RESULTS AND DISCUSSION

Lead is a ubiquitous environmental toxin that induces a broad range of physiological, biochemical and behavioral dysfunctions. Its toxicity has been known from ancient times and many studies have explored the mechanisms and symptoms of this toxicity through the years. Lead is known to cause several pathological conditions to the human population including anemia, mental disorders and at chronic low level of exposure leads to cancer. Hence effective treatment is required to heal the toxicity produced by lead.

Although several hypotheses have been proposed to explain the possible mechanism for lead induced toxicity, the exact mechanism of action is still unclear. Accumulating evidence in support of the role of oxidative stress in the pathophysiology of lead poisoning (Gurer and Ercal, 2000; Hernandez et al. 2001) has prompted the present study. The efficacies of lipoic acid (a well known antioxidant) and DMSA (a metal chelator) against lead induced neurotoxicity have been evaluated in this study.

Body Weight, Food And Water Intake

Table 3.1 shows the body weight, food and water intake of animals. Lead treated rats did not exhibit any significant changes with regard to the body weight, food and water intake.

Lead Levels

Accumulation and excretion profile of lead in the brain regions, blood and in the urine is depicted in the table 3.2. The heavy metal lead was extensively accumulated in the brain and blood of lead administered rats (p<0.05, when compared with control). Of the three brain regions studied, hippocampus was the region wherein high amount of lead was accumulated. 50 % of the brain-lead is stored in the hippocampus. The reason for accumulation of lead mainly in hippocampus remains unknown.
Table 3.1: Body weight, food and water intake of control and experimental animals
(Mean ± S.D for six animals in each group)

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>Group I Control</th>
<th>Group II Pb</th>
<th>Group III LA</th>
<th>Group IV DMSA</th>
<th>Group V LA+DMSA</th>
<th>Group VI Pb+LA</th>
<th>Group VII Pb+DMSA</th>
<th>Group VIII Pb+LA+DMSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>120 ± 10.0</td>
<td>105 ± 9.5</td>
<td>115 ± 10.2</td>
<td>125 ± 10.32</td>
<td>123 ± 11.3</td>
<td>118 ± 21.6</td>
<td>113 ± 18.6</td>
<td>123 ± 16.5</td>
</tr>
<tr>
<td>Food intake</td>
<td>12.3 ± 1.32</td>
<td>9.95 ± 1.02</td>
<td>11.2 ± 1.2</td>
<td>12.4 ± 1.31</td>
<td>11.5 ± 1.3</td>
<td>12.5 ± 1.13</td>
<td>10.3 ± 1.11</td>
<td>13.5 ± 1.23</td>
</tr>
<tr>
<td>Water intake</td>
<td>19.8 ± 1.78</td>
<td>18.6 ± 1.76</td>
<td>20.3 ± 1.87</td>
<td>18.9 ± 1.65</td>
<td>20.6 ± 1.78</td>
<td>19.8 ± 1.5</td>
<td>20.6 ± 1.84</td>
<td>19.2 ± 1.45</td>
</tr>
</tbody>
</table>

Body Weight – gm
Food intake – g/100 g b.wt/day
Water intake – ml/100 g b.wt/day
Table 3.2: Level of Lead accumulation and excretion in control and experimental rats
(mean ± S.D for six animals in each group)

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>Group I Control</th>
<th>Group II Pb</th>
<th>Group III LA</th>
<th>Group IV DMSA</th>
<th>Group V LA+DMSA</th>
<th>Group VI Pb+LA</th>
<th>Group VII Pb+DMSA</th>
<th>Group VIII Pb+LA+DMSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. Cortex</td>
<td>0.10 ± 0.021</td>
<td>2.56 ± 0.23</td>
<td>0.12 ± 0.009</td>
<td>0.08 ± 0.007</td>
<td>0.09 ± 0.009</td>
<td>1.33 ± 0.11 ab</td>
<td>0.52 ± 0.045 abc</td>
<td>0.21 ± 0.022 abcd</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td></td>
<td>a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.07 ± 0.006</td>
<td>2.35 ± 0.24</td>
<td>0.08 ± 0.009</td>
<td>0.06 ± 0.005</td>
<td>0.07 ± 0.008</td>
<td>1.22 ± 0.1 ab</td>
<td>0.44 ± 0.043 abc</td>
<td>0.17 ± 0.02 abcd</td>
</tr>
<tr>
<td></td>
<td>a</td>
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<td>a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.12 ± 0.014</td>
<td>5.62 ± 0.52</td>
<td>0.13 ± 0.012</td>
<td>0.10 ± 0.009</td>
<td>0.11 ± 0.01</td>
<td>3.52 ± 0.31 ab</td>
<td>1.68 ± 0.17 abc</td>
<td>0.67 ± 0.063 abcd</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td></td>
<td>a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>10.15 ± 1.29</td>
<td>97.56 ± 11.1</td>
<td>11.21 ± 1.10</td>
<td>9.43 ± 1.03</td>
<td>8.94 ± 1.15</td>
<td>57.23 ± 5.93 ab</td>
<td>24.16 ± 2.29 abc</td>
<td>15.32 ± 1.73 abcd</td>
</tr>
<tr>
<td></td>
<td>a</td>
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<td>a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>8.12 ± 0.95</td>
<td>252 ± 30.21</td>
<td>10.23 ± 1.23</td>
<td>8.56 ± 1.2</td>
<td>9.35 ± 0.85</td>
<td>440.2 ± 45.3 ab</td>
<td>600 ± 65.6 abc</td>
<td>820.6 ± 75.6 abcd</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td></td>
<td>a</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Lead level in tissues - μg/g tissue
Lead level in blood - μg/100 ml
Lead level in urine - μg/24 hr of urine

a – as compared with Group I; b – as compared with group II; c – as compared with Group VI; d – as compared with Group VII
a, b, c, d – represents statistical significance at p<0.05
Brain capillary endothelial cells are an important component of the blood-brain barrier. These cells have tight junctions that limit the passage of non-lipophilic solutes from blood to brain and vice versa. Many solutes in blood must cross both the luminal and abluminal plasma membranes of capillary endothelial cells to reach the brain.

Lead readily crosses the blood-brain barrier. The mechanisms by which lead is transported across capillary endothelial cell membranes have not been determined. Deane and Bradbury (1990), using in vivo perfusion of Pb²⁺ in rats, found evidence of transport that was passive, pH dependent, and unaffected by the presence of Ca²⁺. They hypothesized that the transported species was PbOH⁺. This group is also responsible for the efflux of Pb²⁺ from brain capillary cells via the Ca²⁺-ATPase. Kerper and Hinkle (1997) showed that Pb²⁺ entry into the brain cell lines was activated by the depletion of intracellular Ca²⁺ stores.

DMSA administration significantly chelated the lead from the brain but lipoate treatment was not as effective as DMSA. The combined treatment produced the best result.

Better effects were seen with the removal of lead from the brain of lead intoxicated rats by DMSA administration rather than lipoic acid, which was evidenced from the increased urinary excretion of lead in group VII. On combined treatment (Group VIII) lead chelation was more significant (p<0.05) in this group when compared to Group VI and Group VII.

Tissue Enzymes

The effect of lead, lipoate and DMSA administrations on brain tissue enzymes is depicted in table 3.3. A significant inhibition (p<0.05), with regard to alkaline phosphatase (ALP) and acid phosphatase (ACP) were observed in all the three brain regions of lead intoxicated rats (Group II), when compared
Table 3.3: Effect of lipoic acid and DMSA on the activities of Tissue enzymes in Control and Experimental animals (mean ± S.D for six animals in each group)

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>Group I Control</th>
<th>Group II Pb</th>
<th>Group III LA</th>
<th>Group IV DMSA</th>
<th>Group V LA+DMSA</th>
<th>Group VI Pb+LA</th>
<th>Group VII Pb+DMSA</th>
<th>Group VIII Pb+LA+DMSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. Cortex</td>
<td>0.14 ± 0.012</td>
<td>0.09 ± 0.007</td>
<td>0.13 ± 0.011</td>
<td>0.15 ± 0.012</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.008</td>
<td>0.13 ± 0.012</td>
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<tr>
<td></td>
<td>a</td>
<td></td>
<td></td>
<td></td>
<td>ab</td>
<td>ab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.19 ± 0.017</td>
<td>0.12 ± 0.01</td>
<td>0.20 ± 0.019</td>
<td>0.19 ± 0.018</td>
<td>0.21 ± 0.022</td>
<td>0.17 ± 0.015</td>
<td>0.15 ± 0.014</td>
<td>0.18 ± 0.017</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td></td>
<td></td>
<td></td>
<td>b</td>
<td>ab</td>
<td>b</td>
<td>bd</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.17 ± 0.014</td>
<td>0.10 ± 0.009</td>
<td>0.16 ± 0.015</td>
<td>0.18 ± 0.016</td>
<td>0.18 ± 0.019</td>
<td>0.15 ± 0.013</td>
<td>0.14 ± 0.015</td>
<td>0.16 ± 0.015</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td></td>
<td></td>
<td></td>
<td>ab</td>
<td>ab</td>
<td>ab</td>
<td>bd</td>
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<tr>
<td>ACP</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. Cortex</td>
<td>6.2 ± 0.56</td>
<td>4.26 ± 0.51</td>
<td>6.3 ± 0.59</td>
<td>6.41 ± 0.62</td>
<td>6.21 ± 0.6</td>
<td>5.32 ± 0.54</td>
<td>5.12 ± 0.50</td>
<td>6.0 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td></td>
<td></td>
<td></td>
<td>ab</td>
<td>ab</td>
<td>ab</td>
<td>bd</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>11.2 ± 0.96</td>
<td>6.05 ± 0.71</td>
<td>10.65 ± 1.02</td>
<td>10.81 ± 1.11</td>
<td>11.3 ± 1.20</td>
<td>9.87 ± 0.81</td>
<td>9.12 ± 0.85</td>
<td>10.87 ± 1.11</td>
</tr>
<tr>
<td></td>
<td>a</td>
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<td></td>
<td></td>
<td>ab</td>
<td>ab</td>
<td>ab</td>
<td>bd</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>8.56 ± 0.82</td>
<td>5.67 ± 0.54</td>
<td>8.44 ± 0.83</td>
<td>8.78 ± 0.94</td>
<td>8.12 ± 0.80</td>
<td>7.25 ± 0.71</td>
<td>6.87 ± 0.66</td>
<td>8.12 ± 0.83</td>
</tr>
<tr>
<td></td>
<td>a</td>
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<td></td>
<td></td>
<td>ab</td>
<td>ab</td>
<td>ab</td>
<td>bd</td>
</tr>
</tbody>
</table>

