction

Lead is a ubiquitous and versatile metal that has been used by human kind for over 7,000 years. It is highly resistant to corrosion, pliable, having high density, low elasticity, high thermal expansion, low MP, easy workability, easily recycled, an excellent antifriction metal and inexpensive. Because of these excellent properties, it is used in acid battery manufacturing, printing press, paints, soldering water distribution pipes, ceramic glazes, paper industry and silver jewellery industries. Today, it is considered as one of the most widely distributed toxins in the environment. Lead and its compounds can enter the environment at any point during mining, smelting, processing, use, recycling and disposal (Leon *et al*., 1994; Curtis *et al*., 2008).

Lead that is present in food, beverages, soil or dust and atmospheric air is absorbed by the gastro intestinal tract (GIT). The metal is rapidly taken up in blood and soft tissue and then to bone. Lead has been shown to cause adverse effects in several organs and organ systems including hematopoietic, nervous, renal, cardio-vascular, reproductive and immune. It is also mutagenic in mice (ATSDR 2005; WHO IPCS 1995; Patil *et al*., 2006).

The biological effect of lead depends upon the level and duration of lead exposure. Lead inhibits three enzymes of heme biosynthesis: δ-Aminolevulinic acid dehydratase, coproporphyrin oxidase and ferrochelatase. Several studies have reported that heavy metals like copper, nickel and lead produce reactive oxygen species (ROS) leading to lipid peroxidation, DNA damage and depletion of cells antioxidant defence system. Erythrocytes are more vulnerable to oxidative damage than other cells. Lead causes an alteration in antioxidant enzyme activity such as SOD, CAT, Glutathione peroxidase, changes concentration of certain antioxidant molecules and thus impacts upon overall antioxidant capacity in lead exposed workers (Monteiro *et al*., 1985; Ito *et al*., 1985; Sugawara *et al*., 1991; Chiba *et al*., 1996).

The automobile workers are exposed to lead by their routine activities like radiator repair, spray painting, battery recharging and recycling in small-scale automobile workshops. The overall activities of these workers serve as a source of lead exposure to their family members also. The drastic increase in the number of automobile vehicles in India in the last two decades has increased the exposure of this labour class to lead. These workers have been found to have high blood lead levels. Therefore, the purpose of this study was to evaluate the activity of the antioxidant enzymes and the concentration of products of ROS in blood with reference to heme biosynthesis related parameters in automobile workers of North Karnataka (India).

## Methods

The study comprised an occupationally lead exposed automobile workers study group (N=30) and a normal healthy non-occupationally lead exposed subjects control

**Table 1.0**
Mean values of PbB, PbU and heme biosynthesis related parameters in automobile workers and control group.

| Sr. No. | Biochemical parameters | Control group (N= 30) | Automobile workers (N= 30) |
|---|---|---|---|
| 1 | PbB µg/dl | 10.2 ± 5.8 (2.0 – 23.0) | 47.37 ± 23.22*** (5.0 – 85.0) |
| 2 | PbU µg/dl | 6.28 ± 3.83 (1.0 – 14.0) | 17.37 ± 12.5*** (1.0 – 41.0) |
| **A** | **Heme Biosynthesis Related Parameters Erythrocyte δ-ALAD (µmol δ-ALA utilised/min/litre of erythrocytes)** | | |
| 3 | Activated δ-ALAD | 19.70 ± 4.96 (4.73 – 28.62) | 17.08 ± 3.75* (14.81 – 24.50) |
| 4 | Non-activated δ-ALAD | 16.31 ± 4.54 (4.03 – 32.70) | 13.29 ± 4.74** (3.46 – 28.39) |
| 5 | Act/N-Act ratio | 1.46 ± 0.83 (0.42 – 2.28) | 2.10 ± 0.99*** (1.27 – 4.05) |
| 6 | U-δ-ALA mg/l | 9.62 ± 5.45 (2.5 – 17.5) | 17.68 ± 4.42*** (4.69 – 27.94) |
| 7 | U-PBG mg/l | 10.10 ± 2.87 (3.5 – 15.87) | 13.84 ± 3.3*** (8.47 – 18.76) |

