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Post natal antioxidant enzyme activity of rat brain regions during developmental lead exposure

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Abstract: *This study reports the effects of low level developmental Pb exposure on specific brain regions like hippocampus, cerebellum and cerebral hemispheres of antioxidant enzyme activities. Wistar dams were exposed to 50 ppm, 100 ppm and 500 ppm of Pb acetate in drinking water during pregnancy and lactation (gestation day 6 through PND 21 (post natal day) and activities of superoxide dismutase (SOD), glutathione peroxidase (GSH–Px) and glutathione reductase (GR) were determined in the hippocampus, cerebellum and cerebral hemispheres of pups during treatment period (PND 7, 14, and 21 days) and also during withdrawl period (PND 35, 45, 60 and 90 days). During treatment period, SOD activity significantly ($p<0.05$) decreased in all regions of all the treated groups with maximum decrease in 500 ppm treated group of 21 days, while GSH–Px and GR activities increased with maximum increase in 21 days aged 500 ppm group. During withdrawl period, the activities of all enzymes were significantly ($p< 0.05$) reversed. Thus the perinatal exposure of dams to variable dosages of low level lead results in characteristic neurochemical allterations in rat brain regions due to impaired antioxidants function.*

Key words: *Oxidative stress, Neurotoxicity, Antioxidants, Pb exposure*

Introduction

The congenital lead poisoning in infants from third world countries is prevalent where lead is still widely used in gasoline, paints, pottery and cosmetics *(*Ghafour, 1984). Environmental exposure to Pb is also known to produce behavioural, physiological and biochemical deficiencies in human (Verity, 1995; Silbergeld, 1992). Chronic low level Pb exposure results in growth retardation, intellectual impairment and hyperactivity (Silbergeld and Goldberg, 1975; Shih and Hanin, 1978). Furthermore, neuronal manifestations are part of the serious consequences of Pb toxicity especially in children (Petit, 1986). The effects of low level lead exposure on the impairment of learning abilities, behaviour, intelligence and motor coordination of children were found (Rutter, 1980; Needleman and Bellinger, 1984; Pocock and Ashby, 1985). However, a longstanding issue has been the lowest level of exposure at which its effects on health can be reliably demonstrated.

The studies on oxidative stress in brain following Pb exposure were carried out in adult animals (Sandhir *et al.,* 1994; Adonaylo and Oteiza, 1999b) or in newborns exposed postnatal in high doses of P b (Valenzuela *et al.,* 1989). A few studies (Moreira *et al.,* 2001) have investigated the occurrence of oxidative stress in weaned and adult rats in some regions of brain at 500 ppm Pb after exposure during pregnancy and lactation. It is also found that lower level in utero lead exposure may be related to deficits in both foetal grown and post natal behavioural development (Bellinger *et al.,* 1986*;* Bornschein, 1986; Dietrich *et al.,*1986). Pb disrupts the normal development of the brain, causing reductions in cellular development in cerebellum (Cookman *et al.*, 1987; Hasan *et al.,* 1989; Regan, 1989), cerebral cortex (McCauley *et al.,* 1982) and hippocampus (Alfano *et al.,* 1982; Campbell *et al.,* 1983; Regan, 1989). However, there is no comprehensive information on effects of variable doses of Pb such as 50, 100 and 500 ppm on hippocampus, cerebellum and cerebral hemispheres in antioxidant enzyme activity.

The present study reports the effect of prenatal and postnatal treatment of variable doses of Pb (50, 100 and 500 ppm) on antioxidant, superoxide dismutase (SOD) glutathione peroxidase (GSH–Px) and glutathione reductase (GR) enzyme activities in hippocampus, cerebral hemispheres and cerebellum of rat at PND 7, 14, 21, as well as after withdrawl of Pb exposure at PND 35, 45, 60 and 90.