ALP - μmoles of phenol liberated/min/mg protein; ACP - μmoles x 10^-2 of phenol liberated/min/mg protein; LDH - μmoles of Pyruvate released/min/mg protein

a - as compared with Group I; b - as compared with group II; c - as compared with Group VI; d - as compared with Group VII

a, b, c, d - represents statistical significance at p<0.05
to control (Group I). On treatment with lipoic acid (Group VI) and DMSA (Group VII) the activities were significantly elevated \((p<0.05)\). On combined therapy the enzymic activities were reverted to near normal levels (Group VIII), illustrating the protectiveness of the combined therapy.

As alkaline phosphatase has been reported to hydrolyse various phosphomonoesters non-specifically (Cathala et al., 1975), the enzyme might be involved in the metabolism of phosphomonoesters such as phosphocholine or glycerophosphate (Klunk et al., 1997). In this context, it is assumed that the loss of alkaline phosphatase activity may promote the pathogenesis of the brain.

Sok (1999) has indicated that brain alkaline phosphatase, glycosylphosphatidylinositol – anchored protein, is among the enzymes highly susceptible to oxidative inactivation by the \(\text{H}_2\text{O}_2/\text{Cu}^{2+}\) system. An earlier study has reported that kidney alkaline phosphatase was inactivated by a mixed function oxidation system (Mordente et al., 1987). Compared with kidney alkaline phosphatase, brain alkaline phosphatase seems to be much more susceptible to oxidative inactivation in several respects; \(~90\%\) of the brain enzyme is rapidly inactivated by the \(\text{H}_2\text{O}_2/\text{Cu}^{2+}\) system (Sok, 1999). The characteristics of oxidative inactivation are similar to those described for the inactivation of superoxide dismutase (Hodgson and Fridovich, 1975) by \(\text{H}_2\text{O}_2\); the effective inactivation by \(\text{H}_2\text{O}_2\) requires the existence of \(\text{Cu}^{2+}\) or a divalent metal. Thus, it appears that hydroxy radicals, generated at a specific \(\text{Cu}^{2+}\) or \(\text{Pb}^{2+}\) binding site of phosphatase, might be responsible for the oxidative inactivation, as exemplified in metal catalyzed oxidation (Stadman and Oliver, 1991).

Earlier, alkaline phosphatase has been reported to interact with divalent metal ions in multiple ways (Brunel and Cathala, 1973; Janeway et al., 1993): the requirement of \(\text{Zn}^{2+}\) for catalysis and the activation and stabilization by
Acid phosphatase is organized in the lysosome and is involved in the process of phagocytosis, dissolution of tissue components and in the metabolism of xenobiotics. Acid phosphatase was lowered in lead administered rats (Group II). Drug supplementation was effective in restoring the enzyme activities to normalcy.

Tissue ATPases

Lipoic acid, DMSA and their combined administrations brought about salient changes in the activities of the tissue phosphohydrolases (Fig. 3.1). Lead caused a significant decrease in the activities of all ATPases, in all the three regions of brain (Group II). Lipoic acid and succimer significantly increased the activities of these enzymes (p<0.05). The efficiency in increasing the activities of these enzymes was more pronounced with combined therapy of lipoic acid and DMSA rather than when given solely.

Lead exerts an inhibitory effect directly on Na⁺,K⁺-ATPase activity. This enzyme plays a vital role in linking the extracellular signals to the intracellular medium in neural tissues, hence the inhibition of this enzyme by lead could be damaging to the brain cells leading to an earlier stage of edema, followed by a later stage of degeneration and necrosis. Several previous works have shown that perinatal exposition to this metal even at lower doses inhibits the synaptosomal Na⁺, K⁺-ATPase (Rafalowska et al., 1996; Antonio et al., 2002). Antonio and Leret (2000) found a significant inhibition of this enzyme in cortex and cerebellum after perinatal exposure to isolated lead. Lead binds to free –SH groups and reversibly inhibits the enzymes Na⁺, K⁺-ATPase and Mg²⁺-ATPase (Fox et al., 1991).
Fig. 3.1: Effect of LA and DMSA on neural membrane bound ATPases in control and experimental animals (Mean ± S.D for six animals)

- **Na⁺K⁺-ATPase**
  - Cerebral Cortex
  - Cerebellum
  - Hippocampus

- **Mg²⁺ ATPase**
  - Cerebral Cortex
  - Cerebellum
  - Hippocampus

- **Ca²⁺ATPase**
  - Cerebral Cortex
  - Cerebellum
  - Hippocampus

Legend:
- ■ control
- □ Lead
- □ LA
- □ DMSA
- □ LA+DMSA
- □ Pb+LA
- □ Pb+DMSA
- □ Pb+LA+DMSA

- a - as compared with Group I; b - as compared with group II;
- c - as compared with Group VI; d - as compared with Group VII
- a, b, c, d - represents statistical significance at p<0.05
The Ca\(^{2+}\) - mimic action of lead (Raunio and Tahti, 2001), alterations in intracellular calcium ion regulation (Cooper and Manalis, 1983) and effects on nerve terminal membrane sulphhydryl groups leads to decreased Ca\(^{2+}\)-ATPase in the nervous tissue. This accentuates the cytotoxic nature of lead.

Lipoic acid and succimer brought about significant changes in the activities of all the ATPases in the brain. The drugs contributed their thiol groups for detoxifying the divalent metal and thereby improved the cell membrane integrity by increasing the activities of the membrane ATPases.

**Glucose Metabolizing Enzymes**

Fig. 3.2 illustrates the activities of glucose metabolizing enzymes. Glucose-6-phosphatase and glycolytic enzymes namely, hexokinase (HK), phosphoglucoisomerase (PGI), aldolase (ALD), glyceraldehydes-3-phosphate dehydrogenase (GAPDH) were found to be inhibited significantly (p<0.05) in the cerebral cortex, cerebellum and hippocampus of lead intoxicated rats. Lipoate and succimer were found to enhance the activities of glucose metabolizing enzymes significantly (p<0.05). Combined therapy with lipoate and succimer was found to be more effective than their individual administrations (Group VI and Group VII) in enhancing the enzyme activities to near normalcy (Group VIII).

Reduction of glucose utilisation and impaired energy production is the general pathogenic mechanism induced by most neurotoxicants (Planas and Cunningham, 1987). Both the cytosolic and mitochondrial neural hexokinase are inhibited by lead (Nehru and Dua, 1997). This inhibition of hexokinases might be extended to other glycolytic enzymes during non-availability of substrates.

Since lead has affinity towards sulphhydryl groups, sulphur containing enzymes namely aldolase and glyceraldehyde-3-phosphate dehydrogenase
Fig 3.2: Effect of LA and DMSA on glucose metabolising enzymes in control and experimental animals (Mean ± S.D for six animals)

HK - nmoles of glucose utilised/; PGI - nmoles of fructose/min/mg protein
GADPH - nmoles of NADH formed/; G6Pase - nmoles of Pi liberated/min/mg protein
Aldolase - nmoles of glyceraldehyde/min/mg protein

a - as compared with Group I; b - as compared with group II;
c - as compared with Group VI; d - as compared with Group VII
a, b, c, d - represents statistical significance at p<0.05
were inhibited (Yun and Hoyer, 2000). This is supported by our results. Inhibition of glycolytic enzymes in the tissue leads to the diminished level of glycolytic intermediates (Takagaki, 1968). Activity of glucose-6 phosphatase also decreased, which might also be attributed to the non-availability of substrate.

