Nutritional strategy to alleviate Pb-mediated neurotoxic response in prepubertal rats: Ameliorative effect of ferulic acid
1.0 INTRODUCTION

Pb is a well-known toxicant causing impairments in both human and experimental animals which causes functional and structural abnormalities in the brain (Lidsky and Schneider, 2003; White et al., 2007). Pb is known to cause, increased production of ROS and alteration of antioxidant defense systems in animals and occupationally exposed workers. Reducing the possibility of Pb interacting with critical biomolecules and inducing oxidative damage, or disrupting the cell’s antioxidant defenses might be associated with the beneficial role of antioxidant nutrients through exogenous supplementation of antioxidant molecules. The potential role of oxidative stress in Pb-induced toxicity suggests that antioxidants may enhance the efficacy of treatments to mitigate Pb-induced neurotoxicity and cell damage (Chang et al., 2012).

Chelation therapy with calcium-disodium ethylenediamine tetra acetic acid (CaNa$_2$EDTA), sodium 2,3-dimercaptopropane 1-sulfonate (DMPS), British Anti Lewisite (BAL), meso 2,3-dimercaptosuccinic acid (DMSA) etc., are considered to be the best known treatment against Pb poisoning (Flora et al., 2006, 2004; Saxena et al., 2005). However, the treatment with these chelating agents is reported to be compromised with number of serious side-effects (Flora et al., 2008). Chelation therapy is the primary means of treating Pb poisoned children, but the application of the conventional chelators in children with low blood lead levels has been somewhat restricted by the reported adverse health effects and the unspecificity in their efficacy in reversing the toxic effects of Pb. A multicenter study has shown that chelation therapy does not significantly reverse Pb-induced cognitive deficits in children (Dietrich et al., 2004). Many researchers have investigated the benefit of antioxidants in preventing lead toxicity, the mechanisms of antioxidant nutrients being effective via rebalancing the impaired prooxidant/antioxidant ratio are not completely clear.

In recent times, natural plant products have gained enormous importance in the prevention or treatment of various chronic diseases (Pocerich et al., 2011). Phenolic compounds widely distributed in plants have been considered to play a vital role as dietary antioxidants for the prevention of oxidative stress mediated
diseases such as cancer, ischemia and neurodegenerative disorders (Sultana et al., 2005; Duffy et al., 2008; Kelsey et al., 2010). Phytochemicals are a heterogenous group of bioactive compounds that are extensively studied for their health promoting potentials in humans (Van Duynhoven et al., 2011). Most of these compounds have the ability to either scavenge free radicals directly or indirectly by up regulation of endogenous cellular antioxidant defense system via up regulation of transcription factors which control he expression of vitagens such as heat shock proteins, thioredoxin, sirutin, glutathione levels and several other proteins (Ren et al., 2011).

Recent studies have investigated the protective effect of a xanthone derivative of *Garcinia mangostana* against Pb-induced acetylcholinesterase (AChE) dysfunction and cognitive impairment in mice by inhibiting oxidative stress (Phyu and Tangpong, 2014). Protective effect of curcumin and tannic acid on aluminum and Pb-induced oxidative neurotoxicity and alteration in NMDA receptors has been recently studied in rats (Tuzmen et al., 2015). Antioxidants such as ascorbic acid ameliorate oxidative damage induced by maternal low-level lead exposure in the hippocampus of rat pups during gestation and lactation (Chang et al., 2012). Recent studies have demonstrated the protective effect of quercetin, a major bioflavonoid abundant in fruits and vegetables against Pb-induced oxidative stress and impairment of synaptic plasticity (Liu et al., 2013). Likewise previously, Gallic acid, a natural hydrolysis product of tannins (found abundantly in grapes, berries, and other fruits as well as in wine) was shown to attenuate Pb-induced oxidative and locomotor damage in rats (Reckziegel et al., 2011). Other natural flavonoids such as puerrarin, gossypin and curcumin, are also reported to protect against Pb-induced perturbations in hepatic antioxidant system, reduce brain oxidative stress and memory deficits in rats (Dairam et al., 2007; Gautam and Flora, 2010; Liu et al., 2011).

Ferulic acid (trans-4-hydroxy-3-methoxycinnamic acid; FA), one of the most-abundant phenolic compounds in the human diet, is synthesized in plants by the metabolism of phenylalanine and tyrosine. FA is present in seed plants (rice, wheat and oat), vegetables (tomato and carrot), and fruits (pineapple and orange) and possess excellent free radical scavenging and antioxidant
properties (Srinivas et al., 2007; Barone et al., 2009; Sultana, 2012). FA possesses high antioxidant potential owing to its resonance stabilized phenoxy radical structure. In the context of neurological disease, intravenous administration of FA has been shown to protect against neuronal cell death induced by cerebral ischemia (Cheng et al., 2008; Koh, 2012). Interestingly, FA is also known to promote neural progenitor cell proliferation in vitro/ in vivo, ameliorate stress-induced depression-like behavior in mice (Yabe et al., 2010) and improves behavioral impairments and Alzheimer-like pathology in transgenic mice (Mori et al., 2013).

Accordingly, the hypothesis that short-term oral supplements of FA may significantly alleviate Pb-induced phenotype, oxidative stress and neurotoxicity was evaluated employing a prepubertal (PP) rats. In the first study, the protective effect of oral FA supplements were assessed in rats provided with Pb acetate in drinking water which were maintained on normoprotein diet (NPD) and the results obtained are presented in Section A. In the subsequent study, adopting a similar experimental design, the efficacy of FA supplements were assessed in rats which were maintained on low protein diet (LPD) and the results obtained are presented in Section B. In both the studies, the efficacy was assessed by its potential to ameliorate oxidative stress response in brain regions, cholinergic function and dopamine (DA) among PP rats subjected to Pb intoxication at the higher dosage. Since Pb exposure is known to cause specific effects on hippocampal function, we also examined the potential of FA to modulate histological lesions in Hc.

2.0 OBJECTIVE

The primary objective of this study was to examine whether short term oral supplements of Ferulic acid (FA), a ubiquitously distributed phenolic acid in staple foods (fruits, vegetables, cereals, coffee etc.) would abrogate Pb-mediated oxidative stress and neurotoxicity in prepubertal rats maintained on either normoprotein (NPD) or low protein diet (LPD).
3.0 EXPERIMENTAL DESIGN

SECTION -A

3.1 Efficacy of Ferulic acid (FA) to attenuate Pb-mediated neurotoxic response in prepubertal (PP) rats fed normoprotein diet (NPD)

3.1.1 Dosage of Pb acetate

Only one dose (3000ppm) was employed in this study. The dosage selection of Pb was based on the first study (Dose standardization study).

3.1.2 FA dosage selection

The criterion of selection of FA dose was based on our preliminary dose finding studies in rats (Denny Joseph and Muralidhara, 2014).

3.1.3 Neuroprotective efficacy of FA against Lead acetate among PP rats

Study 1: Male PP rats were randomly assigned into four experimental groups (n=6) and were designated as follows: Control, ferulic acid (FA), lead acetate (Pb) and Pb + FA (PbFA) groups.

(1) Control group: Rats received Pb-free deionized water
(2) Ferulic acid (FA) group: positive control received daily oral supplements of FA at (25mg/kg bw/d);
(3) Pb group: rats received lead acetate (PbA) 3000 ppm in deionized drinking water
(4) Pb+FA treated group: Rats received PbA in drinking water and daily oral FA supplements.

Initially, rats of all groups were trained in open field box to assess locomotor phenotype. Daily feed intake and water consumption were measured. The duration of the experiment was 5wks.
A diagrammatic representation of the experimental design is presented below.