Materials and Methods

Animals and tissue preparation: Timed pregnant Wistar rats were obtained from NCLAS (National Centre for Laboratory Animal Sciences) of NIN (National Institute of Nutrition), Hyderabad and animals were maintained in polypropylene cages at 25 ± 2°C in 12 hr light/dark cycle and were fed the standard pellet diet for rat (Hindustan Lever Ltd., Lipton India) and water was given ad libitum until autopsy. Pb exposure was initiated on gestation day 6 with the addition of 50, 100 and 500 ppm lead acetate to double distilled water of the dam, and continued through PND 21. To prevent the formation of Pb precipitate double distilled deionized water was used. The treatment lasted throughout the pregnancy and lactation (*i.e.*, from GD 6 through PND 21) and maintained upto day 90 with withdrawl of Pb treatment from day 21 and animals were decapitated on PND 7, 14, 21, 35, 45, 60 and 90 days. Cerebral hemispheres, cerebellum and hippocampal regions of the brain were isolated and used to

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Table - 1(a): SOD activity in cerebral hemispheres of peri natal lead treated (GD 6 to PND 21) rat brain in different time periods

	Age of rats (days)						
	7	**14**	**21**	**35**	**45**	**60**	**90**
Control	0.222 ± 0.00088	0.237 ± 0.00602	0.256 ± 0.00057	0.265 ± 0.00145	0.275 ± 0.00088	0.286 ± 0.00088	0.299 ± 0.00176
50 ppm lead	0.218 ± 0.00057 (-1.80)	0.232 ± 0.00115 (-2.19)	0.249 ± 0.00115 (-2.73)	0.268 ± 0.00117 (1.13)	0.279 ± 0.00115 (1.45)	0.292 ± 0.00088 (2.09)	0.303± 0.00060 (2.67)
100 ppm lead	0.210 ± 0.00145 (-5.40)	0.223 ± 0.00120 (-5.9)	0.237 ± 0.00088 (-7.42)	0.272± 0.00055 (2.64)	0.283 ± 0.00115 (2.9)	0.296 ± 0.00115 (3.49)	0.311 ± 0.00088 (3.67)
500 ppm lead	0.206 ± 0.00348 (-7.20)	0.215 ± 0.00145 (-9.28)	0.222 ± 0.00115 (-13.28)	0.276± 0.0115 (3.77)	0.288 ± 0.00115 (4.72)	0.303 ± 0.00117 (5.59)	0.321 ± 0.00148 (7.35)

(Parenthesis: % variation) ; Values are expressed in mean ± S.E. of six individuals in each age group. The values are significant between groups, days and group Vs days at p<0.05 according to ANOVA

Table - 1(b): Glutathione peroxidase activity in cerebral hemisphere

	Age of rats (days)						
	7	**14**	**21**	**35**	**45**	**60**	**90**
Control	18.054 ± 0.0029	18.404 ± 0.0029	18.86 ± 0.0012	19.14 ± 0.0017	19.46 ± 0.0012	19.62 ± 0.0020	19.91 ± 0.0017
50 ppm lead	18.86 ± 0.00145 (4.48)	19.02 ± 0.00145 (3.2)	19.32 ± 0.00233 (2.6)	18.01 ± 0.00436 (-5.75)	18.15 ± 0.00491 (-6.7)	18.22 ± 0.00433 (-7.04)	18.36 ± 0.00491 (-7.73)
100 ppm lead	20.02 ± 0.00406 (10.80)	20.3 ± 0.00376 (10.32)	20.71 ± 0.00376 (10.10)	18.28 ± 0.00376 (-4.71)	18.35 ± 0.00346 (-5.4)	18.47 ± 0.00491 (-5.76)	18.59 ± 0.00406 (-6.58)
500 ppm lead	22.4 ± 0.00260 (24.01)	22.6 ± 0.00376 (22.82)	22.80 ± 0.333 (21.80)	18.7 ± 0.00173 (-2.09)	18.87 ± 0.00231 (-2.73)	18.94 ± 0.00145 (-3.36)	19.02 ± 0.00145 (-4.42)

(Parenthesis: % variation) ; Values are expressed in mean ± S.E. of six individuals in each age group. The values are significant between groups, days and group Vs days at p<0.05 according to ANOVA