α-Lipoic acid is a potent antioxidant in the brain and might be even more efficacious in nerves (Nickander et al., 1996). α-lipoic acid may be effective in increasing glucose transport, enhancing glucose utilisation (Natraj et al., 1984) and stimulating glycolysis (Singh and Bowman, 1970). Exogenous α-lipoate may in part, act as a cofactor in the multienzyme complexes, namely dehydrogenases which are involved in the glycolytic pathway (Loffelhardt et al., 1995).

Heme Metabolizing Enzyme (ALA-D) And ALA In Brain And Blood

Table 3.4 shows the activity of δ - aminolevulinic acid dehydratase (ALA-D) in the blood and brain regions. The activity of ALA-D was significantly low in blood and brain regions in the lead intoxicated rats (Group II, p<0.05). Due to the inhibition of δ-aminolevulinic acid dehydratase there was an excessive accumulation of δ-aminolevulinic acid (ALA) in the brain tissue. Lipoic acid and succimer significantly increased the activity of ALA-D (Group VI, VII and VIII: p<0.05) thereby minimising the accumulation of ALA in the blood and brain regions.

A major manifestation of lead toxicity is its effect on the hematopoietic system. The ability of lead to impair heme synthesis in erythroid cells is well known, as are the hematologic consequences (Bechara, 1996; Prine et al., 1998). In heme biosynthesis, lead inhibits the conversion of ALA to porphobilinogen, which is catalysed by the enzyme, ALA-D. Lead has also been shown to inhibit the enzyme ferrochelatase, which catalyses the formation of heme from iron and protoporphyrin. Thus, lead poisoning can
### Table 3.4: Effect of Lipoic acid and DMSA on the Heme metabolism in control and experimental groups (mean ± S.D for six animals in each group)

<table>
<thead>
<tr>
<th>Regions</th>
<th>Group I Control</th>
<th>Group II Pb</th>
<th>Group III LA</th>
<th>Group IV DMSA</th>
<th>Group V LA+DMSA</th>
<th>Group VI Pb+LA</th>
<th>Group VII Pb+DMSA</th>
<th>Group VIII Pb+LA+DMSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA-D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>8.32±0.76</td>
<td>5.56±0.51 a</td>
<td>7.65±0.71</td>
<td>8.21±0.78</td>
<td>8.21±0.78</td>
<td>6.68±0.65 ab</td>
<td>6.45±0.54 ab</td>
<td>7.66±0.71 bed</td>
</tr>
<tr>
<td>C. Cortex</td>
<td>4.26±0.41</td>
<td>3.08±0.32 a</td>
<td>4.32±0.44</td>
<td>4.22±0.41</td>
<td>4.13±0.39</td>
<td>3.78±0.33 ab</td>
<td>3.58±0.34 ab</td>
<td>4.11±0.39 bd</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>4.17±0.42</td>
<td>2.89±0.30 a</td>
<td>4.21±0.43</td>
<td>4.11±0.39</td>
<td>4.08±0.41</td>
<td>3.77±0.35 b</td>
<td>3.46±0.36 ab</td>
<td>3.97±0.41 bd</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>4.86±0.44</td>
<td>3.01±0.29 a</td>
<td>5.02±0.51</td>
<td>4.79±0.46</td>
<td>4.78±0.50</td>
<td>4.25±0.44 ab</td>
<td>3.88±0.40 ab</td>
<td>4.75±0.48 bd</td>
</tr>
<tr>
<td>ALA</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>0.28±0.025</td>
<td>0.42±0.036 a</td>
<td>0.25±0.022</td>
<td>0.26±0.023</td>
<td>0.29±0.031</td>
<td>0.33±0.032 ab</td>
<td>0.35±0.031 ab</td>
<td>0.30±0.028 bd</td>
</tr>
<tr>
<td>C. Cortex</td>
<td>0.16±0.014</td>
<td>0.24±0.021 a</td>
<td>0.15±0.013</td>
<td>0.17±0.016</td>
<td>0.18±0.02</td>
<td>0.2±0.018 ab</td>
<td>0.21±0.018 ab</td>
<td>0.18±0.015 bd</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.15±0.013</td>
<td>0.24±0.022 a</td>
<td>0.15±0.016</td>
<td>0.16±0.014</td>
<td>0.14±0.011</td>
<td>0.2±0.02 ab</td>
<td>0.2±0.019 ab</td>
<td>0.17±0.016 bcd</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.18±0.011</td>
<td>0.29±0.025 a</td>
<td>0.16±0.012</td>
<td>0.18±0.019</td>
<td>0.19±0.017</td>
<td>0.23±0.021 ab</td>
<td>0.24±0.024 ab</td>
<td>0.2±0.018 bcd</td>
</tr>
</tbody>
</table>

ALAD - nmole of PBG/hr/mg protein; ALA - μmole/dl
a - as compared with Group I; b - as compared with group II; c - as compared with Group VI; d - as compared with Group VII
a, b, c, d - represents statistical significance at p<0.05
lead to accumulation of protoporphyrin in red blood cells, an increase in ALA in blood and urine, inhibition of blood ALA-D and an increased proportion of immature red cells (reticulocytes and basophilic stippled cells) in blood. The ALA-D activity in red blood cells correlates directly with blood lead levels; hence, inhibition of ALA-D is a sensitive indicator of lead poisoning.

Our findings show that the activity of δ-ALA-D is low in the blood of lead-poisoned rats. Reductions in the blood δ-ALA-D reflect similar reductions in brain-enzyme activity.

The correlation between lead levels and the activity of aminolevulinic acid dehydratase are shown in the figure 3.3. The correlation value between lead levels and ALA-D activity in blood, hippocampus, cerebral cortex and cerebellum are -0.653, -0.711, -0.613 and - 0.609 respectively. The negative correlations provide the evidence that when the level of lead is increased, there will be a lowering in the activity of ALA-D. This suggests a possible mechanism wherein lead may act directly on the sulphydryl group of the enzyme resulting in inhibition of the enzyme. Lead was accumulated in high concentration in the blood and hippocampus than in the other brain regions. Parallel to this, there was much more inhibition of ALA-D activity in hippocampus (38% inhibition) and in blood (44% inhibition) than in cerebral cortex and cerebellum (28% and 31% inhibition respectively).

5-Aminolevulinic acid (ALA) has been shown to undergo iron-catalysed aerobic oxidation with the generation of reactive oxygen species (Monteiro et al., 1986; Monteiro et al., 1989). Similar to α-hydroxycarbonyl compounds like glucose (Thornalley, 1985) and dihydroxyacetone (Mashino and Fridovich, 1987), ALA (an α - aminoketone) undergoes enolization and superoxide-propagated reaction with dioxygen in slightly alkaline medium. Hydrogen peroxide and iron-catalysed HO⁻ radicals, produced via the Haber – Weiss reaction, were demonstrated to be formed during ALA aerobic
Fig 3.3: Correlation between lead levels and amino levulinic acid dehydratase

Cerebral Cortex

Cerebellum

Hippocampus

Blood

$r = -0.6095$

$r = -0.61261$

$r = -0.71067$

$r = -0.65316$
oxidation by the use of scavengers and spin trapping techniques (Monteiro et al., 1989). The cellular events triggered by free radicals leading to the typical syndromes are, however, still obscure.

ALA-promoted TBARS formation was inhibited by α-tocopherol, a well known scavenger of HO⁺ and ROO⁺ radicals. The evidence demonstrated that ALA is capable of promoting lipid peroxidation in liposomes, suggesting that the oxidation of membrane lipids could be an operating event in ALA-mediated cell injury (Princ et al., 1998)

Oteiza and Bechara (1993) found a positive correlation between lipid peroxidation induced by ALA and the permeability of membranes. They observed that ALA caused the oxidation of unsaturated fatty acids in cardiolipin and phosphatidylcholine.

ALA administration to rats is shown to trigger oxidative damage to brain lipids and proteins in parallel with cerebral iron accumulation and GABA binding alteration (Demasi et al., 1996).

Pappas et al. (1995) reported increase in the activity of δ-ALA-D on oral administration of DMSA, indicating that DMSA was effective when challenged with ongoing Pb treatment in water, in reversing the toxic effects of Pb on heme synthesis. As Pb is removed from the enzyme δ-ALA-D, δ-Amino levulinic acid can be utilized by the enzyme thereby restoring its concentrations to normalcy.

Oxidative Stress And Defense Mechanisms

Fig 3.4 depicts lipid peroxidative changes in the brain regions. Lipid peroxides were significantly increased with lead administration (p<0.05, Group II). Cerebral cortex showed about 153% increase in the lipid peroxide content and in cerebellum increase was around 156% increase whereas in
Fig 3.4: Effect of LA and DMSA on lipid peroxidation in the brain regions of control and experimental rats

LPO: nmol of MDA formed /mg protein
a – as compared with Group I; b – as compared with Group II;
c – as compared with Group VI; d – as compared with Group VII
a, b, c, d – represents statistical significance at p<0.05
The increase in lead levels was accompanied by an increase in lipid peroxidation, which is a basic deteriorative process of the cell. Figure 3.5 showed a positive correlation between lead levels and the lipid peroxidation in the blood and brain regions. The correlation value between lead levels and lipid peroxidation are +0.837, +0.813, +0.835, +0.870 in cerebral cortex, cerebellum, hippocampus and blood respectively. Interestingly, the region that accumulated maximum lead i.e., hippocampus, showed a high degree of lipid peroxidation, indicating that this region is most susceptible to the damage by lead.