![Experimental Design Diagram]

**3.1.4 Behavioral assessment: motor activity**

Prior to sacrifice, rats of all groups were subjected to behavioral assessment, open field locomotor phenotype assay (as described in Materials and Methods).

**3.1.5 Biochemical studies**

Terminally, rats of all the groups were sacrificed under mild anesthesia and whole blood was drawn by cardiac puncture in heparinized tubes. Brain was excised and placed in 0.9% ice-cold NaCl solution and cleaned with the physiological saline solution to completely remove blood cells, blotted on filter paper.

**Analysis of Pb markers in whole blood**

Hemoglobin (Hb) levels, ALAD activity and blood Pb levels were measured (as described in materials and methods).

**Biochemical analysis in brain regions**

Further, the brain was sub-dissected into cortex (Ct), cerebellum (Cb), hippocampus (Hc) and striatum. Cytosol and mitochondria were prepared (as described in Materials and Methods) and the following biochemical markers were determined in brain regions.

**Oxidative stress markers**: Markers of oxidative stress viz., ROS, HP level was estimated in the cytosolic fractions of brain regions. The degree of lipid peroxidation was determined as malondialdehyde (MDA) levels in all the brain.
regions. Reduced GSH levels were quantified in all the brain regions. Extent of protein oxidation was determined in all the brain regions.

Antioxidant enzymes: Activities of enzymes viz., SOD, GPx, GR, GST, and TRR were determined in the cytosolic fractions.

Mitochondrial functions: Activities of enzymes viz., NADH-cyt C reductase (complex I-III), succinate-cyt C reductase (complex II-III), and MTT reduction were measured in all the brain regions.

Cholinergic function/dopamine levels: cholinergic function was measured by AChE and BChE activity. Dopamine levels were measured in striatum by HPLC.

Histopathological evaluation: Histopathological examination of hippocampus CA1 and DG region of control, Pb treated and Pb+FA rats was conducted by hematoxylin and eosin staining.

SECTION -B

3.2 Efficacy of Ferulic acid (FA) to attenuate Pb-mediated neurotoxic response in prepubertal (PP) rats fed low protein diet

3.2.1 Dosage of Pb acetate

Only one dosage (2000ppm) was employed in this study. The dosage selection of Pb was based on the previous study (Section-B; Chapter 2-Study 2).

3.2.2 Low protein Diet as a response modifier

Male PP rats were randomly assigned into four experimental groups (n=6) and were designated as follows:

Group I: Low protein Control (LPD)

Group II: Low protein + Ferulic acid (FA, 25 mg/ kg bw/ day oral, 4 weeks)

Group III: Low protein + lead (Pb) acetate (2000ppm in drinking water for 4 weeks)
Group IV: Low protein+ FA + lead (Pb) acetate

Rats from all the groups were housed individually and fed with low protein diet. Control group received deionized drinking water and the treatment group received deionized drinking water with Pb. The duration of the experiment was 5wks. Daily food intake, water intake and weekly body weights were recorded throughout the experimental period of 4 weeks.

3.2.3 Behavioral assessment: motor activity

Prior to sacrifice, rats of all groups were subjected to behavioral assessment, open field locomotor phenotype assay (as described in materials and methods).

3.2.4 Biochemical studies

Various biochemical parameters viz., Oxidative stress markers, antioxidant defenses, redox status, mitochondrial and neurochemical markers were assayed in tissue samples.

Histopathological evaluation in hippocampus was studied as mentioned in Section A, 3.2.3.

4.0 RESULTS

SECTION -A

4.1 Efficacy of Ferulic acid to attenuate Pb-mediated neurotoxic response in prepubertal (PP) rats fed normoprotein diet

4.1.1 Growth characteristics - body weight

Rats administered with lead (Pb) acetate (PbA-3000ppm) in drinking water exhibited significant reduction (17%) in body weight. While ferulic acid (FA) supplements on the Pb-induced decrease in body weight (Data not shown).

4.1.2 Food and water consumption and lead (Pb) intake

Rats provided Pb in drinking water consumed less feed compared to the controls while the feed intake was reduced by 22% during fourth week and further reduction (28%) was evident during 5th week. Further, terminally at the end of 5
weeks the average PbA intake in rats was 55.2mg/day. However, FA supplements had no significant effect either on the feed intake, or lead intake.

4.1.3 Effect on behavior phenotype - locomotor activity

Pb exposure caused a marked increase in crossing (62%) and a decrease in rearing (40%) number in relation to the control group. Only the rearing effects were partially reversed by FA supplements (Fig 3.1A&B)

4.1.4 Effect of FA supplements: Hb levels, ALAD activity and Pb levels

Data on the effect of FA supplements on parameters measured in blood is shown in Fig 3.2. FA had no effect on the Pb-induced decrease in Hb levels (Fig 3.2A). Pb exposure significantly decreased the ALAD activity levels (by 65%) in relation to the control group. FA alone had no effects on this variable, but the FA supplements partially reversed the effect of Pb (Fig 3.2B). As anticipated rats exposed to Pb showed significantly higher blood levels of the metal than the controls. However, FA treatment had no significant effect on the blood Pb levels (Fig 3.2C).

Modulatory effect of FA: biochemical evidence

4.1.5 Effect on oxidative markers in brain regions

Cerebellum and hippocampus: Pb caused differential degree of oxidative stress in brain regions. While the extent of ROS generation was marginal in Cb (16%), it was robust (93%) in Hc clearly suggesting the higher susceptibility of this region. Interestingly, the ROS levels in both regions were restored to normalcy by FA supplements (Fig 3.3A). Further, Pb exposure decreased the HP levels in Cb (18%) and increased the levels in Hc (18%), while FA supplements normalized the levels (Fig 3.3B). Furthermore, Pb caused moderate elevation in MDA levels in both Cb (33%) and Hc (30%), while FA supplements completely normalized the MDA levels (Fig 3.3C). Similarly, Pb also caused a significant increase in protein carbonyl levels in Hc (16%), while FA supplements marginally reduced the levels (Fig 3.3D).
Cortex and striatum: Modulatory effect of FA on the status of oxidative stress markers are presented in Table 3.1. Pb exposure resulted in a significant increase in the HP and MDA levels in cortex. Interestingly, FA supplement normalized the elevated levels to normalcy (Table 3.1). Further, Pb exposure caused significant increase in the oxidative stress markers - ROS, HP and Protein carbonyl levels in striatum. However, FA supplement brought down only the elevated carbonyl levels to normalcy (Table 3.1).

4.1.6 Effect on antioxidant enzyme activities

Cerebellum and hippocampus: Pb exposure resulted in decreased SOD activity in Cb (28%) and Hc (21%), while FA supplements partially restored the levels (Fig 3.4A). Further, Pb exposure resulted in increased glutathione reductase activity in Cb (27%) and Hc (29%). However, FA supplements restored the elevated activity levels to normalcy in both the brain regions (Fig 3.4B). Likewise, Pb exposure caused increased glutathione peroxidase activity in Cb (22%) and decreased the activity levels in Hc (23%). However, FA supplements enhanced the levels in Hc by 1.2 fold and restored the levels in Cb (Fig 3.4C). Interestingly, Pb exposure caused enhanced TRR activity in both Cb and Hc and however, FA supplement had no effect on the TRR activity levels (Fig 3.4D). Among Pb exposed rats, GST activity was enhanced in Cb (45%), and Hc (14%) and FA supplements further enhanced the activity in Hc (Data not shown).

