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Influence of Calcium and Zinc on Lead-induced Alterations in ATPases in the Developing Mouse Brain

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Abstract: Developing nervous system has long been recognized as a primary target for lead (Pb^{2+}) induced toxicity. We have examined the effect of calcium (Ca^{2+}) and zinc (Zn^{2+}) supplementation on Pb^{2+}-induced alterations in adenosine triphosphatase (ATPase) activities in the developing mouse brain from postnatal day (PND) 14 to 90. Albino mice were lactationally exposed to low (0.2%) and high (1%) levels of lead acetate via the drinking water of the mother. Pb^{2+}-exposure was commenced on PND 1, continued up to PND 21 and stopped at weaning. Ca^{2+} and Zn^{2+} (0.02% in 0.2% Pb^{2+}-water or 0.1% in 1% Pb^{2+}-water) were supplemented separately to the mother up to PND 21. The activities of Mg^{2+}ATPase in mitochondrial fraction and Na^+K^+ATPase in P_2-fraction of the cerebral cortex, hippocampus, cerebellum and medulla were assayed. The specific activity of both ATPases increased gradually in an age-dependent manner in the control brain. Pb^{2+}-exposure significantly inhibited the activities of both Mg^{2+}ATPase and Na^+K^+ATPase in different brain regions of the developing brain. The inhibition was more pronounced at PND 28 in the brain regions of mice exposed to 1% Pb^{2+}. Ca^{2+} or Zn^{2+}-supplementation significantly reversed the Pb^{2+}-inhibited activity of both ATPases. These results indicate that dietary Ca^{2+}/Zn^{2+}-supplementation decreases intestinal absorption of Pb^{2+} and there by reduces the neurotoxic effects of Pb^{2+}.

Key words: Calcium-supplementation, zinc-supplementation, lead-exposure, ATPase, Mouse brain

Introduction

Na^+K^+ATPase and Mg^{2+}ATPase have a relatively high sensitivity to certain classes of heavy metals and other pollutants and it has been shown that toxicosis from pollutants may develop primarily from ATPase inhibition (Sushma, 1999). A number of studies have shown that ATPase activity was inhibited by heavy metals such as Pb^{2+} (Singerman, 1976), cadmium (Valle and Ulmer, 1972; Tucker and Matte, 1980) and methyl mercury (Schmidt-Niensen, 1974). Heavy metal binding to sulfhydryl (-SH) groups has often been implicated in the inhibition of Na^+K^+ATPase which maintains cellular fluid balance and provides the electrochemical gradients essential for synaptic potentials and action potentials (Sweadner and Goldin, 1980; Carfagna *et al*., 1996).

Pb^{2+} is known to exert its neurotoxic effects by competing with Ca^{2+} for its receptors coupled with second messenger functions (Hammond *et al*., 1984; Bressler and Goldstein, 1991) and in some

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cases, inhibits the actions of Ca^{2+} as a regulator of cell function (Bressler and Goldstein, 1991). Bogden *et al*. (1992) have demonstrated that a dietary excess of Ca^{2+} reduces Pb^{2+} -absorption. However, this effect is not as dramatic as the effect of Ca^{2+} insufficiency on Pb^{2+} uptake and retention. Therefore, the achievement of adequate rather than excessive dietary Ca^{2+} seems to be more useful in combating Pb^{2+}-intoxication (Peraza *et al*., 1998). Similar to Ca^{2+}, Zn^{2+} also competes for Pb^{2+} and effectively reduces the availability of binding sites for its uptake (Flora and Tandon, 1990). Therefore, present study was designed to examine the influence of Ca^{2+} and Zn^{2+} on Pb^{2+}-induced alterations in the specific activities of Mg^{2+} ATPase and Na^+K^+ ATPase in different regions of developing mouse brain.

Materials and Methods

Chemicals

Chemicals used in this study were obtained from Sigma, St. Louis, MO, USA.

