

Antioxidant Defense and Lipid Peroxide Level in Liver and Kidneys of Lead Exposed Rats

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ABSTRACT : An experiment was carried out with 48 IVRI 2CQ rats 6-8 week old, weighing 50-100 g, to study the effect of lead exposure on antioxidant defense, lipid peroxide level, status of thiol groups and concentration of lead in the liver and kidneys at the end of the exposure and also after withdrawal of lead administration. Twenty four rats were given lead at a daily dose rate of 1 mg lead/2 ml of distilled water/kg body weight as lead acetate solution intraperitoneally for a period of 30 days. Another 24 control rats received 2 ml of sterile normal saline solution (0.85% NaCl)/kg body weight in an identical manner. A many-fold increase in concentration of lead was associated with a non-significant ($p > 0.05$) decrease in the activities of superoxide dismutase (SOD) in the liver (27%) and kidneys (12%) and catalase in kidneys (22%). A significant ($p < 0.05$) increase in lipid peroxide level was recorded in the liver (40%) compared with control values. There were significant ($p < 0.05$) decreases in the total thiol and protein bound thiol contents in liver and an increase in non-protein bound thiol groups in the kidneys of lead exposed rats. During the 10 day observation period after withdrawal of lead administration, no significant change was observed with respect to any of the above parameters indicating that a 10 day withdrawal period was not enough for restoration of normality. It is concluded that the magnitude of response and the resultant changes in the lipid peroxide concentration, and the activities of SOD and catalase were not identical in the liver and kidneys of lead-exposed rats. (*Asian-Aus. J. Anim. Sci.* 2000. Vol. 13, No. 10 : 1433-1439)

Key Words : Lead, Oxidative Stress, Lipid Peroxide, Thiol Groups, Liver and Kidney

INTRODUCTION

Lead is a pervasive environmental pollutant that has aroused serious concerns for researchers and planners (Rehman, 1984; Ercal et al., 1996). Awareness about its deleterious effects on the health of animals and humans is increasing (Jindal and Gill, 1999). To explain its pathotoxicity, various mechanisms have been proposed, but none of them has been fully defined (Ercal et al., 1996). Of late, generation of free radicals, changes in antioxidant defense and lipid peroxidation aftermath of lead exposure have been reported in erythrocytes of lead exposed rats and humans and were claimed to be responsible for the toxic effects of lead (Gurer et al., 1998; Monteiro et al., 1985). Increased lipid peroxidation in the brain following lead exposure and potentiation of lead induced inhibition of the antioxidant defense system by ethanol have been reported in rats, and these changes in the lipid profile were attributed to a higher content of polyunsaturated fatty acids in this vital organ (Rehman, 1984; Jindal and Gill, 1999). The liver and kidney accumulate the maximum concentration of lead per g of wet tissue, and play vital roles in detoxification and elimination of this biotoxicant (Zmudzki et al., 1983), but there appear to be no reports on the effects of subclinical lead exposure on

antioxidant defense, lipid peroxide level and thiol groups in these two organs. Thus, the present work was aimed at evaluating the propensity of lead in disturbing prooxidant and antioxidant balance in the liver and kidney at the end of exposure and to record the post exposure effect.

MATERIALS AND METHODS

Animals

Forty eight IVRI 2CQ rats, weighing between 50-100 g and in the age group of 6-8 weeks, were utilized in the study. They were allowed ad lib laboratory animal feed and water. Animals were acclimatised to the experimental laboratory animal housing environment for a period of one month before the start of the experiment.

Experimental design

The experimental animals were divided into two equal groups (A and B), consisting of 24 rats. The rats of group B were given sterile lead acetate solution intraperitoneally at a daily dose rate of 1 mg of lead/2 ml of distilled water/kg body weight for a period of 30 days. The rats of group A received sterile normal saline solution (0.85% NaCl) in an identical manner to serve as a control. Six rats from each group were sacrificed at the end of the exposure period to assess the effects of lead exposure. In order to find out the post exposure effects, six rats from each group were sacrificed on days 3, 7 and 10 of withdrawal of the lead treatment under light anesthesia,

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induced by inhalation of chloroform. Liver and kidney samples were excised immediately. Half of the liver and one kidney were kept at -20°C for wet digestion. The remaining tissue samples were washed in ice cold normal saline solution, blotted with filter paper and immediately processed for biochemical estimation.