Cortex and striatum: Data on the effect of FA supplements on the status of antioxidant enzymes is presented in Table 3.2. Pb exposure caused a significant decrease in the SOD levels (27%) and TRR activity in Ct. However, FA supplements further increased the activity levels in Ct (Table 3.2). Interestingly, FA supplements further increased the activity levels of GR, GPx, and TRR in Ct (Table 3.2). Further, Pb exposure caused significant reduction SOD levels (43%) in St. FA supplement further decreased the SOD levels (33%) (Table 3.2). Similarly, Pb exposure resulted in increased activity levels of GR (89%), GPx (47%), TRR (27%) and GST (20%) levels. FA supplement restored the activity levels of GR and GPx, the activity levels of TRR was further enhanced, and no effect was evident on GST levels.
4.1.7 Modulatory effect on mitochondrial enzymes in brain regions

*Cortex and Striatum*: Pb caused a significant decrease in the activity levels of complex I-III (37%) and the complex II-III (32%) in Ct. However, FA supplement restored the mitochondrial complex I-III activity levels to near normalcy in Ct (*Table 3.3*). Interestingly, Pb caused a significant increase in the activity levels of complex I-III (45%) and decreased the complex II-III (26%) in St (*Table 3.3*). However, FA supplements restored the activity levels to normalcy suggesting the protective effect of FA in mitochondria. Further, FA supplement among Pb exposed rats, significantly offset the extent of MTT reduction as evident in the MTT reduction assay (*Table 3.3*).

*Cerebellum and hippocampus*: Pb exposure caused a significant decrease in the activity of complex I-III in Cb (31%) and elevated the activity levels in Hc (64%). However, FA supplements restored the activity levels to normalcy in both Cb and Hc (*Fig 3.5A*). In contrast, Pb exposure caused a significant decrease in the activity of complex II-III in Cb (32%) and Hc (15%). However, FA supplements had no effect on decreased activity levels in Cb, but FA supplement further decreased the activity levels in Hc (37%) (*Fig 3.5B*). Mitochondria of Pb exposed rats exhibited a significant reduction in the formation of formazan on exposure to MTT in Hc (23%), and FA supplement offered significant protection in Hc (*Fig 3.5C*).

4.1.8 Effect on cholinergic function and Dopamine levels in striatum

Pb intoxication resulted in a marginal increase in AChE in striatum. While, FA supplements restored the activity levels (*Fig 3.6A*). However, Pb exposure increased the BChE levels by 16%. Interestingly, FA treatment further elevated the activity levels by 23% (*Fig 3.6B*). Among Pb administered rats, DA levels were significantly increased. However, the enhanced levels were completely restored upon FA treatment (*Fig 3.6C*).

4.1.9 Histopathological alterations: modulatory effect of FA supplements

*Histoarchitecture of Hippocampus*: *Fig 3.7&3.8* represents the cornu ammonis area CA1 and the dentate gyrus regions of the hippocampus of control; Pb treated and Pb plus FA-treated groups. The hippocampi of the control group had
a normal architecture, and damaged cells were almost nonexistent in CA1 (Fig 3.7) and DG (Fig 3.7) regions. The number of damaged neurons in the Pb-treated group was markedly increased in the CA1, and DG regions of the hippocampi compared to that of the control group. However, the Pb plus FA-treated group had a significantly reduced number of damaged neurons in CA1 (Fig 3.7) and DG (Fig 3.8) compared to that of the Pb-treated group.

SECTION -B

4.2 Ferulic acid enrichment alleviates Pb-mediated neurotoxic response in PP rats fed low protein diet

4.2.1 Growth characteristics and body weight

LPD Rats administered with lead (Pb) acetate (PbA-2000ppm) in drinking water exhibited marginal reduction in body weight. While ferulic acid (FA) supplements on the Pb-induced decrease in body weight had no effect (Data not shown).

4.2.2 Changes in Food, water and lead intake

The food intake, water intake, and PbA intake of rats were measured daily. PbA administered rats exhibited marginal reduction in the feed intake (Data not shown). Further, terminally at the end of 4 weeks the average PbA intake in rats was 51.6mg/day. However, FA supplement had no effect on the feed intake and lead intake.

4.2.3 Effect on locomotor activity

Pb exposure caused a marked increase in crossing (65%) and a decrease in rearing (43%) number in relation to LPD control. Only the rearing effects were partially reversed by FA treatment group (Fig 3.9A & B)

4.2.4 Effect of FA on Hb and ALAD activity and blood Pb levels

The modulatory effect of FA on Pb induced alterations in blood Hb and ALAD activity are shown in Fig 3.10. FA had no effect on the Pb-induced decrease in Hb levels (Fig 3.10A). Pb exposure significantly decreased blood ALAD activity
(33%) in relation to LPD control. FA alone further decreased the ALAD (33%) activity. Co-treatments Pb plus FA had no effect on the ALAD activity (Fig 3.10B). In Pb treated rats, the blood levels were significantly higher than in the control group, while the blood Pb concentration in the Pb plus FA treated group was not decreased relative to the Pb treated group (Fig 3.10C).

Ameliorative effects of FA on oxidative impairments

4.2.5 Modulatory effect of FA on oxidative markers in brain regions

Cortex and cerebellum: Data on the status of cytosolic oxidative stress markers among control and Pb groups as influenced by FA enrichment is presented in Fig 3.11. Pb exposure resulted in significant increase in the MDA levels in both Ct and Cb (Fig 3.11B). Interestingly, FA supplement completely normalized the MDA levels in Ct and partially reduced the levels in Cb (Fig 3.11B). Further, Pb-induced increase in the HP levels was also restored to normalcy in Ct (Fig 3.11C). However, Pb caused marginal elevation in GSH levels was diminished by FA supplements in Ct (Fig 3.11D). Likewise, FA supplements normalized the Pb-induced decrease in the total thiol levels in Cb (Fig 3.11E). Further, Pb caused significant decrease (19%) in the nitric oxide (NO) levels in Ct and marginal decrease in Cb. Interestingly, FA supplement normalized the NO levels in Ct and Cb (Fig 3.11F).

Hippocampus and striatum: Data on the status of cytosolic oxidative stress markers among control and Pb groups as influenced by FA enrichment is presented in Fig 3.12. Pb exposure decreased the ROS levels in Hc. Interestingly; FA restored the ROS levels to normalcy in Hc (Fig 3.12A). Likewise, FA further decreased the ROS levels in St (Fig 3.12A). Further Pb caused marginal decrease in hydroperoxide levels in St and the levels were further decreased by FA supplement (Fig 3.12C). Likewise, FA supplement further decreased the GSH levels in Hc. However, In St Pb-induced marginal increases in GSH levels were further reduced by FA (Fig 3.12D).

4.2.6 Effect on mitochondrial oxidative markers in cortex and cerebellum

Effect of FA on the status of oxidative markers is presented in Table 3.4. Pb administration caused significant increase in the HP levels in Ct and FA
supplements partially reduced the levels in Ct. However, FA supplement in Pb administered rats further increased the TSH and NO levels in Ct (Table 3.4). Likewise, Pb elevated the NO levels and protein carbonyls in Cb, while FA supplements partially decreased the levels in Cb (Table 3.4).

4.2.7 Effect on antioxidant enzyme activities in brain regions

FA alone in LPD rats significantly increased the antioxidant enzyme activities in Ct and Cb (Fig 3.13). Among Pb administered rats, CAT activity was significantly increased in Ct and Cb and the levels were partially reduced by FA supplements (Fig 3.13A). Likewise, FA supplements normalized the Pb-induced increase in the activity levels of SOD (Fig 3.13B) and GPX in Ct (Fig 3.13C). Interestingly, Pb caused increase in TRR activity levels in Ct and Cb were brought to normalcy by FA supplements in both the regions (Fig 3.13E).