Figures indicate Mean ± SD values and those in parenthesis are range of values.
* P<0.05, **P<0.01, ***P<0.001, •Non-significant as compared to controls.
**PbB** – Blood Lead, **PbU** – Urinary Lead, **Act-ALD** – Activated δ-Aminolevulinic acid dehydratase, **NA-ALD** – Nonactivated δ-Aminolevulinic acid dehydratase, **Act/NA** – Activated δ-Aminolevulinic acid dehydratase/Nonactivated δ-Aminolevulinic acid dehydratase, **U-ALA** – Urinary δ-Aminolevulinic acid, **U-PBG** – Urinary Phorphobilinogen.

group (N=30). All subjects were aged in the range of 20-45 years. Written consent was obtained from all automobile workers and control subjects and before the sample collection, demographic, occupational and clinical data were collected from both groups by questionnaire and interview. Male subjects of average socio-economic status, normal dietary intake, food habits, non-alcoholics and non-smokers, who were exposed to lead for more than six hours per day over 2-15 years, were selected for the study. Most of the workers consumed a mixed type of diet. The subjects who were taking drugs for minor illnesses and had past history of major illness were excluded from the study. The entire protocol was approved by the institutional ethical committee and utmost care was taken during the experimental procedure according to the Helsinki declaration of 1964.

Blood samples were collected by puncturing the antecubital vein in heparinised polypropylene tubes and 2ml blood was also taken in plane tubes. At the time of blood collection, random urine samples also were collected in small dark amber coloured plastic bottles. Collections of 24 hours urine sample from each subject were difficult because all subjects were busy in their routine work.

The blood lead and urinary lead concentrations were measured using a Perkin Elmer model 303 graphite furnace atomic absorption spectrophotometer connected to Hitachi 165 recorder. (Parson *et al.,* 1993)

Erythrocyte δ-aminolevulinic acid dehydratase (ALAD) was estimated by the Chisholm *et al.*, (1986) method. Erythrocyte ALAD acts on aminolevulinic acid (ALA) to form porphobilinogen (PBG), which further reacts with modified Ehrlich's reagent to form a pink coloured compound measured on a spectrophotometer at 555nm. Hg-TCA solution stops the reaction by precipitating the

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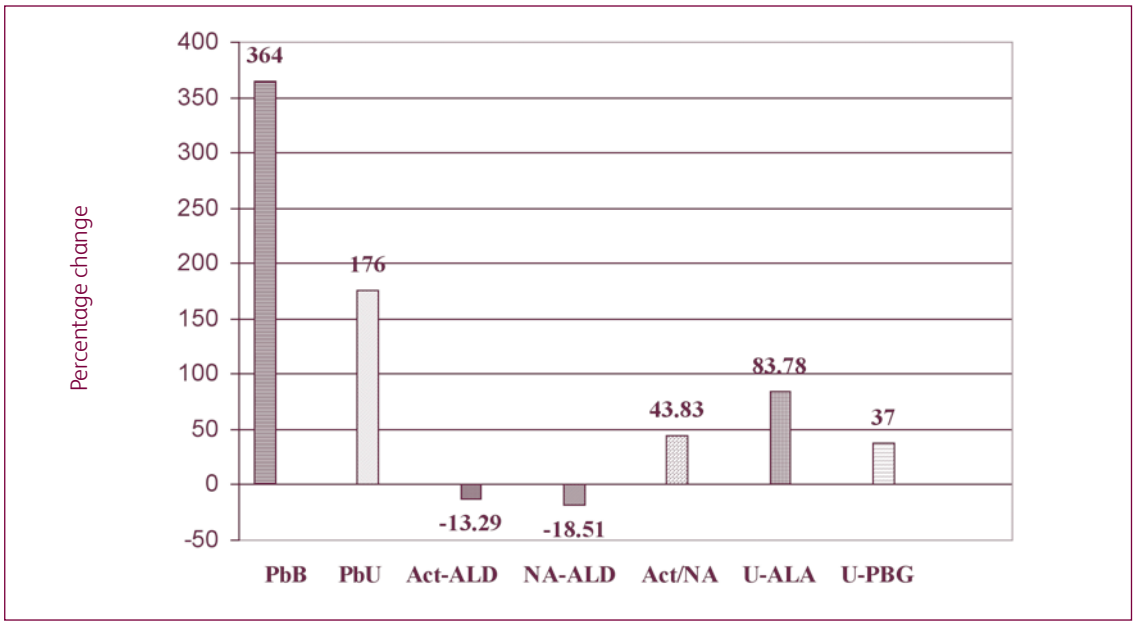


**Figure 1.0**
Percentage change of PbB, PbU and heme biosynthesis related parameters in automobile workers with respect to control group.