Animal Exposure

Swiss albino pregnant mice were purchased from Indian Institute of Science, Bangalore, India. All pups were pooled on PND 1 and new litters consisting of eight males were randomly selected and placed with each dam. Young mice were lactationally exposed to low-level (0.2%) and high-level (1%) Pb^{2+} by adding lead acetate to deionized drinking water of the mother. Pb^{2+}-exposure was commenced on PND 1, continued up to PND 21 and stopped at weaning. Control mice received only deionized water without Pb^{2+}.

Calcium and Zinc-Supplementation

Ca^{2+}/Zn^{2+}-supplemented to Pb^{2+} (0.02% in 0.2% Pb^{2+}-water and 0.1% in 1% Pb^{2+}-water) was separately given to mothers upto PND 21 and stopped at weaning.

Isolation of Tissues

The control, Pb^{2+}-treated and Ca^{2+}/Zn^{2+}-supplemented mice were sacrificed at PND 14, PND 21, PND 28 and at 3 months age. The different brain regions such as cortex, hippocampus, cerebellum and medulla were quickly isolated under ice cold conditions and were stored at -80°C for biochemical analysis.

Preparation of Mitochondrial and P_2 fractions

The mitochondrial and P_2 fractions of cortex, hippocampus, cerebellum and medulla were prepared using ficoll-sucrose gradients as described by Cotman and Matthews (1971).

Determination of ATPase Activity

The specific activities of Na^+K^+ ATPase in P_2 fraction and mitochondrial Mg^{2+}ATPase were determined as described by Tirri *et al*. (1973). The enzyme activity was expressed as μmoles of inorganic phosphate (Pi) formed/mg protein/hour. The Pi was estimated by the method of Fiske and Subba Row (1925). The protein content was estimated by the method of Lowry *et al*. (1951).

Statistical analysis

Standard statistical procedures such as student t-test and ANOVA were used to analyze the data for significance level.

Results

The specific activities of Mg^{2+} ATPase and Na^+ K^+ ATPase in brain regions of the control mice increased with increase in age from PND 14 to 3 months (Table 1 and 2). The cortex region documented higher enzyme activities followed by cerebellum, hippocampus and medulla. Pb^{2+} decreased the specific activities of both Mg^{2+} ATPase and Na^+K^+ ATPase in all the brain regions examined. The inhibition was greater in the brain of mice exposed to 1% Pb^{2+} as compared to the mice exposed to 0.2% Pb^{2+}. The decrease in Mg^{2+} ATPase was more pronounced in the hippocampus of mice exposed to 1% Pb^{2+} where as the maximum inhibition of Na^+K^+ ATPase was observed in cerebellum (Table 1 and 2). The decrease in enzyme activities was found to be higher at PND 14. However, the enzyme activity slowly increased and reached almost control levels in three months old brain.

The data show that the Ca^{2+}/Zn^{2+}-supplementation reduced the Pb^{2+}-inhibition of Mg^{2+} ATPase and Na^+ K^+ ATPase activities observed in all the brain regions of mice (Table 1 and 2). The protective effect of Ca^{2+}/Zn^{2+}-supplementation from Pb^{2+}-toxicity was greater in the mice exposed to 0.2% Pb^{2+}

Table 1: Alterations in Mg^{2+}ATPase activities in brain of young and adult mice exposed to Pb^{2+} and supplemented with different concentrations of Ca^{2+} or Zn^{2+}