Among Pb administered rats, SOD activity was significantly increased in St. While FA supplements restored the activity (Fig 3.14A). Likewise, Pb exposure marginally increased the GPx activity in St. While FA supplement further decreased the levels (Fig 3.14B). Interestingly, Pb-induced increase in the GR activity levels were further decreased by FA treatment in St (Fig 3.14C). Further, Pb-induced marginal increase in the TRR levels were decreased by FA supplements in St (Fig 3.14D). Further, Pb administration had no effect on GST activity levels in Hc and St. However, FA further decreased the GST levels only in St (Fig 3.14E).

4.2.8 Modulatory effect on cholinergic function and striatal dopamine

With Pb exposure, the activity levels of AChE were diminished in Ct, Cb and Hc. While, FA supplements further decreased the activity in both Cb and St (Fig 3.15A&B). Among Pb administered rats, DA levels in St were increased, while FA supplements had no effect (Fig 3.15C).

4.2.9 Effect on mitochondrial function

Pb administration caused significant increase in the mitochondrial complex I-III activity in Ct, Cb and Hc and the activity levels were normalized in Ct (Fig 3.16A) and Hc (Fig 3.16B). However, marginal decrease evident in the complex I-III
activity levels in St were elevated with FA supplements (Fig 3.16B). Further, Pb had no effect on the formation of formazan on exposure to MTT in all the brain regions. However, FA supplements increased the formazan adduct only in Ct (Fig 3.16C).

4.2.10 Effect on Pb-induced histological alterations in the hippocampus

Typical histological photographs of hippocampi regions are presented in Fig 3.17-3.19. Fig 3.17 represents the cornu ammonis area CA1 regions of the hippocampus of LPD control; LPD plus FA; Pb treated and Pb plus FA-treated groups. The hippocampi of the control and FA alone group had a normal architecture, and damaged cells were almost nonexistent in CA1 (Fig 3.17). The number of damaged neurons in the Pb-treated group was markedly increased in the CA1 of hippocampi compared to that of the LPD control group. However, the Pb plus FA-treated group had a significantly reduced number of damaged neurons in CA1 (Fig 3.17).

Further, degenerating neurons were markedly increased in CA3 region of the LPD control. While, FA alone significantly reduced the degenerating cells in LPD rats (Fig 3.18). Pb administration further increased the damaged cells in LPD group. Interestingly, damaged cells were markedly reduced in CA3 region by FA supplements (Fig 3.18). Further, DG region in FA alone group had a normal architecture, and the damaged cells were almost nonexistent compared to LPD control. Further, Pb administration further increased the damaged cells. However, FA treatment had no effect on the damaged cells in DG region (Fig 3.19).
**Fig 3.1**

Modulatory efficacy of ferulic acid (FA) supplements on Pb-induced effects on crossing and rearing activity among rats

Values are mean ± SE (n=6).
Data analyzed by one-way ANOVA followed by post hoc Tukey's test.
* p<0.05, compared to control group; # p<0.05, compared to Pb 3000 group.
CTR-Control; Pb-lead acetate-3,000 ppm, FA-ferulic acid (25 mg/kg bw/day oral for 5 weeks)
A-Crossing activity in open filed box; B-Rearing activity in open filed box

**Fig 3.2**

Modulatory effect of FA supplements on hemoglobin levels and activity of ALAD in prepubertal rats exposed to lead (3000 ppm in drinking water)

Values are mean ± SE (n=6).
Data analyzed by one-way ANOVA followed by post hoc Tukey's test.
* p<0.05, compared to control group; # p<0.05, compared to Pb 3000 group.
CTR-Control; Pb-lead acetate-3000 ppm, FA-ferulic acid (25 mg/kg bw/d oral for 5 wks)
A-Hemoglobin levels
B-Aminolevulinic acid dehydratase activity
C-Blood lead (Pb) levels
Fig 3.3

Effect of FA supplements on oxidative stress markers of PP rats exposed to lead (Pb) acetate (3000ppm in drinking water) maintained on NPD

Values are mean ± SE (n=6)

Data analyzed using one-way ANOVA followed by post hoc Tukey’s test.

*<p<0.05, compared to control group; #p<0.05, compared to Pb 3000 group.
CTR-Control; Pb-lead acetate-3,000 ppm, FA-ferulic acid (25 mg/kg bw/d oral for 5 wks)

Cb-cerebellum, Hc- hippocampus

A-Reactive oxygen species levels; B-Hydroperoxide levels;
C-Malondialdehyde levels; D-Protein carbonyls
Fig 3.4

Modulatory effect of Ferulic acid (FA) supplements on antioxidant enzyme activities in PP rats exposed to lead (Pb) acetate (3000ppm in drinking water) maintained on NPD

Values are mean ± SE (n=6).

Data analyzed by one-way ANOVA followed by post hoc Tukey's test.
*p<0.05, compared to control group; *p<0.05, compared to Pb 3000 alone group.
CTR-Control; Pb-lead acetate-3,000 ppm, FA-ferulic acid (25 mg/kg bw/d oral for 5 wks)

Cb-cerebellum, Hc- hippocampus

A-Superoxide dismutase; B-Glutathione reductase
C-Glutathione peroxidase; D-Thioredoxin reductase
Table 3.1

Modulatory effect of ferulic acid on the activities of antioxidant enzymes in cortex and striatum of PP rats exposed to lead (Pb) acetate (3000ppm in drinking water) maintained on NPD

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>FA</th>
<th>Pb</th>
<th>Pb+FA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROS(^a)</td>
<td>8.7 ± 0.5</td>
<td>8.2 ± 0.2</td>
<td>8.4 ± 0.3</td>
<td>7.8 ± 0.7</td>
</tr>
<tr>
<td>GSH(^b)</td>
<td>3.7 ± 0.08</td>
<td>3.8 ± 0.05</td>
<td>3.8 ± 0.06</td>
<td>3.9 ± 0.02</td>
</tr>
<tr>
<td>HP(^c)</td>
<td>31.6 ± 1.2</td>
<td>26.9 ± 2.0(^*)</td>
<td>40.9 ± 3.3(^*)</td>
<td>29.2 ± 0.3(^#)</td>
</tr>
<tr>
<td>MDA(^d)</td>
<td>2.0 ± 0.0</td>
<td>1.5 ± 0.0(^*)</td>
<td>2.4 ± 0.0(^*)</td>
<td>2.0 ± 0.1(^#)</td>
</tr>
<tr>
<td>PC(^e)</td>
<td>10.6 ± 0.98</td>
<td>11.1 ± 0.15</td>
<td>9.6 ± 9.8</td>
<td>10.24 ± 0.20</td>
</tr>
<tr>
<td><strong>Striatum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROS(^a)</td>
<td>6.65 ± 0.08</td>
<td>5.03 ± 0.3(^*)</td>
<td>9.72 ± 0.24(^*)</td>
<td>7.98 ± 0.53(^#)</td>
</tr>
<tr>
<td>GSH(^b)</td>
<td>3.52 ± 0.01</td>
<td>3.52 ± 0.01</td>
<td>3.18 ± 0.03(^*)</td>
<td>3.44 ± 0.03</td>
</tr>
<tr>
<td>HP(^c)</td>
<td>40.9 ± 0.85</td>
<td>34.3 ± 2.05(^*)</td>
<td>32.8 ± 1.42(^*)</td>
<td>32.1 ± 0.93</td>
</tr>
<tr>
<td>MDA(^d)</td>
<td>1.31 ± 0.00</td>
<td>1.11 ± 0.0</td>
<td>1.30 ± 0.1</td>
<td>1.15 ± 0.0</td>
</tr>
<tr>
<td>PC(^e)</td>
<td>12.80 ± 0.18</td>
<td>9.62 ± 0.39(^*)</td>
<td>17.69 ± 0.41(^*)</td>
<td>14.03 ± 0.79(^#)</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n = 6).