Biochemical assay

Lipid peroxide: The lipid peroxide levels in hepatic and renal tissues were estimated following the method of Ohkawa et al. (1979) and the values were expressed in nmol of malondialdehyde/g (MDA/g) of wet tissue using the extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ (Utley et al., 1967).

Superoxide dismutase (E.C. 1.15.1.1): Superoxide dismutase (SOD) activity was measured in 20% tissue homogenate following the method of Marklund and Marklund (1974) with certain modifications. The homogenate was centrifuged at 4000 rpm for 10 min at 4°C and the supernatant was used for analysis of SOD. Briefly, the assay mixture in a total volume of 3 ml consisted of 50 mM tris cacodylic acid, 1 mM diethylene triamine pentaacetic acid (DTPA), 0.1 mM nitroblue tetrazolium and triton X 0.001%, enzyme preparation after suitable dilution of sample, and 0.2 mM of pyrogallol. In the blank, the enzyme preparation was substituted by an equal quantity of distilled water. The increase in absorbance due to autooxidation of pyrogallol was recorded at 420 nm using a spectrophotometer (Electronic Corporation of India Limited). One unit of SOD activity was defined as the quantity of enzyme that inhibited pyrogallol autooxidation by 50% under the given experimental conditions.

Catalase (E.C. 1.11.1.6): Catalase activity in the above supernatant was estimated by measuring the breakdown of hydrogen peroxide in the reaction mixture. The reaction was started by the addition of 50 μl of suitably diluted supernatant to 3 ml of phosphate buffer H_2O_2 solution. Initial absorbance at 240 nm was read after 20 seconds against a reference cuvette in which same amount of distilled water was added instead of H_2O_2 . The time required for initial absorbance to decrease by 0.05 units was noted. Catalase activity in the sample was calculated and expressed in U/mg of protein (Cohen et al., 1970).

Thiol groups: Total (T-SH), protein bound (P-SH) and non-protein bound (NP-SH) thiol groups in the tissue homogenate were determined as per the method of Sedlak and Lindsay (1968). The molar extinction coefficient of 13,100 at 412 nm was used for the determination of thiol contents and the values were

expressed in $\mu\text{mol/g}$ of wet tissue.

Protein: The protein content of supernatant was measured by the method of Lowry et al. (1952).

Analysis of metal

Tissue samples were wet digested (Kolmer et al., 1951) and the concentrations of lead in the digested samples were measured using an atomic absorption spectrophotometer (AAS 4129, Electronic Corporation of India Limited) at 217 nm wave length and 6 ma current. The values were expressed in ppm.

Statistical analysis

The data were statistically analysed using one way analysis of variance to compare the means at different periods of observation in a particular group and Student 't' test to compare the means between the control and lead-exposed groups at a particular observation period (Snedecor and Cochran, 1979).

RESULTS AND DISCUSSION

Daily intraperitoneal administration of 1 mg of lead/kg body weight as lead acetate solution significantly ($p < 0.05$) increased its accumulation in the liver and kidneys, and the lead concentration in these organs was many-fold higher in the treated group at the end of the exposure period (table 1). After removal of the lead treatment, the concentration of lead continued to remain significantly ($p < 0.05$) higher than the respective control values (figure 1). Lead has been recognized as a biological toxicant and different dose levels have been used experimentally to study its effects (Skoczynska et al., 1993; Chmielnicka et al., 1994; Skoczynska, 1995). The daily dose of 1 mg of lead/kg body weight was given intraperitoneally to simulate more or less the level of exposure in occupational workers, and to eliminate error due to variation in absorption in different individuals through an oral route of exposure. Biokinetic patterns of lead distribution in various tissues varied with route of administration. Lead, given by the intravenous route, accumulates most in the liver and bone marrow followed by kidneys, spleen, lungs and bones. Following oral administration, the major portion of lead (60%) goes to the bone and the rest is distributed in the liver (25%), kidneys (4%), intestinal wall (31%), reticuloendothelial system (3%) and other body tissues (4%) including teeth and hair (Oehme, 1972). However, the concentration of lead in terms of per g of tissue has been reported to be higher in kidneys than in the liver (Zmudzki et al., 1983; Upadhaya and Swarup, 1990). The bioaccumulation of lead in the liver and kidney after intraperitoneal administration was similar to the observation of