Table - 1(c): Glutathione reductase activity in cerebral hemispheres

	Age of rats (days)						
	7	**14**	**21**	**35**	**45**	**60**	**90**
Control	15.144 ± 0.00115	15.204 ± 0.00115	15.343 ± 0.0014	15.388 ± 0.00173	15.416 ± 0.00115	15.54 ± 0.00115	15.7 ± 0.00115
50 ppm lead	15.58 ± 0.00145 (3.17)	15.63 ± 0.00145 (2.82)	15.67 ± 0.00115 (2.15)	15.14 ± 0.00176 (-1.56)	15.01 ± 0.00260 (-2.66)	14.9 ± 0.00173 (-4.5)	14.7 ± 0.00115 (-7.01)
100 ppm lead	16.11 ± 0.00203 (6.4)	16.16 ± 0.00145 (6.3)	16.20 ± 0.00115 (5.61)	14.87 ± 0.00145 (-3.31)	14.9 ± 0.00115 (-3.47)	14.6 ± 0.00173 (-5.8)	14.3 ± 0.00145 (-8.9)
500 ppm lead	17.2 ± 0.00115 (14.8)	17.52 ± 0.00115 (14.5)	17.52 ± 0.00203 (13.4)	14.33 ± 0.00173 (-6.8)	14.36 ± 0.00233 (-6.92)	14.2 ± 0.00145 (-8.3)	14.01 ± 0.00088 (-10.19)

(Parenthesis: % variation) ; Values are expressed in mean ± S.E. of six individuals in each age group. The values are significant between groups, days and group Vs days at p<0.05 according to ANOVA

Table - 2(a): SOD activity in cerebellum of perinatal lead treated (GD 6 to PND 21) rat brain in differential time periods

	Age of rats (days)						
	7	**14**	**21**	**35**	**45**	**60**	**90**
Control	0.301 ± 0.00173	0.310 ± 0.00088	0.322 ± 0.00145	0.331 ± 0.00120	0.344 ± 0.00088	0.362 ± 0.00173	0.387 ± 0.00088
50 ppm lead	0.296 ± 0.00059 (-1.66)	0.303 ± 0.00068 (-2.25)	0.311 ± 0.00067 (-3.10)	0.336 ± 0.00056 (1.20)	0.352 ± 0.00088 (2.41)	0.372 ± 0.00088 (3.59)	0.41 ± 0.00115 (5.94)
100 ppm lead	0.290 ± 0.00114 (-3.65)	0.302 ± 0.00115 (-3.22)	0.309 ± 0.00116 (-4.07)	0.343 ± 0.00088 (3.62)	0.358 ± 0.00088 (4.06)	0.377 ± 0.00115 (4.69)	0.43 ± 0.00058 (11.11)
500 ppm lead	0.284 ± 0.00115 (-5.64)	0.292 ± 0.00058 (-5.80)	0.299 ± 0.00115 (-7.14)	0.351 ± 0.00115 (6.04)	0.367 ± 0.00088 (6.68)	0.386 ± 0.00115 (7.45)	0.451 ± 0.00088 (16.27)

(Parenthesis: % variation) ; Values are expressed in mean ± S.E. of six individuals in each age group. The values are significant between groups, days and group Vs days at $p<0.05$ according to ANOVA