PND	Tissue	Control	0.2% Pb^{2+}	0.2% Pb^{2+}+Ca^{2+}	0.2% Pb^{2+}+Zn^{2+}	1% Pb^{2+}	1% Pb^{2+}+Ca^{2+}	1% Pb^{2+}+Zn^{2+}
14	Cerebral	25.8±1.9	18.0±1.3	22.3±1.5	21.3±1.3	15.8±0.9	21.5±1.3	17.9±0.9
	Cortex		(-30.2)	(-13.5)	(-17.4)	(-38.7)	(-16.6)	(-30.6)
	Hippocampus	22.9±1.6	15.7±1.0	18.2±0.6	16.6±0.9	12.3±0.8	16.7±0.8	14.6±0.6
			(-31.4)	(-20.5)	(-27.5)	(-46.2)	(-27.0)	(-36.2)
	Cerebellum	24.7±1.5	19.6±0.8	21.7±0.9	20.5±1.1	15.1±1.1	20.4±1.6	19.1±1.4
			(-20.6)	(-12.1)	(-17.0)	(-38.8)	(-17.4)	(-22.6)
	Medulla	21.3±1.3	17.2±1.0	18.9±0.9	17.8±0.9	14.5±0.8	16.7±0.9	5.1±0.6
			(-19.2)	(-11.2)	(-16.4)	(-31.9)	(-21.5)	(-29.1)
21	Cerebral Cortex	31.9±2.1	16.4±0.9	24.9±1.1	23.4±1.0	14.4±0.7	4.1±1.6	22.3±1.5
			(-48.5)	(-21.9)	(-26.6)	(-54.8)	(-24.4)	(-30.0)
	Hippocampus	29.3±2.0	14.8±0.9	22.9±1.2	18.1±0.6	11.7±0.5	20.5±1.4	6.3±0.9
			(-49.4)	(-21.8)	(-38.2)	(-60.0)	(-30.0)	(-44.3)
	Cerebellum	30.8±1.8	18.5±1.3	24.6±1.3	21.3±1.0	13.2±0.8	22.4±1.6	0.3±1.2
			(-39.9)	(-20.1)	(-30.8)	(-57.1)	(-27.2)	(-34.0)
	Medulla	27.0±2.0	16.5±1.1	21.9±1.3	19.8±1.4	13.2±0.9	19.8±1.0	8.4±1.3
			(-38.8)	(-18.8)	(-26.6)	(-51.1)	(-26.6)	(-31.8)
28	Cerebral Cortex	33.0±2.3	15.8±0.8	25.6±1.1	24.7±1.6	10.6±0.4	4.4±1.6	23.2±1.2
			(-52.1)	(-22.4)	(-25.1)	(-67.8)	(-26.0)	(-29.6)
	Hippocampus	31.1±2.3	12.2±0.5	23.7±1.7	18.8±0.7	8.5±0.1	21.1±1.3	7.5±1.0
			(-60.7)	(-23.7)	(-39.5)	(-72.6)	(-32.1)	(-43.7)
	Cerebellum	32.6±2.4	16.0±0.3	25.2±0.9	22.5±1.8	11.5±0.9	23.1±1.4	21.3±1.6
			(-50.9)	(-22.6)	(-30.9)	(-64.7)	(-29.1)	(-34.6)
	Medulla	28.7±1.5	16.3±0.9	22.7±1.1	20.9±0.9	11.8±0.7	20.3±1.5	19.5±1.3
			(-43.2)	(-20.9)	(-27.1)	(-58.8)	(-29.2)	(-32.0)
90	Cerebral Cortex	36.8±1.5	23.3±1.1	29.9±1.2	27.7±1.4	21.9±1.3	27.8±1.5	25.6±1.3
			(-36.6)	(-18.7)	(-24.7)	(-40.4)	(-24.4)	(-30.4)
	Hippocampus	34.6±2.4	18.8±0.9	27.5±1.1	23.6±1.5	13.8±0.8	24.8±1.3	22.5±1.1
			(-45.6)	(-20.5)	(-31.7)	(-60.1)	(-28.3)	(-34.9)
	Cerebellum	35.15±1.7	21.2±0.9	27.3±0.7	25.5±1.7	22.0±1.3	25.1±1.4	24.6±1.3
			(-39.6)	(-22.2)	(-27.3)	(-37.3)	(-28.4)	(-29.9)
	Medulla	31.6±2.0	19.7±0.9	26.3±1.2	24.1±0.9	16.9±0.9	23.7±1.5	21.8±1.1
			(-37.6)	(-16.7)	(-23.7)	(-46.5)	(-25.0)	(-31.0)