Data analyzed using one-way ANOVA followed by post hoc Tukey’s test. 
p < 0.05 \(^*\) compared to control; \(^#\) compared to Pb 3000 CTR-Control; Pb-lead acetate- 3000 ppm, FA-ferulic acid (25 mg/kg bw/d oral for 5 wks)

\(^a\) Reactive oxygen species, pmol DCF/min/mg protein.
\(^b\) Reduced glutathione, \(\mu\)gGSH/mg protein (GSH)
\(^c\) Hydroperoxides, nmol hydroperoxides/mg protein
\(^d\) Malondialdehyde, nmol MDA/mg protein
\(^e\) Protein carbonyls, nmol carbonyls/mg protein
Table 3.2

Modulatory effect of ferulic acid on the status of oxidative stress markers and reduced glutathione in cortex and striatum of 
PP rats exposed to lead (Pb) acetate (3000 ppm in drinking water) maintained on NPD

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>FA</th>
<th>Pb</th>
<th>Pb+FA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.1 ± 7.5</td>
<td>94.0 ± 0.7</td>
<td>60.3 ± 4.2*</td>
<td>119.1 ± 4.1#</td>
</tr>
<tr>
<td>GR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.5 ± 1.0</td>
<td>8.4 ± 1.1</td>
<td>11.1 ± 0.2</td>
<td>15.5 ± 0.1#</td>
</tr>
<tr>
<td>GPx&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.6 ± 2.2</td>
<td>34.6 ± 2.2</td>
<td>31.5 ± 0.4</td>
<td>50.8 ± 1.3#</td>
</tr>
<tr>
<td>TRR&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.0 ± 0.4</td>
<td>13.5 ± 0.9*</td>
<td>13.9 ± 0.7*</td>
<td>15.4 ± 0.2</td>
</tr>
<tr>
<td>GST&lt;sup&gt;e&lt;/sup&gt;</td>
<td>116.6 ± 2.9</td>
<td>154.3 ± 5.0*</td>
<td>124.8 ± 0.2</td>
<td>148.1 ± 2.2#</td>
</tr>
<tr>
<td><strong>Striatum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>130.5 ± 4.2</td>
<td>97.08 ± 6.5*</td>
<td>74.52 ± 3.4*</td>
<td>50.21 ± 17.1#</td>
</tr>
<tr>
<td>GR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.6 ± 0.47</td>
<td>5.0 ± 0.53</td>
<td>8.7 ± 0.83*</td>
<td>4.1 ± 0.03#</td>
</tr>
<tr>
<td>GPx&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.5 ± 1.53</td>
<td>28.7 ± 0.09*</td>
<td>34.5 ± 1.65*</td>
<td>29.0 ± 1.12#</td>
</tr>
<tr>
<td>TRR&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.3 ± 1.02</td>
<td>14.1 ± 0.96</td>
<td>16.9 ± 0.12*</td>
<td>20.3 ± 0.13#</td>
</tr>
<tr>
<td>GST&lt;sup&gt;e&lt;/sup&gt;</td>
<td>84.2 ± 0.95</td>
<td>100.8 ± 2.56*</td>
<td>101.0 ± 0.61*</td>
<td>97.7 ± 0.42#</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n = 6).

Data analyzed using one-way ANOVA followed by post hoc Tukey’s test
p < 0.05, * compared to control; # compared to Pb 3000 CTR-Control; Pb-lead acetate- 3000 ppm, FA-ferulic acid 25mg/kg bw/day oral for 5 weeks.

<sup>a</sup> Units/mg protein (SOD)
<sup>b</sup> µmol NADPH oxidized/ min/ mg protein (GR)
<sup>c</sup> nmol NADPH oxidized/ min/ mg protein (GPx)
<sup>d</sup> µmol DTNB oxidized/ min/ mg protein (TRR)
<sup>e</sup> nmol GS-DNB/ min/ mg protein (GST)
Table 3.3

Modulatory effect of ferulic acid on the mitochondrial function in cortex and striatum of PP rats exposed to lead (Pb) acetate (3000ppm in drinking water) maintained on NPD

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>FA</th>
<th>Pb</th>
<th>Pb+FA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex I-III(^a)</td>
<td>55.97 ± 0.5</td>
<td>59.99 ± 1.2</td>
<td>35.35 ± 0.3(^*)</td>
<td>47.07 ± 5.6(^#)</td>
</tr>
<tr>
<td>Complex II-III(^b)</td>
<td>9.75 ± 0.9</td>
<td>8.68 ± 0.1</td>
<td>6.63 ± 0.4(^*)</td>
<td>6.50 ± 0.6</td>
</tr>
<tr>
<td>MTT(^c)</td>
<td>31.83 ± 3.0</td>
<td>29.07 ± 1.1</td>
<td>36.50 ± 0.9</td>
<td>37.58 ± 0.2</td>
</tr>
<tr>
<td><strong>Striatum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex I-III(^a)</td>
<td>50.88 ± 3.08</td>
<td>66.36 ± 3.46(^*)</td>
<td>73.66 ± 0.64(^*)</td>
<td>58.36 ± 5.75(^#)</td>
</tr>
<tr>
<td>Complex II-III(^b)</td>
<td>8.33 ± 0.78</td>
<td>8.96 ± 0.15</td>
<td>6.17 ± 0.14(^*)</td>
<td>8.46 ± 0.39(^#)</td>
</tr>
<tr>
<td>MTT(^c)</td>
<td>30.4 ± 1.18</td>
<td>26.7 ± 0.64</td>
<td>21.8 ± 0.93(^*)</td>
<td>29.6 ± 0.45(^#)</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n = 6).

Data analyzed using one-way ANOVA followed by post hoc Tukey’s test.

\( p < 0.05 \), \(^*\) compared to control; \(^#\) compared to Pb 3000
CTR-Control; Pb-lead acetate- 3000 ppm, FA-ferulic acid (25 mg/kg bw/d oral for 5 wks)

\(^a\) nmol cyt C reduced/ min/ mg protein (Complex)
\(^b\) nmol cyt C reduced/ min/ mg protein (Complex)
\(^c\) Absorbance/ mg protein (MTT)
Fig 3.5

Modulatory effect of Ferulic acid (FA) supplements on NADH- cyt C reductase, succinate- cyt C reductase and MTT reduction in mitochondria of brain regions PP rats exposed to lead (Pb) acetate (3000ppm in drinking water) maintained on NPD

Values are mean ± SE (n=6).

Data analyzed by one-way ANOVA followed by post hoc Tukey’s test.
*p<0.05, compared to control group; #p<0.05, compared to Pb 3000 alone group.
CTR-Control; Pb-lead acetate-3,000 ppm, FA-ferulic acid (25 mg/kg bw/d oral for 5 wks)

Cb-cerebellum, Hc- hippocampus

A-NADH cyt C reductase; B-Succinate cyt C reductase; C-MTT reduction
Fig 3.6

Modulatory effect of Ferulic acid supplements on activity levels of AChE, BChE and dopamine levels in striatum PP rats exposed to lead (Pb) acetate (3000ppm in drinking water) maintained on NPD

Values are mean ± SE (n=6).

Data analyzed by one-way ANOVA followed by post hoc Tukey’s test.

* $p<0.05$, compared to control group; * $p<0.05$, compared to Pb 3000 alone group.