Table - 2(b): Glutathione peroxidase activity in cerebellum

	Age of rats (days)						
	7	**14**	**21**	**35**	**45**	**60**	**90**
Control	10.845 ± 0.00289	11.234 ± 0.00231	11.566 ± 0.00173	11.84 ± 0.00173	12.23 ± 0.00173	12.48 ± 0.00231	12.63 ± 0.00145
50 ppm lead	11.40 ± 0.00115 (5.5)	11.71 ± 0.00145 (4.46)	11.95 ± 0.00115 (3.9)	11.7 ± 0.00203 (-0.8)	11.95 ± 0.00145 (-1.96)	12.16 ± 0.00203 (-2.58)	12.23 ± 0.00145 (-3.16)
100 ppm lead	11.9 ± 0.00145 (10.18)	12.28 ± 0.00120 (9.64)	12.49 ± 0.00173 (8.60)	11.35 ± 0.00145 (-3.81)	11.58 ± 0.00115 (-5.08)	11.67 ± 0.00173 (-6.49)	11.78 ± 0.00115 (-6.73)
500 ppm lead	12.36 ± 0.00145 (14.11)	12.42 ± 0.00173 (10.80)	12.58 ± 0.00115 (9.39)	11.01 ± 0.00231 (-6.77)	11.24 ± 0.00173 (-7.86)	11.37 ± 0.000115 (-8.89)	11.45 ± 0.00145 (-9.34)

(Parenthesis: % variation) **;** Values are expressed in mean ± S.E. of six individuals in each age group. The values are significant between groups, days and group Vs days at $p<0.05$ according to ANOVA

Table - 2(c): Glutathione reductase ctivity in cerebellum

	Age of rats (days)						
	7	**14**	**21**	**35**	**45**	**60**	**90**
Control	21.448 ± 0.00176	21.503 ± 0.00145	21.602 ± 0.00176	21.783 ± 0.0020	21.86 ± 0.00145	21.92 ± 0.00115	22.01 ± 0.00145
50 ppm lead	22.5 ± 0.00173 (5.14)	22.3 ± 0.00145 (3.72)	22.01 ± 0.00088 (1.85)	21.3 ± 0.00176 (-1.84)	21.92 ± 0.00536 (-3.65)	20.81 ± 0.00173 (-5.02)	20.5 ± 0.00115 (-6.81)
100 ppm lead	23.2 ± 0.00058 (10.28)	22.91 ± 0.00145 (6.5)	22.7 ± 0.00173 (5.09)	20.04 ± 0.00145 (-7.64)	19.93 ± 0.00115 (-8.71)	19.72 ± 0.00116 (-10.04)	19.5 ± 0.00115 (-11.3)
500 ppm lead	24.40 ± 0.00115 (14.48)	24.20 ± 0.00173 (12.89)	24.01 ± 0.00115 (11.11)	19.41 ± 0.00115 (-10.5)	19.2 ± 0.00088 (-11.92)	19.01 ± 0.00145 (-13.24)	18.98 ± 0.00088 (-13.72)

(Parenthesis: % variation) ; Values are expressed in mean ± S.E. of six individuals in each age group. The values are significant between groups, days and group Vs days at $p<0.05$ according to ANOVA

Table - 3(a): SOD activity in hippocampus

	Age of rats (days)						
	7	**14**	**21**	**35**	**45**	**60**	**90**
Control	0.572 ± 0.00115	0.581 ± 0.00125	0.588 ± 0.0310	0.598 ± 0.00115	0.621 ± 0.00121	0.644 ± 0.00119	0.661 ± 0.0117
50 ppm lead	0.567 ± 0.00176 (-0.87)	0.572 ± 0.00115 (-1.73)	0.578 ± 0.00145 (-2.06)	0.601 ± 0.00115 (0.50)	0.626 ± 0.00088 (0.80)	0.652 ± 0.00011 (1.24)	0.672 ± 0.00058 (1.66)
100 ppm lead	0.560 ± 0.00117 (-2.09)	0.565 ± 0.00067 (-2.73)	0.571 ± 0.00115 (-2.89)	0.605 ± 0.00173 (1.17)	0.630 ± 0.0115 (1.44)	0.660 ± 0.00115 (2.48)	0.683 ± 0.00115 (3.32)
500 ppm lead	0.552 ± 0.00116 (-3.49)	0.560 ± 0.00115 (-3.61)	0.566 ± 0.00067 (-3.74)	0.612 ± 0.00115 (2.34)	0.641 ± 0.00115 (3.22)	0.674 ± 0.00058 (4.34)	0.695 ± 0.00011 (5.14)

(Parenthesis: % variation) ; Values are expressed in mean ± S.E. of six individuals in each age group. The values are significant between groups, days and group Vs days at $p<0.05$ according to ANOVA