Table 1. Effect of lead on SOD and catalase activities, endogenous lipid peroxide level and thiol groups in liver and kidney of growing rats

| Parameters | Biosamples | Control | Lead exposed | % of control |
|--|------------|--------------|--------------|--------------|
| Lead level (ppm) | Liver | 0.64± 0.08 | 4.63± 0.47* | 723 |
| | Kidney | 0.80± 0.20 | 11.08± 0.21* | 1385 |
| SOD (U/mg of protein) | Liver | 3.35± 0.50 | 2.59± 0.38 | 73 |
| | Kidney | 3.80± 0.58 | 3.34± 0.31 | 88 |
| Catalase (U/mg of protein) | Liver | 9.39± 1.07 | 10.80± 1.71 | 115 |
| | Kidney | 11.97± 2.06 | 9.40± 1.05 | 78 |
| Lipid peroxides level (nmol of MDA/g of wet tissue) | Liver | 270.8 ±30.6 | 378.2 ±36.8* | 140 |
| | Kidney | 229.2 ±16.3 | 309.6 ±33.6 | 135 |
| Thiol group | | | | |
| T-SH (μ mol/g of tissue) | Liver | 11.45± 0.68* | 8.16± 0.47 | 72 |
| | Kidney | 8.52± 0.72 | 6.83± 1.05 | 80 |
| NP-SH (μ mol/g of tissue) | Liver | 2.22± 0.39 | 3.19± 0.49 | 144 |
| | Kidney | 0.85± 0.09 | 1.41± 0.08* | 166 |
| P-SH (μ mol/g of tissue) | Liver | 9.23± 0.70* | 4.96± 0.95 | 54 |
| | Kidney | 7.68± 0.71 | 5.42± 1.04 | 71 |

Lead was administered daily intraperitoneally at 1 mg of Pb/kg b. wt for a period of 30 days. Six rats from both the lead exposed and unexposed groups were sacrificed under light anesthesia. * indicates significantly ($p < 0.05$) higher values than corresponding normal values.

Zmudzki et al. (1983) in cattle following exposure to lead through the oral route. The period required for depletion of excessive quantities of lead from the blood, liver and kidney of animals given variable quantities of lead is unclear (Humphreys, 1991). From

the present study, it is concluded that withdrawal of lead for a period of 10 days is not enough to bring down the bioaccumulation of lead in these two vital organs to the normal level (figure 1 and 2).

Increased lead concentrations were associated with

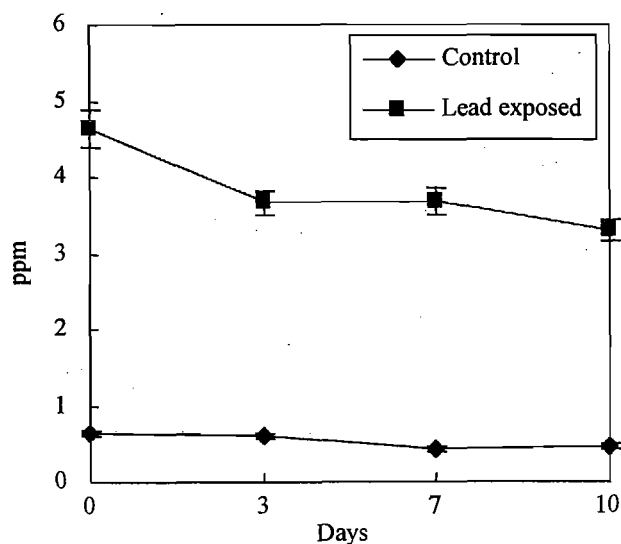


Figure 1. Liver lead level in lead exposed and unexposed rats. Six rats each from both the groups were sacrificed on day 0, 3, 7 and 10 after lead exposure for a period of 30 days at the dose rate of 1 mg of lead/2 ml/kg body weight administered intraperitoneally. Control rats received sterile normal saline solution.