(Each value is mean±6 observations. PND: Postnatal day. Values in parentheses are % change over control. All changes over respective controls are statistically significant ($p<0.5$))

Table 2: Alterations in Na^+K^+ATPase activities in brain of young and adult mice exposed to Pb^{2+} and supplemented with different concentrations of Ca^{2+} or Zn^{2+}

PND	Tissue	Control	0.2% Pb^{2+}	0.2% Pb^{2+}+Ca^{2+}	0.2% Pb^{2+}+Zn^{2+}	1% Pb^{2+}	1% Pb^{2+}+Ca^{2+}	1% Pb^{2+}+Zn^{2+}
14	Cerebral	21.2±1.2	15.3±0.6	16.8±0.7	15.7±0.9	12.4±0.5	15.0±0.8	14.1±0.5
	Cortex		(-27.8)	(-20.7)	(-25.9)	(-41.5)	(-29.2)	(-33.4)
	Hippocampus	19.6±1.3	15.7±0.9	17.4±0.9	16.1±0.8	13.5±0.6	16.0±0.9	14.1±0.6
			(-19.8)	(-11.2)	(-17.8)	(-31.1)	(-18.3)	(-28.0)
	Cerebellum	18.8±1.3	13.8±0.6	16.4±1.0	15.1±0.8	10.6±0.3	15.0±0.9	13.2±0.6
			(-26.5)	(-12.7)	(-19.6)	(-43.6)	(-20.2)	(-29.7)
	Medulla	16.2±0.8	12.7±0.6	14.4±0.7	13.1±0.6	8.9±0.4	13.1±0.7	12.0±0.6
			(-21.6)	(-11.1)	(-19.1)	(-45.0)	(-19.1)	(-25.9)
21	Cerebral	25.1±1.3	12.6±0.8	17.7±0.8	16.5±0.9	10.5±0.7	16.8±0.9	16.0±1.1
	Cortex		(-49.8)	(-29.4)	(-34.2)	(-58.1)	(-33.0)	(-36.2)
	Hippocampus	23.9±1.8	14.4±0.8	20.1±1.4	18.1±0.9	9.8±0.5	18.7±0.7	16.2±1.0
			(-39.7)	(-15.8)	(-24.2)	(-58.9)	(-21.7)	(-32.2)
	Cerebellum	23.6±1.3	10.3±0.6	17.2±1.2	16.4±1.0	8.9±0.4	16.7±0.9	14.4±0.8
			(-56.3)	(-27.1)	(-30.5)	(-62.2)	(-29.2)	(-38.9)
	Medulla	21.4±1.4	10.8±0.6	16.2±0.9	15.8±0.7	7.9±0.3	15.5±1.0	13.3±0.9
			(-49.5)	(-24.2)	(-26.1)	(-63.0)	(-27.5)	(-37.8)
28	Cerebral Cortex	28.5±2.0	11.8±0.4	20.3±1.2	18.7±1.0	8.4±0.3	19.3±1.4	17.9±1.1
			(-58.5)	(-28.7)	(-34.3)	(-70.4)	(-32.2)	(-37.1)
	Hippocampus	27.9±1.7	14.1±0.5	22.3±1.5	21.4±1.3	8.7±0.4	20.9±1.1	18.6±1.3
			(-49.4)	(-20.0)	(-23.2)	(-68.8)	(-25.0)	(-33.3)
	Cerebellum	28.1±1.8	9.4±0.5	19.3±1.1	18.2±1.0	6.2±0.2	17.1±1.4	16.7±0.9
			(-66.5)	(-31.3)	(-35.2)	(-77.9)	(-39.1)	(-40.5)
	Medulla	25.5±1.7	9.7±0.2	17.4±0.9	16.2±0.9	7.2±0.2	16.8±0.7	15.3±1.0
			(-61.9)	(-31.7)	(-36.4)	(-71.7)	(-34.1)	(-40.0)
90	Cerebral Cortex	32.4±2.4	18.7±0.7	24.8±1.6	21.7±1.4	15.3±0.9	23.4±1.5	21.7±1.7
			(-42.2)	(-23.4)	(-33.0)	(-52.7)	(-27.7)	(-33.0)
	Hippocampus	30.9±1.8	17.3±1.0	25.3±1.7	24.2±1.8	13.3±0.7	24.3±1.6	20.7±1.4
			(44.0)	(-18.1)	(-21.6)	(-56.9)	(-21.3)	(-33.0)
	Cerebellum	31.4±2.3	17.3±1.3	27.8±1.6	23.5±1.3	13.2 ± 0.6	25.28±1.8	19.9±1.3
			(-44.9)	(-11.4)	(-25.1)	(-57.9)	(-19.7)	(-36.6)
	Medulla	27.6±1.9	17.1±1.0	20.7 ± 1.4	18.6±0.8	14.2 ± 0.8	19.1±1.0	18.3±0.9
			(-38.0)	(-25.0)	(-32.6)	(-48.5)	(-30.7)	(-33.6)