**PbB** – Blood Lead, **PbU** – Urinary Lead, **Act-ALD** – Activated δ-Aminolevulinic acid dehydratase, **NA-ALD** – Nonactivated δ-Aminolevulinic acid dehydratase, **Act/NA** – Activated δ-Aminolevulinic acid dehydratase/Nonactivated δ-Aminolevulinic acid dehydratase, **U-ALA** – Urinary δ-Aminolevulinic acid, **U-PBG** – Urinary Phorphobilinogen.

proteins. The erythrocyte ALAD activity activated by zinc acetate was also measured and the ratio of activated vs. non-activated ALAD was determined.

Urinary δ-aminolevulinic acid (δALA-U) was estimated by the method of (Osamu *et al*., 1969). δ-aminolevulinic acid reacts with acetyl acetone and forms a pyrrole substance, which reacts with p-dimethyl amino benzaldehyde. The resultant coloured complex was measured spectrophotometrically at 555nm.

Urinary Porphobilinogen (PBG-U) was estimated according to the Mauzerall and Granick (1956) method. Porphobilinogen in urine reacts with p-dimethyl amino benzaldehyde from Ehrlich's reagent in acid solution to form a red compound, which is measured at 555nm exactly after five minutes. The values were calculated according to the Rimington (1971) formula.

Serum lipid peroxide i.e. MDA concentration was measured by the Satoh (1978) method. Serum proteins were precipitated by trichloro acetic acid (TCA) and the mixture was heated for 30 minutes with thiobarbituric acid in 2M sodium sulphate, in a boiling water bath. The resulting chromogen was extracted with n-butyl alcohol and the absorbance of the organic phase was determined at a wavelength of 530nm. The values were expressed in terms of malon dialdehyde (MDA) using 1,1,3,3, tetra ethoxy propane as the standard.

Activity of erythrocyte superoxide dismutase (SOD) was measured by the method of Marklund and Marklund (1988). Superoxide anion is involved in the auto oxidation of pyrogallol at alkaline pH 8.5. The superoxide dismutase inhibits the auto-oxidation of pyrogallol, which can be determined as an increase in absorbance per two minutes at 420nm. The SOD activity was measured as unit $mL^{-1}$ hemolysate. One unit of superoxide dismutase is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol auto oxidation.

Erythrocyte catalase was measured by the method of Aebi (1983). Heparinized blood was centrifuged and the plasma was removed. The erythrocytes were washed two to three times with 0.9% NaCl and lysed in 10 volumes of cold deionized water. The whole mixture was centrifuged further for 10min at 3,000xg. The cell debris was removed and the clear hemolysate was diluted 500 times with

**Table 2.0**
Mean values of lipid peroxide, antioxidants enzymes of automobile workers and control group.
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| Sr. No. | Biochemical parameters | Control group (N= 30) | Automobile workers (N= 30) |
|---|---|---|---|
| **A** | Lipid peroxide [nmol/ml of MDA] | 0.88 ± 0.28 (0.50 – 1.37) | 1.93 ± 0.35*** (1.5 – 2.7) |
| **B** | **Antioxidants Status** | | |
| 1 | RBC – Superoxide dismutase [Unit/ml of hemolysate] | 13.12 ± 4.16 (8.0 – 22.4) | 10.93 ± 3.32* (5.0 – 27.0) |
| 2 | RBC – Catalase (mM $H_2O_2$ decom/mg Hb/min) | 17.32 ± 5.81 (8.5 – 25.35) | 15.33 ± 5.88* (4.2 – 25.35) |
| 3 | Plasma ceruloplasmin [mg/dl] | 29.55 ± 10.3 (18.9 – 57.05) | 20.74 ± 6.95*** (3.5 – 30.65) |
| 4 | Total Antioxidant Capacity µmol/L | 1352 ± 164.4 (1058 – 1878) | 939.34 ± 359.3*** (500 – 1550) |