Table - 3(b): Glutathione peroxidase activity in hippocampus

	Age of rats (days)						
	7	**14**	**21**	**35**	**45**	**60**	**90**
Control	14.19 ± 0.00088	14.324 ± 0.00115	14.46 ± 0.00115	14.83 ± 0.00173	15.16 ± 0.00173	15.47 ± 0.00173	15.72 ± 0.00145
50 ppm lead	14.38 ± 0.00084 (1.9)	14.41 ± 0.00173 (0.83)	14.57 ± 0.00088 (0.76)	14.62 ± 0.00145 (-12.1)	14.9 ± 0.00115 (-1.71)	15.11 ± 0.00145 (-2.32)	15.29 ± 0.00173 (-2.67)
100 ppm lead	14.68 ± 0.00145 (3.45)	14.77 ± 0.00088 (3.10)	14.63 ± 0.00115 (2.5)	14.621 ± 0.00173 (-1.41)	14.78 ± 0.00115 (-2.50)	14.83 ± 0.00115 (-4.13)	14.97 ± 0.00120 (-4.77)
500 ppm lead	14.97 ± 0.00115 (5.49)	15.01 ± 0.0015 (4.8)	15.12 ± 0.00115 (4.56)	14.43 ± 0.00145 (-2.5)	14.57 ± 0.00115 (-3.75)	14.68 ± 0.00116 (-5.10)	14.79 ± 0.00173 (-5.85)

(Parenthesis: % variation) ; Values are expressed in mean ± S.E. of six individuals in each age group. The values are significant between groups, days and group Vs days at $p<0.05$ according to ANOVA

Table - 3(c): Glutathione reductase activity in hippocampus

	Age of rats (days)						
	7	**14**	**21**	**35**	**45**	**60**	**90**
Control	24.114 ± 0.00115	24.254 ± 0.00176	24.367 ± 0.00115	24.586 ± 0.00115	24.77 ± 0.00115	24.923 ± 0.00145	25.041 ± 0.00145
50 ppm lead	24.9 ± 0.00145 (3.31)	24.71 ± 0.00115 (1.85)	24.55 ± 0.00115 (0.77)	24.32 ± 0.00145 (-1.07)	24.06 ± 0.00145 (-2.83)	23.5 ± 0.00173 (-5.62)	23.02 ± 0.00115 (-8.14)
100 ppm lead	25.21 ± 0.00145 (4.56)	25.01 ± 0.00145 (3.09)	24.88 ± 0.203 (2.13)	23.8 ± 0.00173 (-3.17)	23.5 ± 0.00115 (-4.85)	23.1 ± 0.00115 (-7.22)	22.8 ± 0.00203 (-8.94)
500 ppm lead	25.6 ± 0.00173 (6.63)	25.4 ± 0.00115 (4.74)	25.2 ± 0.00115 (3.44)	22.6 ± 0.00145 (-8.05)	22.4 ± 0.00173 (-9.31)	22.3 ± 0.00145 (-10.44)	22.01 ± 0.00145 (-12.64)

(Parenthesis: % variation) ; Values are expressed in mean ± S.E. of six individuals in each age group. The values are significant between groups, days and group Vs days at $p<0.05$ according to ANOVA

estimate the following enzymes. The Pb exposure regimen was chosen based on the previous study (Zawia *et al.,* 1998). Pups were weaned at 22 days of age on tap water.

At 7, 14, 21, 35, 45, 60 and 90 days of age, pups were anesthetized with sodium pentobarbital (50 $mgkg^{-1}$ intraperitonially). After perfusion through the ascending aorta with 140 mM phosphate buffer saline (PBS) pH 7.4, the brain was removed, dissected and different regions were isolated. The tissue was weighed and homogenized (1mg tissue/4 ml buffer) in ice cold 140 mM PBS using 10 stokes in a teflon/glass homogenizer. The tissue was maintained at 0–4 °C throughout the dissection and homogenization procedures. The homogenate was centrifuged for 15 min at 1000 g at 4°C and the supernatant was centrifuged again at 18000 g for 15 min at 4°C.