The peroxidation of membrane phospholipids eventually leads to loss of membrane integrity and finally to cell death. Our results are in agreement with those of Gurer et al. (1999), who observed increased lipid peroxidation in brain following lead exposure. Furthermore, Quinlan et al. (1988) have also reported an enhanced iron-dependent lipid peroxidation with lead, in vitro, in liposomes, erythrocytes and rat liver microsomal fractions.

However, as lead does not undergo oxidation-reduction cycle, it may not have a direct effect on lipid peroxidation. These changes are rather due to an indirect effect of lead on free radical scavenging enzymes and glutathione. This is mainly attributed to the high affinity of lead for sulphhydryl groups or metal co-factor in these enzymes and antioxidant molecules. In addition to the effects of lead on antioxidant enzymes, lead may also enhance lipid peroxidation by elevating intracellular levels of calcium and impairing mitochondrial functions. It is speculated that the increased lipid peroxidation as a result of reduced antioxidant capacity may be the initial event in producing neuronal damage.
Fig 3.5: Correlation between lead levels and lipid peroxidation

Cerebral Cortex

Cerebellum

Hippocampus

Blood

\[ r = 0.83777 \]

\[ r = 0.813327 \]

\[ r = 0.835812 \]

\[ r = 0.870044 \]
The increase in lipid peroxidation was accompanied by a concomitant decrease in the activities of antioxidant enzymes viz., glucose 6-phosphate dehydrogenase, superoxide dismutase, glutathione peroxidase and catalase in all the three regions of brain (shown in Figure 3.6) of lead exposed rats (Group II, p<0.05). Superoxide dismutase is an enzyme responsible for the detoxification of highly reactive and potentially toxic radicals to less toxic hydrogen peroxide (Fridovich, 1983). The results of the present study indicate an inhibition of the enzyme in different brain regions of lead treated animals. These results are in agreement with those of Sandhir et al. (1994) who also observed a decline in the activity of superoxide dismutase. Mylorie et al. (1986) suggested that this decline may be due to the lead induced copper deficiency, as copper plays a catalytic role on this enzyme activity (Fridovich, 1983), hence the decrease in the activity of the enzyme was inevitable.

The seleno-enzyme glutathione peroxidase, catalyses the detoxification of lipid peroxides (Rotruck et al., 1973). From our observations it is evident that exposure to lead leads to an inhibition in the glutathione peroxidase activity in different brain regions. It is speculated that inhibition of the glutathione peroxidase may be due to interaction of lead with the essential selenocysteine moiety of the enzyme. These results are in accordance with those of Valenzuela et al. (1989) who also observed a similar decrease in the enzyme activity.

Catalase, another antioxidant enzyme containing heme as the prosthetic group, is responsible for the decomposition of hydrogen peroxide. The decrease in catalase activity observed in the study may be attributed to the reduced absorption of iron or due to the inhibition of heme biosynthesis as lead has been shown to interfere with both the processes (Dresel and Falk, 1954).

Inhibition of G6PD causes decreased supply of reducing equivalents (NADPH) for the conversion of oxidized glutathione to its reduced form.
Fig 3.6: Antioxidant enzyme activities in discrete brain regions in control and experimental animals (Mean ± S.D for six animals)

- **G6PD**
  - Units/min/mg protein
  - Cerebral Cortex, Cerebellum, Hippocampus

- **SOD**
  - Units/min/mg protein
  - Cerebral Cortex, Cerebellum, Hippocampus

- **GPx**
  - Units/min/mg protein
  - Cerebral Cortex, Cerebellum

- **Catalase**
  - μg of H2O2 consumed/min/mg protein
  - Cerebral Cortex, Cerebellum, Hippocampus

*Key for groups:
  - a - as compared with Group I; b - as compared with group II; c - as compared with Group VI; d - as compared with Group VII
  - a, b, c, d - represents statistical significance at p<0.05
Under conditions of oxidative assault, the NADP/NADH ratio will switch in favour of NADP indicating decreased G6PD activity.

Effect of lead on the level of non-enzymic antioxidants are shown in figure 3.7. The antioxidants were significantly decreased in lead treated groups (Group II p<0.05). Administration of drugs, lipoate and DMSA significantly elevated the levels. Supplementation of combined drug appreciably brings back the diminished antioxidant levels to near normalcy (Group VIII).

The affinity of lead for sulphydryl groups makes it an attractive mechanism for detoxification (Jacobson and Turner, 1980). Glutathione, a naturally occurring antioxidant, protects the membrane-polyunsaturated fatty acids from free radical-mediated lipid peroxidation by abstracting the hydrogen of sulphydryl group instead of methylene hydrogen of unsaturated lipids (Kosower and Kosower, 1979). Thus, the tissue level of glutathione is considered to be a critical determinant for the threshold of tissue injury caused by environmental chemicals (Meister and Tate, 1976).

GSH is also proposed to protect the cell against metal toxicity through a chelation mechanism, removal of generated reactive species and maintenance of the redox state of other thiols (Romero et al., 1990; Meister, 1991). Experimental studies on Pb toxicity with different species, methods and doses of Pb delivery, have reported both increases (Struzynska et al., 2001) and decreases in tissue GSH content. In our laboratory, we have found that lead decreased the thiol content and antioxidant levels in the kidney of lead exposed rats (Sivaprasad et al., 2002). Presence of GSH was found to reduce the accumulation of Pb in astrocytic culture (Lindahl et al., 1999). When neuroblastoma cells were exposed to lead, ROS production was greatly amplified and intracellular GSH level was reduced (Naarala et al., 1995).

Vitamin E is considered as the most efficient biological lipid soluble antioxidant. Vitamin E, a chain breaking antioxidant, is the first antioxidant
Fig 3.7: Effect of LA and DMSA on the levels of non-enzymic antioxidants in the brain regions of control and experimental animals (Mean ± S.D for six animals)

Vitamin C

Vitamin E

GSH

- as compared with Group I; b - as compared with group II; c - as compared with Group VI; d - as compared with Group VII

a, b, c, d - represents statistical significance at p<0.05
present in the cell membrane that counteracts the entry of the metal-induced free radicals into the cell and ultimately gets transformed into tocopheroxyl radical (Shukla and Chandra, 1989). Vitamin C, the major water soluble and nonenzymic primary preventive antioxidant in the cells and body fluids, scavenges the reactive oxygen species produced by lead. It demonstrates a synergistic interaction with the tocopheroxyl radical, resulting in the regeneration of α-tocopherol. Ascorbate imparts its protection by undergoing oxidation ultimately forming dehydroascorbate. GSH is required for the reduction of dehydroascorbate back to ascorbate. When there is a reduction in the level of GSH, this conversion is affected and hence, vitamin C level is lowered.

LA and DMSA administrations increased the content of GSH, vitamin E and vitamin C in the tissue. LA could either mitigate GSH consumption by acting as an alternate ROS scavenger or increase GSH levels by stimulating its biosynthesis. Packer et al. (1995 & 1998) suggested that LA administration could induce increases in GSH levels by facilitating transport of cystine, the limiting factor in GSH synthesis, into the cells. Once LA is taken up by the cell it is immediately reduced to DHLA, which is then released. The released DHLA induces a chemical reduction of extracellular cystine to cysteine. Cysteine can be taken up rapidly (10 times more) by the cells than cystine and can then be used in the biosynthesis of GSH.

DMSA administration is able to produce an increase in the amount of GSH, which is in accordance with the view that both the reversal of ALA-D inactivation and rapid removal of lead could be largely assigned to glutathione availability (Flora et al., 1994; Flora et al., 1995). Further, rapid restoration of lead sensitive biochemical variables such as blood ALA-D is also accompanied by rapid depletion of blood lead.
Drug Metabolising Enzymes

Figure 3.8 and Table 3.5 present the activities of drug metabolizing enzymes in the brain regions and liver tissue respectively. In all the tissues these drug metabolizing enzymes showed significant decrease (p<0.05). With lipoate, DMSA and their combined administrations, the activities of these enzymes were increased (p<0.05) as a tissue response to dispose the lead.

A substantial fraction of the heme synthesized in the liver serves as the prosthetic group of the microsomal hemeprotein, cytochrome P-450. This hemeprotein is the terminal oxidase of the mixed-function oxidase system in the liver, which is involved in the hepatic biotransformation of medicaments, hormones and many environmental chemicals.

Acute administration of lead may exert a direct action on the hepatic mixed function oxidase system since there is a suggestion of involvement of sulphydryl groups in the integrity and electron-transfer function of cytochrome P-450 (Tsai et al., 1970), as well as, of NADPH cytochrome C reductase activities (Phillips and Langdon, 1962). Inhibition of cytochrome P-450 may be due to the lowered activities of ALA-D and ferrochelatase in erythrocytes (Alvares et al., 1976), both of which are key enzymes in the biosynthesis of heme, the prosthetic group of cytochrome P-450.