Figures indicate Mean ± SD values and those in parenthesis are range of values.
* P<0.05, ** P<0.01, ***P<0.001, as compared to controls.
**LP** – Lipid peroxide, **SOD** – Superoxide Dismutase, **CAT** – Catalase, **CP** – Ceruloplasmin, **TAC** – Total antioxidant capacity.

phosphate buffer (60mM) pH 7.4. Catalase decomposes hydrogen peroxide ($H_2O_2$) to form water and molecular oxygen. In the UV range, $H_2O_2$ shows a continual increase in absorbance with decreasing wavelength. At 240nm, $H_2O_2$ absorbs maximum light. When $H_2O_2$ is decomposed by catalase then the absorbance decreases. The decreased absorbance was measured at 240nm at 15 second intervals up to 1min and the difference in absorbance (ΔA at 240nm) per unit time was taken to be a measure of the catalase activity. The unit of catalase activity was expressed as mM of $H_2O_2$ decomposed $mg^{-1}$ Hb $min^{-1}$.

Plasma ceruloplasmin was measured by the method of Herbert A Ravin (1961). Ceruloplasmin oxidizes p-phenylenediamine in the presence of oxygen to form a purple coloured oxidized product. The ceruloplasmin concentration was determined from the rate of oxidation of p-phenylenediamine at 370°c at pH 6.0, measured at an absorption peak at 530nm.

The plasma total antioxidant capacity (TAC) was estimated by FRAP assay method of Miller (1997). The antioxidant power of plasma converts ferric ions to ferrous ions at low pH forming a pink coloured ferrous tripyridyl triazine complex. Ferrous reducing antioxidant power values were obtained by comparing the change in the absorbance at 593nm in mixture with those of ferrous ion of known concentration. The TAC in serum was expressed as µmol $L^{-1}$. The statistical analysis was done by student 't' test.

## Results

The mean and SD values of lead in blood [PbB – 47.37 ± 23.2µg/dl (364 %, P < 0.001)], and urine [PbU 17.37 ± 12.5µg/dl (176%, P < 0.001)] in automobile workers were significantly increased as compared to the control group (PbB 10.2 ± 5.8µg/dl PbU 6.28 ± 3.83µg/dl). Non-activated erythrocytes δ-ALAD (P<0.01, -18.51%), activated δ-ALAD (P<0.05, -13.29) levels were significantly decreased and the ratio of activated vs. non activated δ-ALAD (P<0.001, 43.83%) was also significantly increased in automobile workers as compared to the control subjects. Excretions of δ-ALA (P<0.001, 83.78%) and PBG (P<0.001, 37%) in urine were significantly increased in the study group as compared to the controls (Table 1.0 and Figure 1.0).

Serum lipid peroxides i.e. MDA concentration (P<0.001, 19.31%) levels were significantly increased in automobile workers as compared to the controls. Activities of antioxidant enzymes such as RBC-SOD (P<0.05, -16.69), RBC- CAT (P<0.05, -11.48), plasma ceruloplasmin (P<0.001, -29.81%), and Plasma TAC