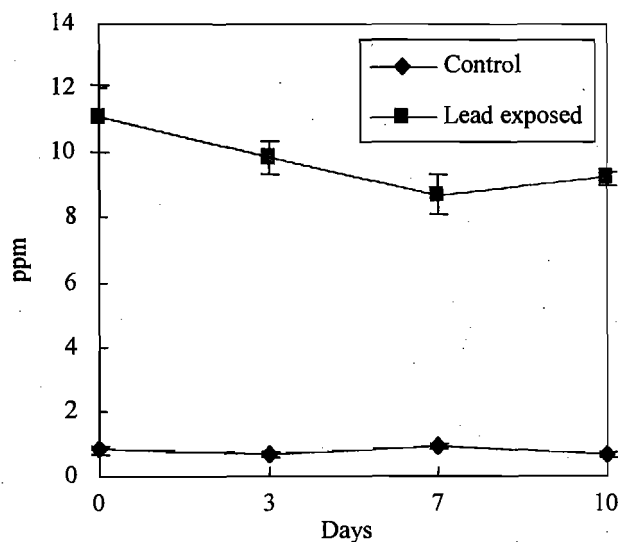


Figure 2. Kidney lead level in lead exposed and unexposed rats. Six rats each from both the groups were sacrificed on day 0, 3, 7 and 10 after lead exposure for a period of 30 days at the dose rate of 1 mg of lead/2 ml/kg body weight administered intraperitoneally. Control rats received sterile normal saline solution.

non-significant ($p>0.05$) decreases in the activity of SOD in liver (27%) and kidneys (18%), as well as an increase in the catalase activity in the liver (15%) and a decrease in the kidney (22%). Superoxide dismutase, a Cu-Zn containing enzyme, is extensively used as a biochemical indicator of pathological states associated with oxidative stress (Patra et al., 1998). It is responsible for the dismutation of superoxide radicals to hydrogen peroxide (H_2O_2), which is further detoxified by the antioxidant enzyme, catalase. Thus, these enzymes serve as the first line of defense against oxidative stress conditions. Inhibition of the activities of SOD and catalase in the rat brain were reported following exposure to a massive dose of lead (50 mg/kg body weight) intragastrically for a period of 8 wk (Jindal and Gill, 1999). Ito et al. (1985) reported the inhibition of superoxide dismutase activity in the blood of workers occupationally exposed to lead, and in rats injected with a single dose of 10 or 20 mg of lead/kg body weight as lead acetate solution. Tissue specific changes in antioxidant enzymes have been reported following administration of a single dose of lead in developing chick embryos (Somashékaraiah et al., 1992). Although the kidney accumulates the maximum concentration of lead per g weight (Zmudzki et al., 1983) and liver acts as the major detoxifying organ, no reports are available with

which to compare the present findings. Withdrawal of lead did not bring about an appreciable change in antioxidant enzyme status until day 10 of the observation period (figure 3, 4, 7 and 8). This might be due to persistence of significantly higher lead levels in liver and kidneys (figures 1 and 2).

Changes in the activities of SOD and catalase were associated with a significant ($p<0.05$) increase in lipid peroxide levels in liver (40%) and a non-significant ($p>0.05$) increase in the kidney (35%). Lipid peroxidation is a basic change of the aging process, oxidative damage to tissues due to pollutants and oxygen toxicity (Somashékaraiah et al., 1992). Measurement of malondialdehyde (MDA) continues to be an useful method for determination of lipid peroxidation. Enhanced lipid peroxidation in the rat brain following acute and chronic exposure to lead, were attributed to depletion of antioxidant enzymes and richness of polyunsaturated fatty acids in brain tissue (Rehman, 1984; Jindal and Gill, 1999). Increased lipid peroxide levels in the serum were also reported in rats, following oral exposure to lead (Skoczynska et al., 1993), and in occupational workers (Ito et al., 1985). However, Lawton and Donaldson (1991) did not notice hepatic lipid peroxidation in chicks fed a diet containing less than 1000 ppm of lead. Similarly, Gelman and Michaelson (1979) concluded that the neurotoxic effects of lead in neonates are not associated with malondialdehyde

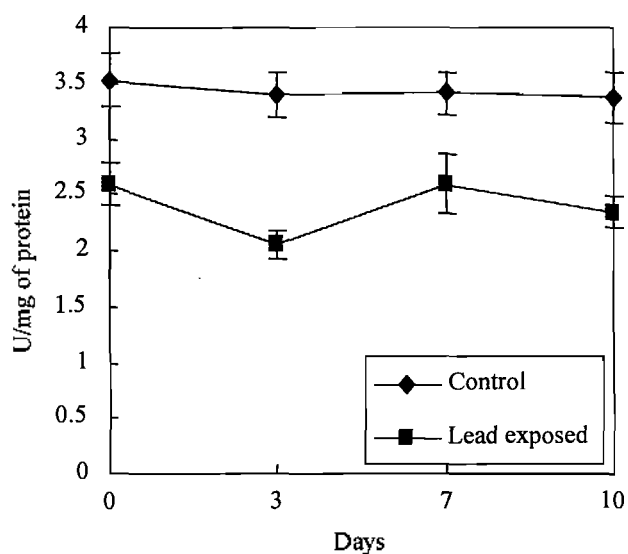


Figure 3. Liver SOD activities in lead exposed and unexposed rats. Six rats each from both the groups were sacrificed on day 0, 3, 7 and 10 after lead exposure for a period of 30 days at the dose rate of 1 mg of lead/2 ml/kg body weight administered intraperitoneally. Control rats received sterile normal saline solution.