The reduced activities of glutathione transferase and glutathione reductase may be due to inhibition by lead at their sulphydryl groups. Glutathione reductase is responsible for the reduction of oxidised glutathione to glutathione (reduced). Glutathione reductase possesses a critical sulphydryl group at its active site which participates in the reduction of oxidised glutathione (Gerson and Shaikh, 1984) and lead may interact with this residue, thereby resulting in the inhibition of the enzyme. The inhibition of glutathione reductase is responsible for altered glutathione status, which ultimately affects the thiol/disulphide ratio of cellular proteins.
Fig 3.8: Effect of LA and DMSA on drug metabolising enzymes in the brain regions of control and experimental animals (Mean ± S.D for six animals)

**Uracilinos Reductase**

- Cerebral Cortex
- Cerebellum
- Hippocampus

**GST**

- Cerebral Cortex
- Cerebellum
- Hippocampus

**Cyt-P450**

- Cerebral Cortex
- Cerebellum
- Hippocampus

GST - moles of CDNB-GSH conjugates for 10 min/mg protein; GR - moles of NADPH utilized/min/mg protein; Cyt-P450 - moles/mg protein.

a - as compared with Group I; b - as compared with group II;

c - as compared with Group VI; d - as compared with Group VII

a, b, c, d - represents statistical significance at p<0.05
Table 3.5: Effect of Lipoic acid and DMSA on the Drug metabolizing enzymes in the liver of control and experimental groups. (Mean ± S.D for six animals in each group)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I Control</th>
<th>Group II Pb</th>
<th>Group III LA</th>
<th>Group IV DMSA</th>
<th>Group V LA+DMSA</th>
<th>Group VI Pb+LA</th>
<th>Group VII Pb+DMSA</th>
<th>Group VIII Pb+LA+DMSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST</td>
<td>150.35±13.53</td>
<td>120.36±10.3</td>
<td>146.3±15.3</td>
<td>152.8±16.2</td>
<td>149.7±14.5</td>
<td>144.6±14.5</td>
<td>136.5±12.6</td>
<td>148.5±13.6</td>
</tr>
<tr>
<td>GR</td>
<td>22.36±2.34</td>
<td>16.5±1.54</td>
<td>21.6±2.36</td>
<td>22.54±2.13</td>
<td>21.8±2.2</td>
<td>20.12±1.98</td>
<td>18.56±1.64</td>
<td>21.88±2.06</td>
</tr>
<tr>
<td>Cyt-P450</td>
<td>18.97±1.65</td>
<td>14.56±1.56</td>
<td>19.12±1.87</td>
<td>18.21±1.75</td>
<td>20.11±1.84</td>
<td>17.23±1.45</td>
<td>16.41±1.45</td>
<td>18.54±1.75</td>
</tr>
</tbody>
</table>

GST – nmoles of CDNB-GSH conjugates formed/min/mg protein; GR – nmoles of NADPH utilized/min/mg protein; Cyt-P450 – nmoles/mg protein.

a – as compared with Group I; b – as compared with group II; c – as compared with Group VI; d – as compared with Group VII
a, b, c, d – represents statistical significance at p<0.005
Results and Discussion

Lipoate is effectively reduced to its dithiol form in vivo by lipoamide dehydrogenase and glutathione reductase systems (Constantinescu et al., 1995) which resulted in increased GST and GR activities by converting them into their active form with a mixed disulphide bond formation (Masakawa and Iwata, 1986). Chelation of lead by DMSA and the antioxidant potential of lipoic acid resulted in increased activity of ALA-D. This increased the availability of heme for the biosynthesis of cyt-450.

Bioamines and Acetyl Choline Esterase

Figure 3.9 indicates the changes in the level of bioamines. Levels of biogenic amines – norepinephrine, dopamine and serotonin in the neural tissues (p<0.05) were decreased. Figure 3.10 and 3.11 depicts the changes of acetylcholine esterase and urinary 5-HIAA. Acetylcholine esterase activity was diminished significantly (p<0.05) in the neural tissues of lead administered rats and increased excretion of 5-HIAA was seen in the urine of lead poisoned rats (p<0.05, Group II).

During lipoate and DMSA treatments, the disturbed levels of the bioamines, AchE and 5-HIAA were restored, to near normal levels (p<0.05) when compared to lead treated group (Group II).

It was found that lead readily enters the immature and adult brain (Bradbury and Deane, 1993). Long term exposure to low levels of Pb²⁺ was found to alter the synaptic transmission in specific brain regions (Kala and JadHAV, 1995). Calcium is an essential nutrient, which is required in multiple cellular and physiological functions, such as cell adhesion, signal transduction and neurotransmission. Lead can substitute it in calmodulin and alter its correct function, inhibit the Ca²⁺ pump and channels, and replace it in various calcium receptors. The interaction with Ca²⁺ in the neurotransmission process or the impairment in its homeostasis, have been often implicated in the
Fig 3.9: Effect of LA and DMSA on neurotransmitters in control and experimental animals. (Mean ± S.D for six animals)

**Dopamine**
- Cerebral Cortex
- Cerebellum
- Hippocampus

**Serotonin**
- Cerebral Cortex
- Cerebellum
- Hippocampus

**Nor-epinephrine**
- Cerebral Cortex
- Cerebellum
- Hippocampus

- control
- Lead
- LA
- DMSA
- LA+DMSA
- Pb+LA
- Pb+DMSA
- Pb+LA+DMSA

a - as compared with Group I; b - as compared with group II; c - as compared with Group VI; d - as compared with Group VII

a, b, c, d - represents statistical significance at p<0.05
Fig 3.10: Effect of LA and DMSA on Acetyl Choline Esterase in the brain regions of control and experimental animals
(Mean ± S.D for six animals)

Fig 3.11: Effect of LA and DMSA on the urinary excretion of 5-HIAA in the control and experimental animals
(Mean ± S.D for six animals)

a - as compared with Group I; b - as compared with group II;
c - as compared with Group VI; d - as compared with Group VII
a, b, c, d - represents statistical significance at p<0.05
Ca\textsuperscript{2+} homeostasis is also deregulated by a number of Pb\textsuperscript{2+}\textendash Ca\textsuperscript{2+} interactions leading to an inhibition of Ca\textsuperscript{2+} and stimulation of Pb\textsuperscript{2+}-influx rates (Simons, 1993; Audesirk, 1993). Lead is capable of substituting calcium at high affinity carboxyl binding sites on intracellular Ca\textsuperscript{2+}-binding proteins. The Pb\textsuperscript{2+}/Ca\textsuperscript{2+} interaction very likely reduces the release rates of several neurotransmitters including dopamine and serotonin (Kala and Jadav, 1995). Scortegagna and Hanbauer (1997) also reported that glial cells on exposure to lead showed decrease in dopamine uptake by 50%.

Urinary 5-HIAA, a degradative metabolite of serotonin (Tozer et al., 1966) was increased in lead intoxicated rats.

One of the principal constellation of Pb\textsuperscript{2+} toxicity includes alterations in motor coordination and cortical function and, as such, draws attention to Pb\textsuperscript{2+} mediated impairments in cholinergic neurotransmission. Cholinergic function requires adequate amounts of acetylcholine, and is contingent also on expression and turnover of acetylcholinesterase.

Acetyl cholinesterase, an integral membrane-bound enzyme responsible for splitting acetylcholine at the nerve terminal was measured to monitor changes in membrane fluidity and integrity. It is well documented that acetylcholinesterase activity is influenced by changes in the membrane microenvironment (Ott, 1985). Mazzanti et al. (1986) have demonstrated that ketamine anesthesia affects synaptic membrane fluidity and kinetics of acetylcholinesterase. Luo and Berman (1997) reported that acetylcholine esterase was inhibited by Pb\textsuperscript{2+} exposure.

The inhibition of the enzyme may be either due to the direct effect of lead or due to alterations in the membrane fluidity as a result of peroxidative damage. Cook et al. (1987) have demonstrated alterations in membrane and
phospholipids composition in erythrocyte membranes from lead exposed workers. The peroxidation of membrane lipids will gradually result in alterations in membrane integrity, which may be of dramatic consequence for the functional proteins in the membranes like receptors, ion channels and enzymes e.g. acetylcholinesterase. Since structural and functional integrity of neuronal membranes is crucial for normal functioning, any alteration in these might have adverse consequences on neurotransmission and may eventually lead to nervous system dysfunction.

On drug treatment, the disturbed levels of the biogenic amines, AchE and 5-HIAA were restored to near normal levels (P<0.05) when compared to toxic group (Group II).

Hematological Changes

As is evident from the data (Table 3.6), lead administration brought about a marked decline in Hb levels and Ht (p< 0.05). Treatment with LA (Group VI) and DMSA (Group VII) as sole agents brought about a significant (p<0.05) improvement in the Hb and Ht levels. However combined treatment with LA and DMSA (Group VIII) was more effective when compared with that of DMSA alone.