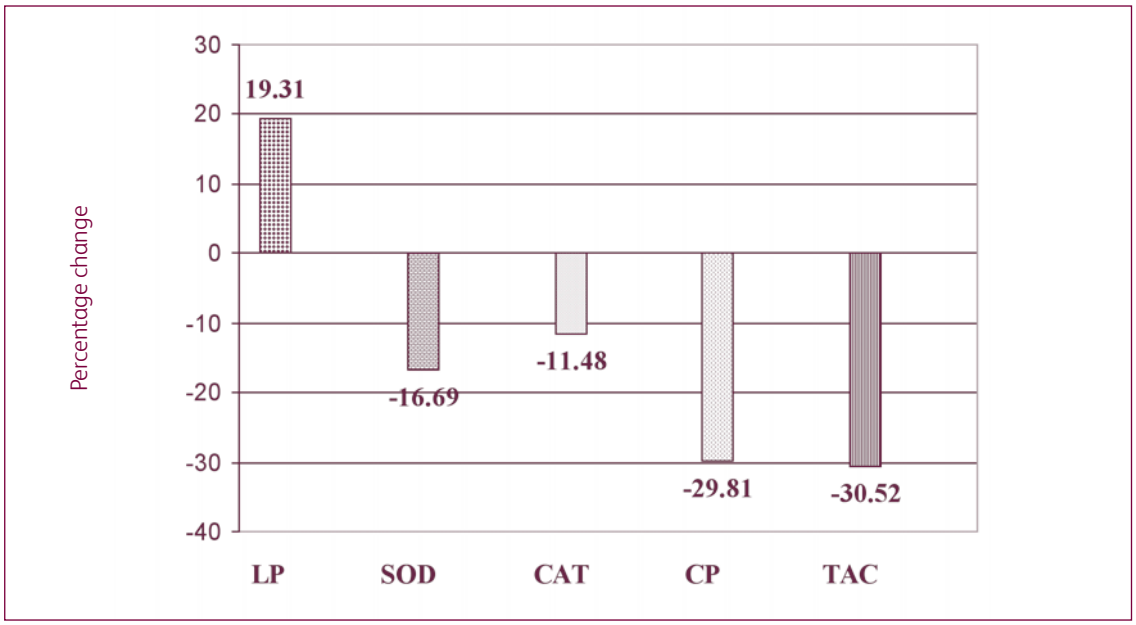


**Figure 2.0**
Percentage change of mean values of lipid peroxide, antioxidants enzymes of Automobile workers with respect to control group.

**LP** – Lipid peroxide, **SOD** – Superoxide Dismutase, **CAT** – Catalase, **CP** – Ceruloplasmin, **TAC** – Total antioxidant capacity.

(P<0.001, -30.52%) were significantly decreased in automobile workers as compared to the control group (Table 2.0, Figure 2.0)

## Discussion

Blood lead (364%, P<0.001) and urinary lead (176%, P<0.001) levels were significantly increased in automobile workers as compared to control subjects, indicating more exposure of lead. Lead absorption results in its rapid urinary excretion. PbB levels generally reflect acute (current) exposure because of short half-life of lead in blood (28 to 36 days), but it is also influenced by previous storage. The PbB level is the best and most sensitive biomarker for identifying lead pollution, human exposure and its adverse effects. Single PbB measurement does not show the body burden of lead, because the metal is circulating in three compartments i.e. blood, bone and soft tissues (ATSDR, 2005). Erythrocytes activated δ-aminolevulinic acid dehydratase (-13.29% P<0.05), non-activated δ-aminolevulinic acid dehydratase (-18.5% p<0.01) activity were significantly decreased and ratio of Act/NA ALAD was significantly increased (43.83% p<0.001) as compared to control subjects, indicating that lead inhibits the activity of δ-aminolevulinic acid dehydratase enzyme in these workers. The level of δ-ALAD decreases as early as the fourth day after the exposure begins. Once the δ-ALAD level is reduced, persistence of abnormality correlates with the amount of lead in body tissues (body burden), so that the δ-ALAD level remains reduced as long as significant quantities of lead remain. Therefore, after chronic lead exposure, low δ-ALAD values may persist for years even though exposure has ceased. The level of δ-ALAD is also a very sensitive indicator of lead toxicity and is usually reduced to 50% or less of normal activity when blood lead values are in the 30-50µg/dl range. Unfortunately, the δ-ALAD level reaches a plateau when marked reduction takes place, so it cannot be used to quantify the degree of lead exposure (WHO 1995). Decreased δ-ALAD activity caused by lead can be reversed by adding Zn or dithiothreitol (DTT) or by heating (Sakai T, 1980). Possible mechanisms of reactivation include reduction of sulfhydryl groups, which are essential for enzyme activity, or, in the case of DTT, chelation of lead from binding sites on the enzyme. Exposure to lead does not decrease the concentration of δ-ALAD in erythrocytes, but substantially decreases δ-ALAD activity (Kajimoto M. 1982), as well as other tissues (Schlick E 1983). Measurement of δ-ALAD activity in the erythrocytes offers a good and simple method of evaluation of lead poisoning.