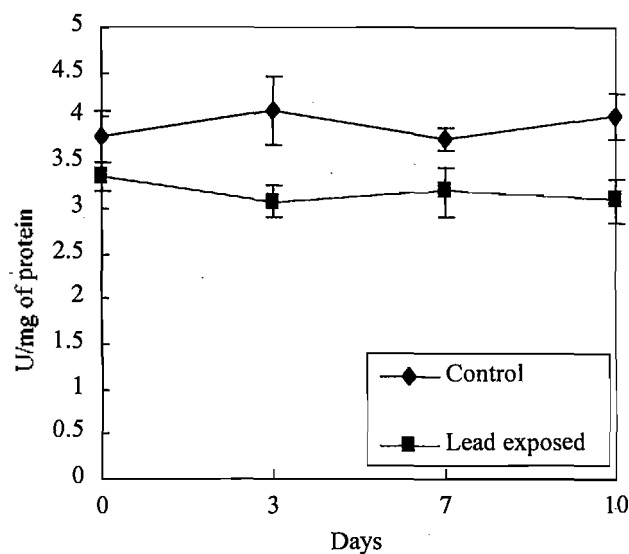


Figure 4. Kidney SOD activities in lead exposed and unexposed rats. Six rats each from both the groups were sacrificed on day 0, 3, 7 and 10 after lead exposure for a period of 30 days at the dose rate of 1 mg of lead/2 ml/kg body weight administered intraperitoneally. Control rats received sterile normal saline solution.

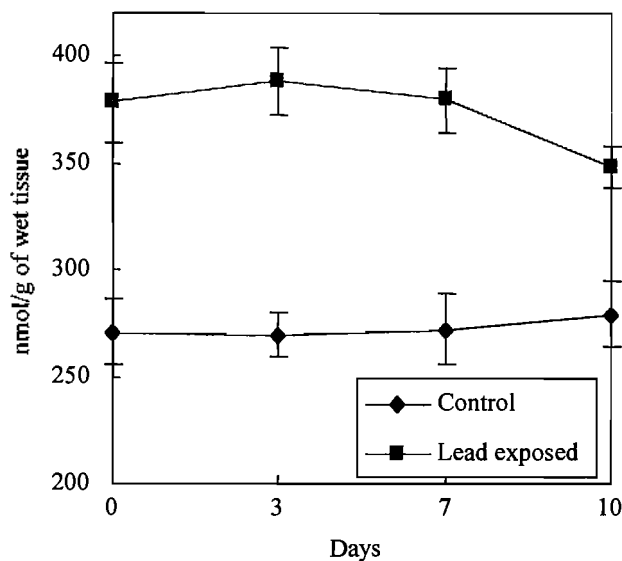


Figure 5. Lipid peroxides level in liver of lead exposed and unexposed rats. Six rats each from both the groups were sacrificed on day 0, 3, 7 and 10 after lead exposure for a period of 30 days at the dose rate of 1 mg of lead/2 ml/kg body weight administered intraperitoneally. Control rats received sterile normal saline solution.