Anemia observed during lead poisoning is thought to result from the inhibition of heme synthesis and a decreased life span of the erythrocytes. The replacement of Zn in numerous enzymes and the decrease in the availability of iron could be one of the mechanism by which Pb exert its toxic effects, as is the case of the anemia associated with its poisoning (Goyer, 1995). The shortening of the life span of RBC is probably due to the direct toxic effect of lead upon the cell membrane (Klassen, 1996). Elevated blood Pb levels proved to be a causative factor for lead-induced anemia. Moreover, oxidative stress is known to cause changes in the structure and function of Hb (Chiu et al., 1982). Therefore, the formation of free radicals in the presence of heavy metals
Table 3.6: Effect of lipoic acid and DMSA on hematological indices in control and experimental groups (mean ± S.D for six animals in each group)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (Pb)</th>
<th>Group III (LA)</th>
<th>Group IV (DMSA)</th>
<th>Group V (LA+DMSA)</th>
<th>Group VI (Pb+LA)</th>
<th>Group VII (Pb+DMSA)</th>
<th>Group VIII (Pb+LA+DMSA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>15.61 ± 1.36</td>
<td>7.36 ± 0.93 a</td>
<td>15.86 ± 1.42</td>
<td>15.58 ± 1.61</td>
<td>15.77 ± 1.23</td>
<td>12.84 ± 1.13 ab</td>
<td>10.36 ± 1.33 abc</td>
<td>13.54 ± 1.42 bd</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>51.45 ± 4.37</td>
<td>28.72 ± 2.63 a</td>
<td>52.71 ± 4.85</td>
<td>51.66 ± 5.40</td>
<td>52.45 ± 5.16</td>
<td>42.18 ± 3.87 ab</td>
<td>39.27 ± 4.01 ab</td>
<td>44.86 ± 4.13 bd</td>
</tr>
</tbody>
</table>

Hemoglobin - g/dL; Hematocrit - %

a – as compared with Group I; b – as compared with group II; c – as compared with Group VI; d – as compared with Group VII
a, b, c, d – represents statistical significance at p<0.05
(Jendryczko, 1998) also contribute to Hb denaturation and precipitation leading to anemia. Other reports lend credence to the decrease in Hb levels during chronic lead exposure (Terayama K, 1993; El-Missiry, 2000). Terayama et al. (1986) has already reported alterations, in the Ht during lead poisoning. Our study is in consonance with that of Richter et al. (1980), who have reported a weak association between Hb depression and rise in BPb levels.

**Lipid Peroxidation and Antioxidant Status**

Table 3.7 depicts the effects of LA and DMSA on lead induced erythrocyte membrane lipid peroxidation and enzymatic antioxidant status. Significant (p<0.05) increase in MDA levels in lead exposed rats (Group II) suggests the fact that RBCs are more vulnerable to oxidative damage. The combined effect of LA and DMSA produced a significant decline (P<0.05) in MDA level indicating the regulatory influence of dithiols on erythrocyte membrane.

Table 3.8 shows the effect of LA and DMSA on nonenzymatic antioxidant status in the hemolysate of lead poisoned rats. Group II animals showed a marked decline (p<0.05) in the thiol capacity of the cell as evidenced by decreased GSH levels. Levels of vitamin C and vitamin E were significantly decreased in the lead intoxicated rats (Group II, p<0.05).

Evaluation of the enzymatic antioxidants namely CAT, SOD and GPx showed depressed activities in the hemolysate of lead intoxicated rats. The combinational aid of LA along with DMSA (Group VIII) paved the way for an improved reductive status of the cell by significantly (p<0.05) increasing the GSH and vitamin levels and the activities of CAT, SOD and GPx. The sole administration of LA and DMSA also brought about a significant (p<0.05) improvement in the cells antioxidant defences but was not as effective when compared to the combined treatment group (Group VIII).
Table 3.7: Effect of lipoic acid and DMSA on enzymic antioxidant status in hemolysate of control and experimental animals. (mean ± S.D for six animals in each group)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II (Pb)</th>
<th>Group III (LA)</th>
<th>Group IV (DMSA)</th>
<th>Group V (LA+DMSA)</th>
<th>Group VI (Pb+LA)</th>
<th>Group VII (Pb+DMSA)</th>
<th>Group VIII (Pb+LA+DMSA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO</td>
<td>7.28 ± 0.63</td>
<td>11.34 ± 0.85</td>
<td>7.29 ± 0.83</td>
<td>7.26 ± 0.70</td>
<td>7.27 ± 0.64</td>
<td>8.49 ± 0.89 ab</td>
<td>8.52 ± 0.83 ab</td>
<td>7.40 ± 0.68 bcd</td>
</tr>
<tr>
<td>CAT</td>
<td>4.93 ± 0.38</td>
<td>2.97 ± 0.26 a</td>
<td>4.98 ± 0.40</td>
<td>4.94 ± 0.45</td>
<td>4.95 ± 0.33 ab</td>
<td>4.29 ± 0.38 ab</td>
<td>4.25 ± 0.41 ab</td>
<td>4.88 ± 0.50 bcd</td>
</tr>
<tr>
<td>SOD</td>
<td>1.86 ± 0.19</td>
<td>1.34 ± 0.14 a</td>
<td>1.89 ± 0.20</td>
<td>1.85 ± 0.17</td>
<td>1.86 ± 0.16 ab</td>
<td>1.56 ± 0.15 ab</td>
<td>1.57 ± 0.16 ab</td>
<td>1.79 ± 0.17 bcd</td>
</tr>
<tr>
<td>GPx</td>
<td>0.45 ± 0.05</td>
<td>0.24 ± 0.03 a</td>
<td>0.44 ± 0.03</td>
<td>0.42 ± 0.04</td>
<td>0.45 ± 0.05 ab</td>
<td>0.33 ± 0.03 ab</td>
<td>0.30 ± 0.03 ab</td>
<td>0.42 ± 0.04 bcd</td>
</tr>
</tbody>
</table>

LPO – nmole of MDA/min/mg protein
CAT – µ moles of H₂O₂ consumed per min per mg protein; SOD – units per g Hb (one unit is equal to the amount of enzyme required to inhibit the autoxidation of pyrogallol by 50%); GPx – µmoles of GSH per min per mg protein.

a – as compared with Group I; b – as compared with Group II; c – as compared with Group VI; d – as compared with Group VII
a, b, c, d – represents statistical significance at p<0.05
Table 3.8: Effect of lipoic acid and DMSA on non-enzymic antioxidant status in hemolysate of control and experimental animals. (mean ± S.D for six animals in each group)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I Control</th>
<th>Group II Pb</th>
<th>Group III LA</th>
<th>Group IV DMSA</th>
<th>Group V LA+DMSA</th>
<th>Group VI Pb+LA</th>
<th>Group VII Pb+DMSA</th>
<th>Group VIII Pb+LA+DMSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit C</td>
<td>1.72±0.13</td>
<td>1.32±0.07</td>
<td>1.85±0.16</td>
<td>1.68±0.12</td>
<td>1.77±0.15</td>
<td>1.59±0.13</td>
<td>1.47±0.12</td>
<td>1.70±0.16</td>
</tr>
<tr>
<td>Vit E</td>
<td>1.62±0.18</td>
<td>1.22±0.11</td>
<td>1.58±0.14</td>
<td>1.66±0.15</td>
<td>1.63±0.13</td>
<td>1.49±0.13</td>
<td>1.4±0.11</td>
<td>1.60±0.15</td>
</tr>
<tr>
<td>GSH</td>
<td>21.69 ± 1.65</td>
<td>10.36 ± 0.95</td>
<td>23.25 ± 2.14</td>
<td>23.4 ± 1.96</td>
<td>23.96 ± 2.30</td>
<td>16.92 ± 1.7</td>
<td>15.86 ± 1.50</td>
<td>22.34 ± 1.84</td>
</tr>
</tbody>
</table>

GSH – mg/dl; Vit E and Vit C – mg/dl

a – as compared with Group I; b – as compared with group II; c – as compared with Group VI; d – as compared with Group VII

a, b, c, d – represents statistical significance at the level of p<0.05
Oxidative damage to RBC membrane has been suggested to be one of the possible mechanisms for lead-induced toxicity. According to Chiu et al. (1982), erythrocyte membrane lipids are more prone to peroxidation and would alter membrane permeability and lead to hemolysis. In the lead intoxicated rats augmented peroxidation was observed in the erythrocyte membrane. The oxidative stress created by the metal favours increased production of superoxides rendering rat RBC membrane more susceptible to oxidative damage.

CAT catalyses the dismutation of H$_2$O$_2$ and the decrease in its activity could be due to increase in MDA production in RBC’s, which can crosslink with the amino group of protein to form intra and inter molecular crosslinks thereby inactivating several membrane bound enzymes (Kikugawa, 1984). Moreover, Rister and Bachner (1976) have speculated that during oxidative stress, CAT activity decreases, H$_2$O$_2$ accumulates and thereby the peroxidation of lipids is favoured.

SOD, which requires copper and zinc for its activity, was found to be decreased in lead administered rats. Myoloriet et al. (1986) suggested that this could be due to the lead-induced copper deficiency. Minami et al. (1982) showed a 15-40% decrease in the activity of SOD in erythrocytes of rats exposed to fumes of heavy metals like lead, cadmium and mercury. The low activity of SOD could also be due to the inactivation of the enzyme by cross-linking or damage to DNA (Pfafferoat, 1982) by LPO leading to altered gene expression. Patra and Swarup (2000) apparently reported decreased levels of CAT and SOD in the erythrocytes of lead-exposed calves, which corroborates with our results.