(Each value is mean ± 6 observations. PND: Postnatal day. Values in parentheses are % change over control. All changes over respective controls are statistically significant ($p<0.5$))

as compared to 1% Pb^{2+}. The mice supplemented with Ca^{2+} exhibited greater recovery of Pb^{2+}-inhibited ATPase activity as compared to the mice supplemented with Zn^{2+}. The alterations in both the enzyme activities were found to be statistically significant (Table 1 and 2).

Discussion

Heavy metal cations such as Pb^{2+} can bind to a number of sites on proteins including imidazole, histidyl, carbonyl and especially -SH side chains (Winder and Kitchen, 1984). Pb^{2+} has been reported to inhibit Na^+K^+ ATPase of mammalian tissues (Cardone *et al*., 1971) and also interferes with mitochondrial function and blocks the O_2 uptake. In the present study, lactational Pb^{2+}-exposure exerted inhibitory effect on both Mg^{2+} ATPase and Na^+K^+ ATPases in the developing mouse brain in an age- and dose-dependent manner. The decrease in the Mg^{2+}ATPase activity could be attributed to the Pb^{2+}-induced modulations in the oxidative metabolism (Boyer, 1977). Alterations in mitochondrial structure and in neurotransmission were observed in rats chronically exposed to low-doses of Pb^{2+} (Jablonska *et al*., 1994; Struzynska *et al*., 1994). These changes were correlated with changes in energy metabolism of synaptic mitochondria during Pb^{2+}-toxicity.

The data from the present study suggest that Pb^{2+} significantly inhibited the specific activities of Mg^{2+} ATPase as well as Na^+K^+ ATPase which also support the findings of Cardone *et al*. (1971). Earlier studies have linked the inhibition of ATPases to the binding of heavy metal cations to -SH groups (Barcellos *et al*., 1994; Battacharya *et al*., 1997). Chetty *et al*. (1990) have reported that Pb^{2+} has high affinity for free –SH groups in enzymes and proteins and its binding can alter their functions.

Pb^{2+} may exert an inhibitory effect directly on Na^+K^+ ATPase. It is known that brain Na^+k^+ ATPase is among several enzymes particularly affected by Pb^{2+} (Siegel *et al*., 1977; Fox *et al*., 1991; Struzynska *et al*., 1994). Our earlier studies reported that the decrease in Na^+K^+ ATPase activity can change the gradients of Na^+ and K^+ across the cell membrane and alter the neurotransmitters levels (Reddy *et al*., 2003; Devi *et al*., 2005). Pb^{2+} is known to produce oxidative stress by generating ROS. Na^+K^+ ATPase was reported to be inhibited by Reactive Oxygen Species (ROS) in the brain (Rohn *et al*., 1993; Sagara *et al*., 1996). Free radicals might destroy –SH groups or may act as inhibitors for these enzymes resulting in decreased enzyme activity.