Enzyme assays: The final supernatant was used for the evaluation of the activity of SOD (enzyme activity was assayed according to the method of Marklund and Marklund (1974)), GSH–Px activity was measured as per the method of Reddy *et al.* (1981) and GR activity was measured according to the method of Corlberg and Mannervik (1981). Protein content was determined by the method of Lowry *et al.* (1951). Activity of SOD was expressed as units/mg protein/ min. The GSH–Px enzyme activity was expressed as moles of NADPH oxidized/mg protein/min. Activity of the GR enzyme was expressed as μ moles of NADPH oxidized/ mg protein/min.

Statistical treatment of the data: The experimental data was analyzed statistically, mean and S.E. were calculated. Three ways ANOVA was used to assess the antioxidant enzyme activities. Level of statistical significance was set at 0.05 % and analyzed the conformation among variable dosage, age dependent and tissue specific effects.

Results and Discussion

Antioxidant enzyme activities of cerebral hemispheres: The effect of Pb exposure during pregnancy and lactation on the antioxidant enzymes activity of cerebral hemispheres is shown in Table 1. The three way ANOVA indicated an interaction between age and variable dosage of exposure in activities of SOD ($p<0.05$), GSH–Px ($p<0.05$), and GR ($p<0.05$). Post hoc analysis showed that SOD decreased ($p<0.05$) in all the Pb exposed groups in all regions with highest decrement in 500 ppm treated 21 days groups followed by 100 ppm and 50 ppm groups. Where as, this decrement reversed during withdrawl period in all groups of all regions. GSH–Px and GR activities increased ($p<0.05$) during Pb treatment period with highest increment observed in 500 ppm lead treated 21 days group and this activity reversed during withdrawl period in all the treated groups and regions with the maximum recovery ($p<0.05$) observed in 50 ppm lead treated 90 days group followed by 100 and 500 ppm groups.

Antioxidant enzyme activities of cerebellum: The effect of Pb exposure during pregnancy and lactation on the antioxidant enzymes activity of cerebellum is shown in Table 2. The three way ANOVA indicated a significant effect of dose and age in activities of SOD, GSH–Px and GR ($p<0.05$). SOD activity decreased in all the treated groups during treatment period with highest decrement in 500 ppm treated groups followed by 100 ppm and 50 ppm groups ($p<0.05$) and increased during withdrawl period with highest increment in 50 ppm group followed by 100 ppm and 500 ppm groups. Where as, GSH–Px and GR activities increased during treatment period with maximum increase in 500 ppm lead treated group followed by 100 ppm and 50 ppm groups ($p<0.05$) and decreased during withdrawl period with maximum decrease observed in 50 ppm lead treated 90 days group ($p<0.05$) followed by 100 ppm and 500 ppm groups.

Antioxidant enzyme activities of hippocampus: The effect of Pb exposure during pregnancy and lactation on the antioxidant enzymes activity of hippocampus is shown in Table 3. The three way ANOVA indicated a significant effect of dose and age in the activities of SOD, GSH–Px and GR ($p<0.05$). SOD activity ($p<0.05$) decreased in all the treated groups with highest decrement observed in 500 ppm lead treated groups of 21 days followed by 100 ppm and 50 ppm groups and the enzyme activity reversed during withdrawl period in all groups with maximum increase in 50 ppm treated 90 days groups followed by 100 ppm and 500 ppm groups. GSH-Px and GR activities increased ($p<0.05$) in all the treated groups during treatment period with maximum increment observed in 500 ppm lead treated group followed by 100 ppm and 50 ppm groups of 21 days and decreased during withdrawl period in all groups with maximum decrement in 50 ppm treated 90 days group followed by 100 ppm and 500 ppm treated groups ($p<0.05$).