Decreased GPx activity observed in our study could be due to exhaustion or inactivation of the enzyme by ROS since oxidative damage to Hb and cell membrane has been reported to reduce the activity of GPx (Svadlenka et al., 1975).
GSH plays a pivotal role in defending against oxidative hemolysis. Conditions that perturb intracellular levels of GSH have been shown to result in significant alterations in cellular metabolism. Lead binds exclusively to thiol groups (Christie and Costa, 1984) which decreases the GSH levels (Korsrud and Meldrum, 1988) thereby interfering with the antioxidant activity. Gurur et al. (1998) has apparently reported a decline in GSH levels upon lead intoxication.

In lipid membranes, vitamin E is the most prominent antioxidant. It reacts fast with radicals and the vitamin E radical thus formed is fairly stable. This radical, situated within the lipid membrane, is thought to react with vitamin C or with GSH located in the cytosol. Since lead reduced the GSH level, there was also a decline in the level of vitamins.

The usefulness of the antioxidant LA in conjunction with DMSA was very effective in rebalancing the impaired prooxidant/antioxidant ratio. Exogenous administration of LA and DMSA separately brought about an improvement in the cells antioxidant defences due to their antioxidative property (Gurer and Ercal, 2000).

ATPases

Lead-induced peroxidation of membrane lipids was coupled with significant (P < 0.05) inhibition of erythrocyte membrane Na⁺, K⁺ - ATPase, Mg²⁺ - ATPase and Ca²⁺ - ATPase (Table 3.9). During therapy using LA plus DMSA, significant (p<0.05) rise in the activities of the trans membrane enzymes were noted. Mitigation of lead induced inhibition of the membrane bound ATPase by either LA or DMSA as the sole agent was not very effective as compared to the combined treatment (Group VIII).

Erythrocyte membrane bound enzymes are sensitive indices of altered cellular environment. Erythrocyte membrane ATPases are responsible for unidirectional activated transport of Ca²⁺ and Mg²⁺ ions. Na⁺ ions are extruded
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Control)</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>Group VI</th>
<th>Group VII</th>
<th>Group VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺:K⁺-ATPase</td>
<td>1.84 ± 0.16</td>
<td>1.15 ± 0.12</td>
<td>1.88 ± 0.17</td>
<td>1.93 ± 0.18</td>
<td>1.50 ± 0.17</td>
<td>1.47 ± 0.16</td>
<td>1.79 ± 0.15</td>
<td>bod</td>
</tr>
<tr>
<td>K⁺-ATPase</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>ab</td>
<td>ab</td>
<td>bod</td>
<td>bod</td>
</tr>
<tr>
<td>Mg²⁺-ATPase</td>
<td>0.29 ± 0.03</td>
<td>0.12 ± 0.01</td>
<td>0.26 ± 0.02</td>
<td>0.27 ± 0.03</td>
<td>0.29 ± 0.03</td>
<td>0.22 ± 0.02</td>
<td>0.20 ± 0.01</td>
<td>bod</td>
</tr>
<tr>
<td>ATPase - µ moles of phosphorous liberated/mg protein</td>
<td>1.27 ± 0.12</td>
<td>0.68 ± 0.16</td>
<td>1.30 ± 0.13</td>
<td>1.29 ± 0.12</td>
<td>1.28 ± 0.09</td>
<td>1.07 ± 0.14</td>
<td>1.06 ± 0.10</td>
<td>1.23 ± 0.09</td>
</tr>
</tbody>
</table>

ATPases - µ moles of phosphorous liberated/mg protein.

a - as compared with Group I, b - as compared with group II, c - as compared with Group VI, d - as compared with Group VII.

a, b, c, d - represents statistical significance at p<0.05.

Table 3.9: Effect of lipoic acid and DMSA on erythrocyte membrane bound ATPase in control and experimental groups (mean ± S.D for six animals in each group).
against concentration gradient. Due to peroxidation of membrane lipids the osmotic stability of the erythrocytes is altered in the presence of the divalent metal. The causative factor for the shortened survival and decreased deformability can be closely related to inhibition of the membrane bound ATPases.

Our observations are compatible with an earlier report by Raghavan et al. (1981) stating the inhibition of Na⁺, K⁺ - ATPase in the erythrocytes of lead exposed workers. Ca²⁺ - ATPase and Mg²⁺ - ATPase of the erythrocyte membrane were also markedly inhibited due to the divalent cation. Mas-Oliva (1989) has related the inhibitory action of lead on ATPase to essential sulphhydryl groups present in the enzyme.

Serum Marker Enzymes

Enhanced levels of SGOT, SGPT and LDH (p<0.05) were observed in the lead administered rats (Table 3.10), which were reverted to normal levels upon treatment with LA and DMSA (p<0.05). Combined effect of the two drugs was more effective in bringing down the levels of the serum aminotransferases than the individual treatments.

The influence of lead toxicity revealed elevated levels of serum aminotransferases (SGOT and SGPT) revealing leakage of these enzymes from damaged liver cells suggesting the toxic effect of lead on other organs. Tandon et al. (1997) observed similar increments in the levels of SGOT and SGPT in the serum of lead poisoned rats.

Lactate dehydrogenase (LDH), a cytoplasmic enzyme, catalyzes the conversion of lactate to pyruvate and vice versa with the mediation of NAD⁺ as a hydrogen acceptor. LDH also serves as a marker for membrane integrity and is a regulator of many biochemical reactions. Increase in the activity in serum with lead treatment indicates damage of the tissue cell.
Table 3.10: Effect of lipoic acid and DMSA on the activities of serum enzymes in control and experimental groups (mean ± S.D for six animals in each group)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I Control</th>
<th>Group II Pb</th>
<th>Group III LA</th>
<th>Group IV LA+DMSA</th>
<th>Group V LA+DMSA</th>
<th>Group VI Pb+LA</th>
<th>Group VII Pb+DMSA</th>
<th>Group VIII Pb+LA+DMSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT</td>
<td>74.20 ± 6.36</td>
<td>99.29 ± 8.25a</td>
<td>75.30 ± 7.30</td>
<td>76.18 ± 6.85</td>
<td>75.62 ± 5.65</td>
<td>86.61 ± 8.10 ab</td>
<td>88.85 ± 7.43</td>
<td>75.47 ± 6.67bcd</td>
</tr>
<tr>
<td>SGPT</td>
<td>68.36 ± 4.97</td>
<td>89.23 ± 7.14a</td>
<td>69.52 ± 6.30</td>
<td>68.79 ± 5.85</td>
<td>68.63 ± 4.90</td>
<td>79.82 ± 6.36 ab</td>
<td>80.56 ± 7.43</td>
<td>69.17 ± 6.83bcd</td>
</tr>
<tr>
<td>LDH</td>
<td>0.89 ± 0.09</td>
<td>1.75 ± 0.21 a</td>
<td>0.95 ± 0.09</td>
<td>0.87 ± 0.84</td>
<td>0.93 ± 0.07</td>
<td>1.36 ± 0.18 ab</td>
<td>1.24 ± 0.13</td>
<td>1.05 ± 0.11bcd</td>
</tr>
</tbody>
</table>

SGOT and SGPT - units/ml; LDH - μmoles of pyruvate formed/min/mg protein

a – as compared with Group I; b – as compared with group II; c – as compared with Group VI; d – as compared with Group VII
a, b, c, d – represents statistical significance at p<0.05
APOPTOTIC STUDIES

Apoptosis is an integral part of the developmental program and is frequently the end-result of a temporal course of cellular events; it is sometimes referred to as programmed cell death (PCD). It is an active form of cell death in which the cell participates in its own demise via a cascade of molecular interactions. It is morphologically distinct form of cell death (necrosis) and is mediated by outside influences.

Several lines of evidence support the notion that neurotoxic agents may cause neuronal death in the brain by two mechanisms: 1) apoptosis (Martin et al., 1988; Oppenheim, 1991; Franklin and Johnson, 1992; Raff et al., 1993), or 2) necrosis (Manev et al., 1990; Koh et al., 1995). Necrosis is a form of Ca²⁺-dependent neuronal death that may be prompted by an excessive and protracted stimulation of specific glutamate receptors.

Apoptosis, on the other hand, is a form of neuronal death that occurs during development and can be triggered experimentally by drugs or toxins that decrease the availability of neurotrophic factors.

The effect of lead on the neuronal death may be due to the interference of this metal with calcium-dependent cellular processes due to similarities between Ca²⁺ and Pb²⁺ in aqueous solutions (Simons, 1986, 1993). Even at a low dosage of 1μM, Pb²⁺ promotes neuronal apoptosis, as characterized morphologically by cell shrinkage and chromatin condensation, biochemically by the typical internucleosomal DNA fragmentation, and functionally by dependence on new synthesis of macromolecules (Oberto et al., 1996).

It is useful to consider the process of PCD as involving two phase 1) an activation phase, consisting of the intracellular signaling pathways that activate PCD, and 2) an execution phase, consisting of the molecular machinery that is necessary for PCD to occur. Although ROS seems not to be
required in the execution phase, they can be involved in the activation phase, for example, as intracellular signaling molecules.