The high specific activity of ATPases in the cortex, cerebellum and hippocampus regions of the brain suggests the involvement of these regions in different behavioral functions. It is known that the activity levels of ATPases parallel the metabolic demands of different regions of mouse brain and the differential sensitivity to Pb^{2+}-induced neurotoxicity in these brain regions is not due to a preferential Pb^{2+}-accumulation, but possibly could be due to alteration of biochemical or cellular processes that are uniquely associated with, or greatly enhanced in a particular region (Widzowski and Cory-Slechta, 1994; Moreira *et al*., 2001).

The greater inhibition of ATPase activities in young (up to PND 28) mice could be due to the fact that Pb^{2+} passes through the blood-brain barrier easily during the early developmental period. The permeability of blood brain barrier is over 1,000 times greater for Pb^{2+} than for Ca^{2+} (Bradbury and Deane 1993). A significant decrease in ATPase activities in adult mice even after the withdrawal of Pb^{2+}-exposure may be due to the already accumulated Pb^{2+} in these regions of brain.

Pb^{2+} and Ca^{2+} interactions occur at the cellular and molecular levels and Pb^{2+} has the ability to mimic or displace Ca^{2+} during specific physiological processes. Pb^{2+} competes with Ca^{2+} resulting in: a) inhibition of neurotransmitter release, b) alterations in the regulation of cell metabolism by binding to second messenger Ca^{2+} receptors, c) blocking channels and d) inhibition of ATP-dependent Ca^{2+}- and Na^+-pumps (Luthman *et al*., 1994; Bettaiya *et al*., 1996). Ca^{2+}/Zn^{2+}-supplementation reduced the Pb^{2+}-effects on both ATPases. It is likely that the supplemented Ca^{2+}/Zn^{2+} may compete for similar binding sites as that of Pb^{2+}. The use of Ca^{2+} supplements to Pb^{2+}-exposed women during lactation has been shown to blunt the blood Pb^{2+} levels in children (Pires *et al*., 2002). In our recent study (Prasanthi *et al*., 2005), we have also reported the protective effect of Ca^{2+}/Zn^{2+} against Pb^{2+}-toxicity in mouse brain. It has been shown that supplementation with Ca^{2+}/Zn^{2+} decreases gastrointestinal absorption of Pb^{2+} and reduces its tissue accumulation (Cerlewski and Forbes, 1976; Peraza *et al*., 1998). Thus, Ca^{2+} and Zn^{2+} replace Pb^{2+} in the body and thereby reduce the Pb^{2+}-burden in the body.

Greater recovery of Pb^{2+}-inhibited ATPase activity in mice exposed to 0.2% Pb^{2+} suggests that the detoxification mechanisms in mice were strong enough to counter the effects of low-level Pb^{2+}-exposure as compared to the mice exposed to 1% Pb^{2+}. From the present study, it is evident that developmental Pb^{2+}-exposure inhibited the Mg^{2+}ATPase and NA^+K^+ ATPase enzymes in a dose-dependent manner and Ca^{2+}/Zn^{2+}-supplementation significantly reversed the Pb^{2+}-induced alterations in brain Mg^{2+}ATPase and Na^+K^+ ATPase activities.

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References

Barcellos, C.K., M.R.C. Schetinger, A.M.O. Battastini, L.B. Silva, R.D. Dias and J.J.F. Sarkis, 1994. Inhibitory effect of cadmium acetate on synaptosomal ATP diphosphohydrolase from adult rat cerebral cortex. Braz. J. Med. Biol. Res. 27: 1111-1115.

Bertoni, J.M. and P.M. Sprenkle, 1991. Low level lead inhibits the human brain cation pump. Life Sci., 48: 2149-2156.

Bettaiya, R., P.R. Yallapragada, E. Hall and S. Rajanna, 1996. *In vitro* effect of lead on Ca^{2+}-ATPase in synaptic plasma membranes and microsomes of cerebral cortex and cerebellum. Ecotoxicol. Environ. Safety, 33: 157-162.