During treatment period, 500 ppm lead treated group showed maximum decrease in SOD activity in 21 days followed by 100 ppm and 50 ppm groups respectively. Where as, 500 ppm treatment resulted in increment of enzyme activity in GSH–Px and GR activities with maximum increase in 21 days age group followed by 100 ppm and 50 ppm groups. Hippocampus accounted for highest SOD activity followed by cerebellum and cerebral hemispheres. GSH–Px showed maximum activity in cerebral hemispheres followed by hippocampus and cerebellum where as, GR accounted for maximum activity in hippocampus followed by cerebellum and cerebral hemispheres. GR activity followed by GSH–Px and SOD observed in all the treated regions showed tissue specific changes during treatment and withdrawl period in all the enzymes evaluated.

Neurotoxic chemicals that may have temporary ill effect on an adult brain can cause enduring damage to a child's developing brain (NRC, 1993; Needleman *et al.,* 1990). The immaturity of children's internal systems especially the first few months of life, affects their ability to neutralize and to get rid off certain toxics from their bodies. If cells in the developing brain are destroyed by lead (ATSDR, 1993), mercury or other

neurotoxic chemicals or if vital connections between nerve cells fail to form, the damage is likely to be permanent and irreversible. This may mean a loss of intelligence and alteration of normal behaviour (ATSDR, 1993; Gilbert *et al.,* 1995). Adverse effects produced during pregnancy and childhood or developmental toxicity are an obvious societal and public health concern.

The enzymes of antioxidant system in three brain regions (cerebral hemispheres, cerebellum and hippocampus) of weaned and adult rats exposed during pregnancy and lactation to low level variable dosages of Pb were chosen to evaluate instead of whole brain because different regions may respond differently to oxidative stress (Sandhir *et al.,* 1994). In weaned rats, effects of Pb exposure induced statistically significant changes in all enzymes evaluated in all regions. The observed enzyme activities indicate that free radical generation is progressively increased with the increase in exposure spans in all the regions during treatment period but decreased during withdrawl period. During treatment period, SOD activity indicated a decrement in all days of exposure in all the treated regions suggesting the responsiveness of SOD to the oxyradicals generated by lead during treatment period. Reversal of SOD activity during withdrawl period suggests the lesser body burden of lead due to withdrawl of treatment. Where as, GSH–Px and GR activities increased in all the treated groups during treatment period which indicates their responsiveness during the lead intoxication.

The GSH–Px as a selenium containing protein, in which selenium is known to play a very important role in increasing the catalytic efficiency of GSH–Px. During the lead toxicosis, the accumulated lead ions are expected to replace or interact with selenium of GSH–Px thereby increasing its catalytic action. Another reason for the increased GSH–Px activity is due to the possibility of formation of GSH–Px enzyme cofactor metal ion complex in which heavy metals like lead competitively inhibits the activity of GSH–Px. The increase of GR activity could also be considered as one of reasons for the increase of GSH–Px activity in addition to the suspected replacement of selenium by lead. Similar reports of heavy metals interaction with selenium in GSH–Px resulting in its inactivation circle reported (Splittgerber and Tappel, 1979). Therefore, the lead interaction with the metalions or cofactors associated with the enzymes may disturb the catalytic action of GSH–Px.

The activity of GSH–Px needs continuous supply of reduced glutathione. The continuous supply of glutathione is augmented by the glutathione reductase (GR) activity. In the present study, the GR activity increased significantly during the increased exposure dose in all the tissues. This increase in GR activity leads to increased availability of glutathione. Therefore, the increment of GR activity could also be considered as one of the reasons for the increment of GSH–Px activity with the suspected replacement of selenium by lead. Where as, this GR activity remained unaffected in liver, kidney and erythrocytes during lead poisoning (Hsu, *1981*) in newborns exposed postnatal to high doses of lead. Hermes Lima *et al.* (1991) and Sandhir *et al. (*1994*)* reported increase in glutathione activity supporting the results of present study.

Therefore, it may be concluded that lead intoxication induced dose dependent and region specific variation in three regions of brain during application and withdrawl of doses. The dose dependent variations could be attributed to the relative bioaccumulation of lead in different regions of brain depending upon the exposure period and characteristic region of brain and also its specificity.

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