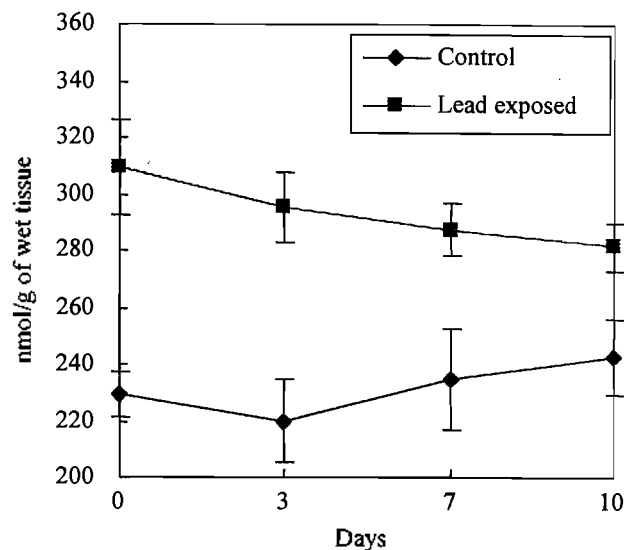


Figure 6. Lipid peroxides level in kidneys of lead exposed and unexposed rats. Six rats each from both the groups were sacrificed on day 0, 3, 7 and 10 after lead exposure for a period of 30 days at the dose rate of 1 mg of lead/2 ml/kg body weight administered intraperitoneally. Control rats received sterile normal saline solution.

formation. The present finding of an enhanced lipid peroxidation might have been due to enhanced generation of superoxide radicals (Monteiro et al., 1986), elevation of intracellular levels of calcium and an impairment of mitochondrial functions (Sandhir et al., 1994), and the inhibitory effects of lead on the activities of antioxidant enzymes. Removal of lead exposure did not reduce lipid peroxide level to normal even by day 10 of withdrawal (figure 5 and 6). Contrary to the present finding, Somashekarai et al. (1992) recorded a decrease in lipid peroxide (LPO) levels to a normal level in the liver, heart and brain of chick embryos after 72 hr of administration of a single dose of lead. In the present study, the duration of exposure was longer and the tissue level of lead continued to remain significantly higher until the last observation recorded. Further studies are required to be carried out for a longer period of time to find out the minimum post exposure period required to restore the alternation to normality.

Lead exposed rats revealed significant ($p < 0.05$) decreases in total and protein bound thiol groups in the liver (38% and 46%). Although decreases in these parameters were recorded in the kidney (20 and 39%), the changes were statistically non-significant ($p > 0.05$). Different biomolecules, including protein containing -SH groups, are capable of binding toxic metals and thus neutralize their toxic effects (Jimenez et al., 1997). The changes observed in the present study might be

due to interaction between lead and thiol groups in the liver and kidney. Our results are in agreement with those of Jindal and Gill (1999) who observed decreased -SH groups in brain following exposure to lead. Decreased thiol groups in liver following oral administration of lead and nickel are also on record (Vodichenska, 1992). Non-significant improvements in the total (T-SH) and protein bound thiol (P-SH) levels following withdrawal of the lead exposure (table 2) might have been due to reduced availability of unbound lead in the tissues.

Contrary to total and protein bound thiol groups, a reverse trend was observed with respect to non-protein bound thiol level. It increased significantly ($p < 0.05$) in the liver (66%) and non-significantly in the kidney (44%). Reduced glutathione is a tripeptide Glu-Cys-Gly which is present in millimolar concentration in most mammalian cells and plays a major role in detoxification and protects the cell from oxidative damages (Reed, 1990). Decreased glutathione levels were reported in the brain following experimental lead exposure in rats (Gill and Jindal, 1999). Sandhir et al. (1994) also reported a decline in NP-SH levels in the brain after 8 wk of oral administration of lead at a dose rate of 50 mg/kg body weight. However, increased glutathione levels were also reported in animal tissues following exposure to lead (Donaldson and Knowles, 1993). The enhanced NP-SH level in the present study, is in agreement with the findings of

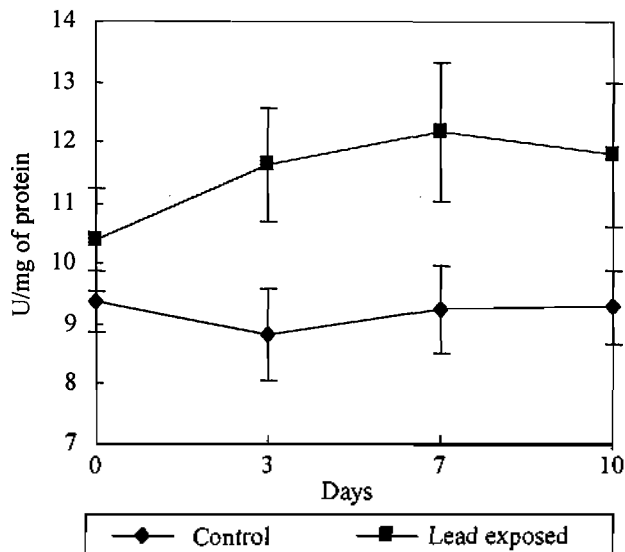


Figure 7. Liver catalase activity in lead exposed and unexposed rats. Six rats each from both the groups were sacrificed on day 0, 3, 7 and 10 after lead exposure for a period of 30 days at the dose rate of 1 mg of lead/2 ml/kg body weight administered intraperitoneally. Control rats received sterile normal saline solution.