Bhattacharya, S., S. Bose, B. Mukhapadhyay, D. Sarkar, D. Das, J. Bandyopadhyaya, R. Bose, C. Majmudar, S. Mondal and S. Sen, 1997. Specific binding of inorganic mercury to Na^+K^+-ATPase in rat liver plasma membrane and signal transduction. Biometals, 10: 157-162.

Bogden, J.D., S.B. Gertner, S. Christakos, F.W. Kemp, Z. Yang, S.R. Katz and C. Chu, 1992. Dietary calcium modifies concentrations of lead and other metals and renal calbidin in rats. J. Nutr., 122: 1351-1360.

Boyer, P., 1977 In: The Enzymes. 3rd Edn. Vol. 13. Academic press, NY.

Bradbury, M.W.B. and R. Deane, 1993. Permeability of the blood brain barrier to lead. Neurotoxicology, 14: 131-136.

Bressler, J.P. and G.W. Goldstein, 1991. Mechanisms of lead neurotoxicity. Biochem. Pharmacol., 41: 479-484.

Cardone, E., M.A. Lessler and G.P. Brierley, 1971. Mitochondrial oxidative phosphorylation Interaction of lead and inorganic phosphate. Proc. Soc. Exp. Biol. Med., 136: 300-312.

Carfagna, M.A., G.D. Ponsler and B.B. Muhoberac, 1996. Inhibition of ATPase activity in rat synaptic plasma membranes by simultaneous exposure to metals. Chem. Biol. Interact., 100: 53-65.

Cerklewski, F.L. and R.M. Forbes, 1976. Influence of dietary zinc on lead toxicity in the rat. J. Nutr., 106: 689-696

Chetty, C.S., B. Rajanna and S. Rajanna, 1990. Inhibition of rat brain microsomal Na^+K^+-ATPase and ouabain binding by mercuric chloride. Toxicol. Lett., 51: 109-116.

Cotman, C.W. and D.A. Matthews, 1971. Synaptic plasma membranes from rat brain synaptosomes: Isolation and partial characterization. Biochem. Biophys. Acta, 249: 380-394.

Devi, C.B., G.H. Reddy, R.P.J. Prasanthi, C.S. Chetty and G.R. Reddy, 2005. Developmental lead exposure alters mitochondrial monoamine oxidase and synaptosomal catecholamine levels in rat brain. Intl. J. Dev. Neurosci ., 23: 375- 381.

Fiske, C.H. and Y. Subba Row, 1925. The colorimetric determination of phosphates. J. Biol. Chem., 66: 375-400.

Flora, S.J.S. and S.K. Tandon, 1990. Beneficial effects of zinc-supplementation during chelation treatment of lead intoxication in rats. Toxicology, 64: 129-139.

Fox, D.A., S.D. Rubinstein and P. Hsu, 1991. Developmental lead exposure inhibits adult rat retinal, but not kidney, Na^+ K^+ATPase. Toxicol. Appl. Pharmacol., 109: 482-493.

Hammond, P., R.L. Bornschein, H. Zenick, 1984. Toxicology considerations in the assessment of lead exposure. Neurotoxicology, 5: 33-66.

Jablonska, L., M. Walski and U. Rafalowska, 1994. Lead as an inductor of some morphological and functional changes in synaptosomes from rat brain. Cell Mol. Neurobiol., 14: 701-709.

Lowry, O.H., N.J. Rosenbrough, A.L. Farr, R.J. Randal, 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Luthman, J., E. Lindqvist, G.A. Gerhardt, L. Olson, B.H. Hoffer, 1994. Alterations in central monoamine systems after postnatal lead acetate treatment in rats. Environ. Res., 65: 100-118.

Moreira, E.G., I. Vassilieff and V.S. Vassilieff, 2001. Developmental lead exposure: Behavioral alterations in the short and long term. Neurotoxicol. Teratol ., 23: 489-495.