CTR-Control; Pb-lead acetate-3,000 ppm, FA-ferulic acid (25 mg/kg bw/d oral for 5 wks)

A-Acetylcholinesterase
B-Butyrylcholinesterase
C-Dopamine
Fig 3.7

Modulatory effect of Ferulic acid (FA) supplements on the histoarchitecture of hippocampus in PP rats exposed to lead (Pb) acetate (3000ppm in drinking water) maintained on NPD

Hematoxylin and eosin stained sections of hippocampi of rats (5 week study). Control (CTR); Pb exposure (Pb 3,000 ppm); Pb+ Ferulic acid (25 mg/kg bw). Degenerating neurons with shrunken and dark nuclei were markedly increased in cornu ammonis CA1 regions of Pb exposed rats. The numbers of degenerating neurons were decreased among Pb+FA rats.
Fig 3.8

Modulatory effect of Ferulic acid (FA) supplements on the histoarchitecture of hippocampus in PP rats exposed to lead (Pb) acetate (3000ppm in drinking water) maintained on NPD.

Hematoxylin and eosin stained sections of hippocampi of rats (5 week study). Control (CTR); Pb exposure (Pb 3,000 ppm); Pb+Ferulic acid (25 mg/kg bw). Degenerating neurons with shrunken and dark nuclei were markedly increased in dentate gyrus (DG) hippocampal regions of Pb exposed rats. The numbers of degenerating neurons were decreased among Pb+FA rats.
Fig 3.9

Efficacy of ferulic acid supplements on crossings and rearing activity of PP rats exposed to lead (Pb) acetate (2000ppm) and maintained on low protein diet (LPD)

Values are mean ± SE (n=6).
Data analyzed by one-way ANOVA followed by post hoc Tukey’s test.
LPD-low protein group control; FA-Ferulic acid 25mg/kg bw/d oral; Pb-lead acetate 2000ppm.
* p<0.05, compared to LPD control group
#p<0.05, compared to Pb 2000 alone group
A-Crossing activity in open filed box
B-Rearing activity in open filed box

Fig 3.10

Efficacy of ferulic acid supplements on hemoglobin levels, ALAD activity and blood Pb levels in of PP rats exposed to lead (Pb) acetate (2000ppm) and maintained on LPD

Values are mean ± SE (n=6).
Data analyzed by one-way ANOVA followed by post hoc Tukey's test.
LPD-low protein group control; FA-Ferulic acid 25mg/kg bw/d oral; Pb-lead acetate 2000ppm.
* p<0.05, compared to LPD control group
# p<0.05, compared to Pb 2000
A-Hemoglobin levels; B-Aminolevulinicacid dehydratase activity; C-Blood lead (Pb) levels
Fig 3.11

Modulatory effect of ferulic acid (FA) supplements on oxidative stress markers in cortex and cerebellum of PP rats exposed to lead (Pb) acetate (2000ppm in drinking water) maintained on LPD

Values are mean ± SE (n=6).

Data analyzed by one-way ANOVA followed by post hoc Tukey’s test.

LPD-low protein group control; FA-Ferulic acid 25mg/kg bw/d oral; Pb-lead acetate 2000ppm. *compared to LPD control p<0.05; # compared to Pb 2000 p<0.05

A-Reactive oxygen species; B-Malondialdehyde; C-Hydroperoxides, D-Glutathione; E-Total thiols; F-Nitric oxide
Fig 3.12

Modulatory effect of ferulic acid (FA) supplements on oxidative stress markers in hippocampus and striatum of PP rats exposed to lead (Pb) acetate (2000ppm in drinking water) maintained on LPD

Values are mean ± SE (n=6).

Data analyzed by one-way ANOVA followed by post hoc Tukey’s test.
LPD-low protein group control; FA-Ferulic acid 25mg/kg bw/d oral; Pb-lead acetate 2000ppm.
*compared to LPD control p<0.05; # compared to Pb 2000 p<0.05

A- Reactive oxygen species; B-Malondialdehyde; C-Hydroperoxides,
D-Glutathione; E-Total thiols; F-Nitric oxide
Table 3.4

Modulatory effect of ferulic acid (FA) supplements on oxidative stress markers in mitochondria of cortex and cerebellum of PP rats exposed to lead (Pb) acetate (2000ppm in drinking water) maintained on LPD

<table>
<thead>
<tr>
<th>Group/Regions</th>
<th>LP</th>
<th>LP+FA</th>
<th>LP+Pb</th>
<th>LP+Pb+FA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP(^a)</td>
<td>114.5 ± 8.8</td>
<td>194.0 ± 0.7(^*)</td>
<td>256.0 ± 12.4(^*)</td>
<td>229.5 ± 4.8(^#)</td>
</tr>
<tr>
<td>TSH(^b)</td>
<td>666.3 ± 9.6</td>
<td>789.3 ± 20.3(^*)</td>
<td>701.1 ± 3.9</td>
<td>927.0 ± 57.3(^#)</td>
</tr>
<tr>
<td>NO(^c)</td>
<td>123.0 ± 1.5</td>
<td>146.0 ± 5.0(^*)</td>
<td>116.0 ± 1.(^#)</td>
<td>162.3 ± 0.6(^#)</td>
</tr>
<tr>
<td>PC(^d)</td>
<td>27.23 ± 1.28</td>
<td>27.19 ± 0.62</td>
<td>16.46 ± 0.3(^*)</td>
<td>25.97 ± 1.4(^#)</td>
</tr>
<tr>
<td><strong>Cerebellum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP(^a)</td>
<td>100.1 ± 0.7</td>
<td>104.0 ± 7.4</td>
<td>112.0 ± 6.1</td>
<td>166.5 ± 2.6(^#)</td>
</tr>
<tr>
<td>TSH(^b)</td>
<td>641.9 ± 19.8</td>
<td>769.7 ± 7.1(^*)</td>
<td>796.4 ± 35.9(^*)</td>
<td>750.4 ± 2.3</td>
</tr>
<tr>
<td>NO(^c)</td>
<td>137.1 ± 1.7</td>
<td>152.3 ± 4.1(^*)</td>
<td>174.6 ± 2.4(^*)</td>
<td>155.8 ± 1.0(^#)</td>
</tr>
<tr>
<td>PC(^d)</td>
<td>17.50 ± 0.66</td>
<td>23.97 ± 0.51(^*)</td>
<td>28.21 ± 0.4(^*)</td>
<td>20.44 ± 0.6(^#)</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n=6).

Data analyzed by one-way ANOVA followed by post hoc Tukey’s test.
LPD-low protein group control; FA-Ferulic acid 25mg/kg bw/d oral; Pb-lead acetate 2000ppm.
*compared to LPD control p<0.05
\(^#\) compared to Pb 2000 p<0.05

\(^a\) Hydroperoxides, nmol HP/mg protein
\(^b\) Total thiols, nmol DTNB/mg protein
\(^c\) Nitric oxide, nmol nitrite/mg protein
\(^d\) Protein carbonyls, nmol carbonyls/mg protein
Modulatory efficacy of ferulic acid supplements on catalase, SOD and GPx activities in cortex and cerebellum of PP rats exposed to lead (Pb) acetate (2000ppm in drinking water) maintained on LPD

Values are mean ± SE (n=6)

Data analyzed by one-way ANOVA followed by post hoc Tukey’s test.
LPD-low protein group control; FA-Ferulic acid 25mg/kg bw/d oral; Pb-lead acetate 2000ppm. *p<0.05, compared to LPD control group; *p<0.05, compared to Pb 2000ppm

Ct-Cortex; Cb-Cerebellum

A-Catalase; B-Superoxide dismutase
C-Glutathione peroxidase; D-Glutathione reductase
E-Thioredoxin reductase; F-Glutathione-S-transferase
Fig 3.14

Modulatory efficacy of ferulic acid supplements on SOD and GPx activity in hippocampus and striatum of PP rats exposed to lead (Pb) acetate (2000ppm in drinking water) maintained on LPD

Values are mean ± SE (n=6).