Urinary excretion of ALA (83.78%, P<0.001) and PBG (37%, P<0.001) increased significantly in automobile workers as compared to the control group, indicating that there is inhibition of the enzymes of the heme biosynthetic pathway resulting in the accumulation and increased excretion of the intermediate in the biosynthesis in the heme biosynthetic pathway namely ALA and PBG. Lead interferes with the biosynthesis of heme by altering the activity of three enzymes ALAS, ALAD and Ferrochelatase. Lead indirectly stimulates the mitochondrial enzyme ALAS, which catalyses the condensation of glycine and succinyl COA to form ALA. The activity of ALAS is the rate limiting step in heme biosynthesis; increase of ALAS activity occurs through feedback depression. Lead inhibits the zinc-containing cytosolic enzyme ALAD that catalyses the condensation of two units of ALA to form porphobilinogen. This inhibition is non-competitive and occurs through the binding of active site of ALAD. Lead bridges the vicinal sulfhydryls whereas zinc, which is normally found at the active site, binds to only one of these sulfhydryls. Inhibition of ALAD and feedback depression of ALAS results in accumulation of ALA. Estimation of urinary ALA and PBG are also useful markers for screening lead exposed workers.

The levels of lipid peroxide (19.31%, P<0.001) were significantly increased with the significant decrease in the activity of antioxidant enzymes like SOD (-16.69%, P<0.05), CAT (-11-48%, P<0.05), plasma ceruloplasmin (CP) (-29.81%, P<0.001) and decreasing the overall plasma total antioxidant capacity (TAC) (-30.52%, P<0.001). This indicates increased oxidative stress owing to exposure to lead and its compounds in these workers while engaged in workplace activities including radiator repairing, battery repairing or replacing/recharging and spray-painting. The workplaces were unhygienic; the workers ate food and drank water in the same atmosphere. Also, their hands and clothes were not clean while taking food. This might lead to an increase in the ingestion of food contaminated by lead particles. These workers were unaware of the side effects.

Erythrocytes Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione peroxide (GPx) are enzymes that scavenge free radicals during lipid peroxidation. The cytotoxicity of the molecular oxygen is checked by the delicate balance between the rate of generation of the partially reduced oxygen species and the rate of their removal by different defence mechanisms. A shift in this delicate balance can lead to cellular damage. In the lead exposed occupational workers, the decreased activities of SOD, CAT, GSHPx, CP and the primary antioxidant enzymes suggest an interaction between the accumulated free radicals and the active amino acids of these enzymes (Amrita Das Gupta *et al*., 2007).

Catalase is a heme containing protein. The biosynthesis of heme is inhibited by lead, resulting in decreased erythrocyte catalase activation. Ceruloplasmin is a copper containing glycoprotein with enzymatic activity as ferroxidase. It plays a crucial role in iron metabolism, whereby it assists the release of iron from cells before its uptake by transferrin. (Osaki *et al*., 1971). The enzyme converts $Fe^{2+}$ to $Fe^{3+}$ and removes $Fe^{2+}$ from the blood, which could otherwise become involved in the generation of harmful ROS.

Plasma total antioxidant capacity (TAC) was decreased (-30.52%, P<0.001) in the automobile workers in this study as compared to the control. Serum has a number of low molecular weight antioxidant molecules either water or lipid soluble. Evaluation of the TAC gives more biological relevant information than that of the individual levels of specific antioxidants of a given body fluid such as plasma. The overall TAC considers the cumulative effect of all antioxidant (known and unknown, measurable and not measurable) present in plasma/serum of several physiological conditions in humans and animals. (Mohammadi *et al*., 2006). Decreased TAC in this study is owed to the adverse effect of lead in these workers to compensate the increased oxidative stress.

## Conclusion

- Increased blood lead levels may arise in automobile workers in small scale workshops owing to greater lead exposure.
- Despite modern technical advancements considerable lead hazards still exist in this industry.
- An increased blood lead level in automobile workers affects the heme biosynthesis by inhibiting the three enzymes (δ-ALAD, Coproporphyrinogen oxidase and heme synthase), resulting in increased urinary δ-ALA, and PBG.
- Increased lipid peroxidation and several impaired antioxidant enzymes in this study might be owed to increased blood lead, which impairs the oxidant/pro-oxidants balance of cells resulting in oxidative damage. Therefore, the supplementation of

antioxidant vitamins could be beneficial to the lead exposed workers.

- Estimations of urinary δ-ALA, PBG and erythrocyte δ-ALAD are most valuable in screening for occupational lead exposure.
- Medical examination and estimations of blood and urinary lead should be done not only at the pre-employment stage but also at regular intervals during their service to identify workers with potential lead toxicity.
- The implementation of modern risk assessment techniques could improve the preventative element in the workshops.

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