There is compelling evidence that ROS can activate the cell death programme. The most straightforward example is induction of PCD by treatments with ROS themselves (Lennon et al., 1991; Sandstrom et al., 1994). Lead directly or indirectly through ALA accumulation produces a large amount of ROS, which will lead to the stimulation of apoptosis.

In our present study, there was remarkable DNA-strand break in the leucocytes (Figure 3.12) in lead treated animals (Alkaline single cell gel electrophoresis, comet assay). The tail length of DNA-strand break of leucocytes and brain regions is shown in Figure 3.13. In lead treated rats there was significant increase in the tail length (p<0.05). On treatment with LA and DMSA, DNA-strand break was minimized (p<0.05, Group VIII).

Lead promotes apoptosis by several mechanisms including generation of reactive oxygen species, inducing the expression of c-fos and activation of nuclear transcription factor (NF-κB), activator protein – 1 (AP-1), c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase kinase (MAPKK) and caspases in rat brain (Ramesh et al., 2001; Kim et al., 1997; Pyatt et al., 1996).

A significant increase in the level of TNF-α is observed in the leucocyte and brain regions of lead intoxicated rats (Group II, p<0.05, Figure 3.14). Lipoic acid, DMSA and their combined therapy markedly reduced the levels (p<0.05, Group VI, VII and VIII respectively). Figure 3.15 shows immunoblotting of TNF-α in leucocyte of experimental rats.

Immunofluorescent studies (Figure 3.16) showed the presence of TNF-α in the brain tissue of experimental rats. The fluorescence in the brain tissue of group II animals depicted the accumulation of TNF-α in the neurons. The intense of fluorescence in the brain of lead exposed rats (Group II) was high.
Fig 3.12: Alkaline single cell gel electrophoresis of leucocytes of control and experimental animals
Fig 3.13: Effect of LA and DMSA on DNA-strand breaks in leucocytes and brain regions of control and experimental animals (Mean ± S.D for six animals)

- a - as compared with Group I;
- b - as compared with group II;
- c - as compared with Group VI;
- d - as compared with Group VII

a, b, c, d - represents statistical significance at p<0.05
Fig 3.14: Effect of LA and DMSA on the level of TNF-α in leucocytes and brain regions of control and experimental animals (Mean ± S.D for six animals)

a – as compared with Group I; b – as compared with group II; c - as compared with Group VI; d – as compared with Group VII

a, b, c, d - represents statistical significance at p<0.05
Figure 3.15: Effect of lipoic acid and DMSA on TNF- expression in the leucocyte of control and experimental animals

Lane 1: Marker
Lane 2: Control
Lane 3: Pb induced
Lane 4: Lipoic acid + Pb
Lane 5: DMSA + Pb
Lane 6: Lipoic acid + DMSA + Pb
Fig 3.16: Immunofluorescent studies with TNF-α in the brain regions of control and experimental animals
compared to control. In drug treated groups (Group VI, VII and VIII), the intensity of fluorescence was less. This proved the hypothesis that lead stimulates TNF-α levels and on treatment with an effective antioxidant and chelator, the levels were significantly reduced.

TNF-α primarily produced by activated macrophages, is an immunomodulatory and pro-inflammatory cytokine that induces a variety of cellular responses including apoptosis (Shackelford et al., 1997). TNF-α production is tightly regulated at the transcriptional and at its translational levels, and the gene expression is mainly regulated by binding of the transcription factor NF-κB. Alterations in the binding of transcription factors were most likely due to increased production of ROS upon stimulation of the cells with lead compounds (Naarala et al., 1995; Savolainen et al., 1998).

Activation of many transcription factors, nuclear factor -κ B (NF-κB) in particular, is known to be regulated by the redox state of the cell (Dalton et al., 1999). A transcriptionally active NF-κB complex is formed by two p50 homodimers or a p50/p65 heterodimer bound to a member of κ B family.

In the brain, glutathione is known to interact with the NMDA receptor, leading to NF-κB activation (Uberti et al., 2000). There are reports suggesting that lead can directly interact with the NMDA receptor (Guilarte et al., 1995; Guilarte, 1997), making it quite possible to activate NF-κB through a mechanism very similar to that of glutamate.

Increase in the production of reactive oxygen species during lead metabolism leads to activation of NF-κB and increase in the synthesis of TNF-α. The reduction in TNF-α in lipoic acid and succimer treated rats, showed their ability to reduce the ROS or their ability to inactivate NF-κB and TNF-α. The increased TNF-α observed in this study may lead to the activation of a cascade of reactions including caspase family, which will lead to cell death.
The DNA ladder formation can be considered as the biochemical indicator of apoptotic cell death. DNA fragmentation analysis illustrates the DNA ladder formation upon agarose gel electrophoretic separation of DNA, indicating DNA fragmentation. Fig 3.17 depicts the agarose gel electrophoretic pattern of DNA fragmentation of leukocytes in the control and experimental rats. DNA fragmentation in terms of percentage is shown in Figure 3.18. We found that there was an increase in the DNA fragmentation in lead treated rats. Upon treatment with lipoic acid, succimer and their combinational therapy, there was significant reduction in the fragmentation of DNA (p<0.05) showing their ability to reduce the toxicity produced by the lead.

Loikkanen et al. (1998), reported that lead alone and lead combined with glutamate induced DNA fragmentation in neuroblastoma cells after 24, 48 and 72 hours after the beginning of exposure. Thus, lead induced neuronal cell death may, at least partially, be mediated through apoptosis.

Administration of lipoic acid and succimer significantly reduced the extent of apoptosis and DNA fragmentation. They inhibit free radical mediated apoptosis by directly eliminating them and also by eliminating lead itself from the environment, thereby minimising the production of proapoptotic factors by lead. Apart from the free radical scavenging property, antioxidants (including lipoic acid) are known to regulate the expression of a number of genes and signal regulatory pathways and thereby may prevent the incidence of cell death (Allen and Tresini, 2000).

Histopathological Study

Figure 3.19a, 3.19b and 3.19c depicts the histopathological changes of cerebral cortex, cerebellum and hippocampus of experimental groups. Control rat neural tissues showed normal architecture. On administration of lead, cerebral tissue showed large perineuronal space with
Fig 3.17: Effect of LA and DMSA on DNA fragmentation in the leukocyte of control and experimental groups

Lane 1: Control
Lane II: Pb induced
Lane III: LA + Pb
Lane IV: DMSA + Pb
Lane V: LA + DMSA + Pb
Lane VI: DNA marker
Fig 3.18: Effect of LA and DMSA on DNA fragmentation in leucocytes and brain regions of control and experimental animals (Mean ± S.D for six animals)

- a – as compared with Group I; b – as compared with group II;
- c - as compared with Group VI; d – as compared with Group VII
- a, b, c, d – represents statistical significance at p<0.05
results and discussion

vessels enlarged, and astrocytes proliferation with nuclear condensation. Cerebellum showed a decrease in granular cell layer with demyelinated white matter and neurons showing chromatolysis, haemorrhage and congestion. In hippocampus, there was a large number of neurons showing chromatolysis and increased perineuronal space. On treatment with lipoic acid and DMSA, there was no major damage seen in the tissues and was comparable with the control histology.
Control - Cerebral cortex showing normal architecture  

Pb - showing large perineuronal space with vessels  
       Enlarged and astrocytes proliferation with nuclear  
       Condensation  

Pb + LA - Showing normal architecture  

Pb + DMSA - Showing minimal astrocyte proliferation  

Pb + LA + DMSA - Showing normal architecture
Fig 3.19a: Histopathological changes in cerebral cortex of experimental animals
<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Description</th>
<th>Magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>Cerebellum showing normal architecture</td>
<td>H &amp; E x 150</td>
</tr>
<tr>
<td>Pb</td>
<td>-</td>
<td>Showing decrease in granular cell layer with demyelinated white matter and neurons showing chromatolysis, haemorrhage and congestion</td>
<td>H &amp; E x 150</td>
</tr>
<tr>
<td>Pb + LA</td>
<td>-</td>
<td>Showing normal architecture</td>
<td>H &amp; E x 150</td>
</tr>
<tr>
<td>Pb + DMSA</td>
<td>-</td>
<td>Showing normal architecture</td>
<td>H &amp; E x 150</td>
</tr>
<tr>
<td>Pb + LA + DMSA</td>
<td>-</td>
<td>Showing normal architecture</td>
<td>H &amp; E x 150</td>
</tr>
</tbody>
</table>
Fig 3.19b: Histopathological changes in the cerebellum of experimental animals.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
<th>Magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Hippocampus showing normal architecture</td>
<td>H &amp; E x 150</td>
</tr>
<tr>
<td>Pb</td>
<td>large neurons showing chromatolysis and increased perineuronal space</td>
<td>H &amp; E x 150</td>
</tr>
<tr>
<td>Pb + L.A</td>
<td>showing normal architecture</td>
<td>H &amp; E x 150</td>
</tr>
<tr>
<td>Pb + DMSA</td>
<td>Showing normal architecture</td>
<td>H &amp; E x 150</td>
</tr>
<tr>
<td>Pb + LA + DMSA</td>
<td>Showing normal architecture</td>
<td>H &amp; E x 150</td>
</tr>
</tbody>
</table>
Fig 3.19c: Histopathological changes in the Hippocampus of experimental animals