Data analyzed by one-way ANOVA followed by post hoc Tukey’s test.
LPD-low protein group control; FA-Ferulic acid 25mg/kg bw/d oral; Pb-lead acetate 2000ppm.
*p<0.05, compared to LPD control group; #p<0.05, compared to Pb 2000ppm

Hc-Hippocampus; St-Striatum

A-Superoxide dismutase; B-Glutathione peroxidase; C-Glutathione reductase;
D-Thioredoxin reductase; E-Glutathione-s-transferase
Fig 3.15

Effect of ferulic acid supplements acetylcholinesterase and dopamine levels (Striatum) in of PP rats exposed to lead (Pb) acetate (2000ppm in drinking water) maintained on LPD

Values are mean ± SE (n=6).

Data analyzed by one-way ANOVA followed by post hoc Tukey's test.
LPD-low protein group control; FA-Ferulic acid 25mg/kg bw/d oral; Pb-lead acetate 2000ppm *p<0.05, compared to LPD control group; #p<0.05, compared to Pb 2000ppm

Ct-Cortex;Cb-Cerebellum;Hc-Hippocampus;St-Striatum

A & B-Acetylcholinesterase activity; C-Dopamine levels in striatum
**Fig 3.16**

Effect of ferulic acid supplements on mitochondrial complex-I-III activity and MTT reduction in cortex and cerebellum of PP rats exposed to lead (Pb) acetate (2000ppm in drinking water) maintained on LPD

Values are mean ± SE (n=6).

Data analyzed by one-way ANOVA followed by post hoc Tukey’s test.

LPD-low protein group control; FA-Ferulic acid 25mg/kg bw/d oral; Pb-lead acetate 2000ppm

*"p<0.05, compared to LPD control group; "#p<0.05, compared to Pb 2000ppm

Ct-Cortex; Cb-Cerebellum; Hc-Hippocampus; St-Striatum

A & B-Complex I-III; C & D-MTT reduction
Fig 3.17

Hematoxylin and eosin stained sections of hippocampi of rats (4 week study). The number of degenerating neurons with shrunken and dark nuclei in cornu ammonis CA1 region was decreased among LPD+Pb+FA group.
Fig 3.18

Hematoxylin and eosin stained sections of hippocampi of rats (4 week study). The number of degenerating neurons with shrunken and dark nuclei in cornu ammonis CA3 region was decreased among LPD+Pb+FA group.
Fig 3.19

Hematoxylin and eosin stained sections of hippocampi of rats (4 week study). The number of degenerating neurons with shrunken and dark nuclei in dentate gyrus region was decreased among LPD+Pb+FA group.
5.0 DISCUSSION

Pb toxicity is a persistent public health problem throughout the world and children are more susceptible than adults owing to their hand to mouth activity, increased respiratory rates and higher gastrointestinal absorption per unit body weight (Ahamed and Siddiqui, 2007). Epidemiological studies have shown that Pb exposure is associated with significant deficits in intelligence quotient of children and is associated with attention deficit hyperactivity disorder (Lanphear et al., 2005). Currently treatment of Pb poisonings has primarily relied on specific chelating agents viz., calcium EDTA, 2,3–dimercaptopropanol (BAL) and meso 2, 3 dimercaptosuccinic acid (DMSA) which form stable complexes with lead, increasing excretion and thus minimizing its toxicity (Sanchez et al., 1995; Markowitz et al., 1997; Ahamed and Siddiqui, 2007). However the use of chelating agent is related to the development of various side effects.

Hence, there is always a need to develop strategies which can significantly reduce the toxic implications of chronic Pb intoxication in human populations especially for children. In this regard, nutritional approaches offer attractive alternatives and in view of this, researchers are exploring, several approaches such as chelation, antioxidant nutrients and their combination have been attempted to alleviate Pb-associated toxic effects (Chang et al., 2012; Liu et al., 2013; Reckziegel, 2011). It is in this context that we examined the potential of FA supplements to ameliorate Pb-induced toxic effects employing a short-term dosing regimen. The criteria for using PP rats was based on the evidence that children exhibit enhanced susceptibility to Pb exposure and the brain is still in the process development of new interneuronal connections which continue till the adult architecture is established by about 6–7 weeks. Hence, first we assessed the pattern of susceptibility of PP rats to varying doses of Pb in terms of phenotype and induction of oxidative stress in brain regions. Further, with the objective of developing a pharmacological intervention strategy, the propensity of FA, a ubiquitous naturally occurring antioxidant to ameliorate Pb-induced toxicity was investigated.
Efficacy of Ferulic acid (FA) supplements to alleviate Pb-induced oxidative stress and neurotoxicity in PP rats

Natural plant products have enormous potential either in the prevention or treatment of various chronic diseases including toxicant induced neuronal dysfunctions. In recent times, FA has been attracting attention since it possess free radical scavenging activity towards hydroxyl radical, peroxynitrite, superoxide radical, and oxidized low–density lipoproteins (Kikuzaki et al., 2002, Kanski et al., 2002). Further, FA has been shown to protect biological membrane from lipid peroxidation and neutralize peroxyl and alkoxyl radicals (Trombino et al., 2004). FA has been approved as a antioxidant additive and food preservative in Japan (Graf, 1992). Interestingly, sodium ferulate, a salt of FA has been employed as a traditional medicine and is approved by state administration of China for the treatment of cardiovascular and cerebrovascular diseases (Wang and Yang, 2005). In view of the above considerations, FA appears to be a good candidate to be studied for its neuromodulatory propensity in the Pb model of neurotoxicity in PP rats.

In the present study, Pb exposure caused a significant reduction in the growth of rats, in agreement with our own findings (chapter 2) and previous reports (Toscano and Guilarte, 2005). Further, in the co-treatment paradigm, FA only marginally improved the reduction in the bodyweight. Pb exposure caused significant inhibition of ALAD activity, as shown previously (Pande and Flora, 2002). ALAD is one of the most-reliable indicators of Pb intoxication, whose inhibition contributes to the development of oxidative stress due to the accumulation of ALA, which is a substrate for ALAD. In the present study, FA treatment partially reversed Pb-induced inhibition of ALAD activity. In contrast, FA supplements had no significant effect on the elevated blood Pb levels. Previously, several workers have adopted various strategies employing chelators to treat Pb poisoning and suggested that combinational therapy of antioxidants along with chelating agents may be a better treatment strategy than monotherapy to counter Pb-induced oxidative stress (Velaga et al., 2014).
Evidence of oxidative damage associated with Pb exposure in the brain clearly suggests a possible role of free radicals in the pathogenesis of Pb neurotoxicity (Toscano and Guilarte, 2005; Vaziri et al., 2003). FA, a phenolic acid with pleiotropic biological activity and exhibits a wide range of pharmacological effects including anti-ageing, anti-inflammatory, anticancer, antidiabetic, antiapoptotic and neuroprotective (Srinivasan et al., 2007). It is an effective scavenger of free radicals and has been approved in certain countries as a food additive to prevent LPO (Adam et al., 2002). It also acts as an antioxidant against peroxyl radical generator (Kansi et al., 2002). Hence, it was reasoned that it may be relevant to understand the neuroprotective effects of FA under Pb intoxication in vivo.

In the PP model, significant elevation of ROS and MDA levels were evident in Cb, Hc and St of Pb administered rats. Accumulating evidence suggests that Pb causes oxidative stress and that MDA levels strongly correlate with Pb concentration in the brain of rats (Nehru and Kanvar, 2004; Zhang et al., 2004, 2009). It is well established that Pb alters the lipid metabolism and enhances lipid peroxidation and increases brain TBARS through the inhibition of SOD in all brain regions (Wang et al., 2006). Interestingly, FA supplements at the administered dose caused a significant decline of ROS and MDA levels in Cb and Hc. Consistent with this the neuroprotective effect of epigallocatechin gallate (EGCG), the green tea polyphenol against Pb-induced neuronal damage is reported both in animal and cell model (Yin et al., 2008). Earlier studies have shown that pre and neonatal Pb exposure altered the expression of antioxidant enzymes in the brain (Baranowska et al., 2012).