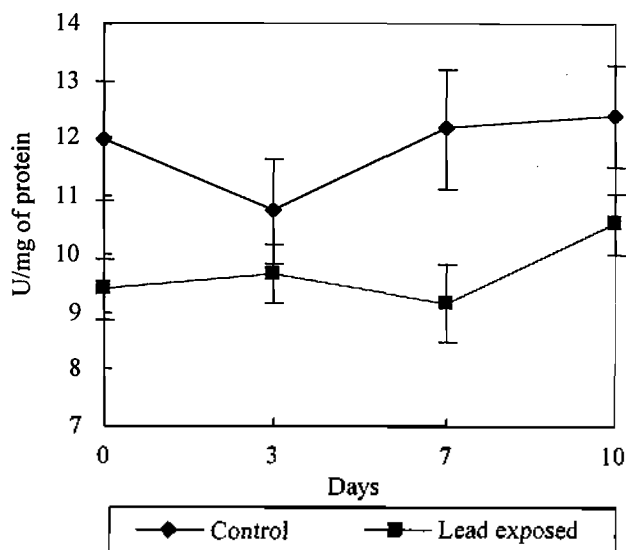


Figure 8. Kidney catalase activity in lead exposed and unexposed rats. Six rats each from both the groups were sacrificed on day 0, 3, 7 and 10 after lead exposure for a period of 30 days at the dose rate of 1 mg of lead/2 ml/kg body weight administered intraperitoneally. Control rats received sterile normal saline solution.

Table 2. Status of thiol groups ($\mu\text{mol/g}$ of wet tissue) in liver and kidney of rats on days after the end of the lead exposure

| Parameters | Samples | Group | Days | | | | SEM |
|------------|---------|-------|-------|-------|-------|-------|------|
| | | | 0 | 3 | 7 | 10 | |
| T-SH | Liver | C | 11.45 | 11.15 | 11.37 | 11.23 | 0.62 |
| | | E | 8.16 | 8.23 | 10.18 | 10.83 | 1.13 |
| | Kidney | C | 8.52 | 8.67 | 8.93 | 8.64 | 0.66 |
| | | E | 6.83 | 6.96 | 8.41 | 8.74 | 0.94 |
| NP-SH | Liver | C | 2.22 | 2.25 | 2.25 | 2.26 | 0.33 |
| | | E | 3.19 | 2.60 | 2.55 | 2.62 | 0.72 |
| | Kidney | C | 0.85 | 0.88 | 0.95 | 0.98 | 0.11 |
| | | E | 1.41 | 1.17 | 1.22 | 1.30 | 0.11 |
| P-SH | Liver | C | 9.23 | 8.90 | 9.12 | 8.79 | 0.54 |
| | | E | 4.96 | 5.62 | 7.62 | 8.21 | 1.17 |
| | Kidney | C | 7.68 | 7.79 | 8.01 | 7.66 | 0.65 |
| | | E | 5.42 | 5.79 | 7.34 | 7.44 | 0.97 |

Six rats were sacrificed from each group on day 0, 3, 7 and 10 after cessation of lead exposure. Lead was administered intraperitoneally at 1 mg/2 ml/kg b. wt. daily for a period of 30 days. Control rats received sterile normal saline solution at 2 ml/kg b. wt in an identical manner. C - Control, E - Lead exposed. * $p < 0.05$.

Donaldson and Knowles (1993). Cytoprotective effects of cells to synthesize more NP-SH, following exposure to very low doses of lead, for chelation of this biotoxicant and scavenging of free radicals (Ito et al., 1985; Skoczynsko et al., 1993) might have been responsible for the present observation.

In conclusion, our results indicate that the

magnitude of response with respect to oxidative stress indices following lead exposure was different between the liver and the kidney, and the withdrawal period of 10 d, following the present level of exposure, was not enough to restore the alterations to normality. The present finding contributes towards understanding of the pathotoxicity of lead and has therapeutic

implications for effective management of its toxicity in livestock.

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