Peraza, M.A., F. Fierro, D.S. Barber, E. Casarez and L.T. Rael, 1998. Effects of micronutrients on metal toxicity. Environ. Hlth. Perspect. 106: 203-216.

Pires, J.B., N. Mickeley and C.M. Donangelo, 2002. Calcium supplementation during lactation blunts erythrocyte lead levels and aminolevulinic acid dehydratase zinc reactivation in women non-exposed to lead and with marginal calcium intakes. Toxicology, 175:247-255.

Prasanthi, R.P.J., G.H. Reddy, C.B. Devi and G.R. Reddy, 2005. Zinc and calcium reduce lead induced perturbations in the aminergic system of developing brain. BioMetals, 18: 615-626.

Reddy, G.R., M.D. Riyaz Basha, C.B. Devi, A. Suresh, J.L. Baker, A. Shafeek, J. Heinz and C.S. Chetty, 2003. Lead induced effects on acetylcholinesterase activity in cerebellum and hippocampus of developing rat. Intl. J. Dev. Neurosci., 21: 347-352.

Rohn, T.T., T.R. Hinds and F.F. Vincenzi, 1993. Ion transport ATPases as targets for free radical damage. Protection by an aminosteroid of the Ca^{2+} pump ATPase and Na^+/K^+ pump ATPase of human red blood cell membranes. Biochem. Pharmacol., 46: 525-534.

Sagara, Y., R. Dargusch, F.G. Klier, D. Schubert and C. Behl, 1996. Increased antioxidant enzyme activity in amyloid beta protein-resistant cells. J. Neurosci., 16: 497-505.

Schmidt-Niensen, B. ,1974. Osmoregulation effect of salinity and heavy metal. Fed .Proc. Fed. Am. Soc. Exp. Biol., 46: 2301-2315.

Siegel, G.J., S.K. Fogt and M.J. Hurley, 1977. Lead Action on Sodium plus Potassium Activated Adenosine Triphosphatase from Electroplax, Rat Brain and Rat Kidney. In: Eds. Miller, M.W. and A.E. Shamoo, Membrane Toxicity. Plenum Press, New York, pp: 465-493.

Singerman, A., 1976. Clinical Signs vs Biochemical Effects for Toxic Metals. In: Effects of Dose and Response Relationships for Toxic Metals. Ed. Nordberg EF, Elsevier. 207.

Struzynska, L., B. Dabrowska-Bouta, J. Lenart, J. Zborowski and U. Rafalowska, 1994. Some metabolic effects in rat brain synaptosomes after exposure to lead *in vivo* and *in vitro*. Bull. Pol. Acad. Sci. Biol. Sci., 42: 55-62.

Sushma, N.J., 1999. Impact of Aluminium acetate on albino mice with special reference to haematological, histological and selected biochemical parameters. Doctoral Dissertation. S.V. University, Tirupati. India.

Sweadner, K.J. and S.M. Goldin, 1980. Active transport of sodium and potassium ions: Mechanism, function and regulation. N. Engl. J. Med., 302: 777-783.

Tirri, R., K.Y.H. Lagerspetz and J. Kohnen, 1973. Temperature dependence of the ATPase activites in brain homogenates during the postnatal development of the rat. Comp. Biochem. Physiol., 44B: 473-481.

Tucker, R.K. and A. Matte, 1980. *In vitro* effects of cadmium and lead on ATPases in the gill of rock crab, Cancer teroratus. Bull. Environ. Contam. Toxicol., 24: 847-852.

Valle, B.L. and D.D. Ulmer, 1972. Biochemical effects of mercury, cadmium and lead. Ann. Rev. Biochem., 41: 91-99.

Widzowski, D.V. and D.A. Cory-Slechta, 1994. Homogeneity of regional brain lead concentrations. Neurotoxicology, 15: 295-307.

Winder, C. and I. Kitchen, 1984. Lead neurotoxicity: A review of the biochemical, neurochemical and drug induced behavioral evidence. Prog. Neurobiol., 22: 59-87.