In the present model, Pb exposure caused significant perturbations in antioxidant enzyme activities such as SOD, catalase, and GPx, and changes in the concentrations of glutathione. SOD is considered as a first line of defense mechanism against ROS generation. Interestingly, FA supplements resulted in varying degree of restoration of SOD activity in Cb, which is in accordance with earlier results wherein FA effectively antagonizes the oxidative and nitrosative stress and inflammation as evidenced by down-regulated nitrite, LPO, IL-1b, TNF-a, and up-regulated GSH and SOD (Xu et al., 2013). In the present study,
Pb exposure resulted in altered GPx activity in Cb and Hc, which also corroborated with previous findings of decreased activity and protein expression of GPx in the frontal cortex, Cb and Hc. Furthermore, Pb exposure induced altered activity levels of GR, TRR and GST, while FA supplements significantly restored the antioxidant enzyme activities clearly suggesting that its protective effects may be mediated through regulating oxidant/antioxidant defense, inflammatory and apoptotic signaling pathways. In the present study, Pb caused increased protein carbonyl levels in brain regions, viz., Cb, Hc, and St suggesting elevated protein oxidation as reported by previous workers (Reckziegel et al., 2011). FA supplements reduced the protein carbonyl levels only in the striatal region of the brain suggesting a specific effect of FA, which merits further study. In contrast, previously the antioxidant, gallic acid failed to reverse the Pb-induced protein carbonyl levels in rats (Reckziegel et al., 2011).

Despite intensive research efforts, the cellular mechanisms underlying the clinical manifestation of low-level Pb-induced neurotoxicity have remained elusive. Earlier studies have shown the role of neurotransmitter systems in Pb-induced behavioral perturbations and alterations in the properties of glutamatergic, cholinergic, and dopaminergic neurotransmitter function and signal transduction (Cory-Slechta et al., 2010; Basha et al., 2012; Lasley and Gilbert, 2010). In the present study, Pb exposure resulted in increased cholinergic activity and enhanced DA levels in the striatal region, as reported previously (Nowak et al., 2010). Similarly, the locomotor activity and exploratory behavior were altered significantly among Pb-exposed animals corresponding to the alterations observed in cholinergic and aminergic systems (Basha et al., 2012). Interestingly, FA supplements significantly altered both cholinergic and dopaminergic activity in PP rats. Further, recent studies have also shown that FA treatment reversed reserpine-induced behavioral abnormalities and decreased norepinephrine, serotonin and DA levels in the Hc and frontal cortex (Xu et al., 2013) and alleviated both learning/memory deficits in vascular dementia model of rats (Luo et al., 2012).
Cerebral cortex and basal ganglia are brain areas involved in motor control and are intensely affected by Pb poisoning (Prasanthi et al., 2010; Moreira et al., 2001; Ramesh et al., 2001). In view of this, in animal models, locomotor activity in open-field is often employed to assess the Pb-induced behavioral phenotype. PP rats exposed to Pb presented increased motor activity as observed by the increased number of crossings and decreased rearing movements, which are related to locomotor and exploratory activities, respectively. Interestingly, FA supplements could partially reverse the locomotor and exploratory movements. The neuronal damage induced by Pb exposure is known to result in a reduction of the catecholaminergic transmission, either by inhibition of the DA synthesis and its release in the synaptic cleft or oxidative damages to the postsynaptic membranes (NourEddine et al., 2005; Cory-Slechta et al., 2010).

Since dopamine systems are important for central regulation of motor activity, we speculate that the observed behavioral phenotype reflects the effect of Pb on dopamine levels and its consequent oxidative stress. Oral administration of FA has been demonstrated to increase the number of newly generated cells in the DG of the Hc of corticosterone treated mice, indicating that FA enhances the proliferation of neural stem/progenitor cells(NSC/NPCs) in vitro and in vivo (Yabe et al., 2010). Further FA was shown to increase cAMP response element binding protein (CREB) phosphorylation and brain-derived neurotrophic factor (BDNF) mRNA level in the Hc and ameliorative effect on the stress-induced depression-like behavior of mice. In the present model, evidence obtained data from histopathologic analysis indicated that supplementation of FA protected hippocampal neurons in the CA1 and DG regions against Pb-induced damage. Although speculative this specific effect of FA may be largely responsible for the ameliorative effect of FA against Pb-induced oxidative stress in the PP rat brain and may alleviate the associated cognitive deficits.
Dietary protein levels and Efficacy of FA supplements to attenuate Pb-induced neurotoxic implications in PP rat model

Since one of the main concerns addressed in this investigation was to understand if FA supplements would offer a similar degree of protection against Pb–mediated effects under low protein dietary regimen, it is imperative to compare the observed effects. Although it is difficult to quantitatively assess the differences, certain generalities may be drawn. i) In general, FA supplements significantly offset Pb-induced oxidative impairments among both dietary groups. ii) However, several qualitative differences were evident in terms of various biochemical parameters evaluated. iii) Although the effects of Pb among PP rats fed LPD is relatively higher, FA supplements had a similar effect on locomotor phenotype and also markedly attenuated the oxidative impairments in all the brain regions studied.

Taken together, these findings clearly suggest that FA supplements could significantly ameliorate Pb-induced oxidative stress damage and reverse the altered antioxidant/mitochondrial enzyme activities in PP rats. Although the precise mechanisms responsible for the neuroprotective efficacy of FA during PP stage warrants further investigation, these data obtained for the first time, demonstrate its efficacy to attenuate Pb mediated oxidative stress in brain regions and neurotoxicity. Since oral FA supplementation is effective in reducing oxidative damage in brain regions and induces modifications in pathophysiological status under Pb-exposure, it is tempting to propose its use as a potential complementary compound in the treatment of low-level Pb intoxication. Since FA is ubiquitously present in a variety of commonly consumed foods, increased consumption of FA-rich foods may reduce the neurotoxic implications of Pb among children.
6.0 SUMMARY

1. Oral supplements of ferulic acid, (FA) in prepubertal rats significantly modulated the endogenous oxidative markers in brain regions.

2. Among PP rats of NPD group, FA supplements partially reversed the locomotor phenotype induced by Pb intoxication.

3. In the NPD group, FA supplements had no effect on Pb-induced alterations in Hb levels, and blood Pb levels, while only marginal reduction was evident with ALAD activity.

4. Further, FA supplements had differential protective effect on oxidative impairments in brain regions of PP rats under Pb intoxication.

5. FA supplements significantly alleviated the Pb induced enhanced oxidative stress in cerebellum and hippocampus as evidenced in terms of ROS generation, lipid peroxidation and protein carbonyl levels.

6. Interestingly in the NPD group, FA markedly restored the Pb-mediated perturbations in the GSH levels and activity levels of antioxidant enzymes.

7. The protective effects of FA supplements were also discernible in striatum in terms of diminished oxidative stress, restored cholinergic activity and dopamine levels.

8. FA supplements significantly attenuated the Pb –mediated perturbations in mitochondrial complex I-III activity in cerebellum and hippocampus.

9. FA supplements were also effective in reducing the histological lesions in hippocampus of Pb –intoxicated rats as evident in the decreased number of damaged cells in CA1 area and dentate gyrus.

10. Pb exposure caused a marked increase in ambulatory and a decrease in rearing number in relation to LPD control. Only the rearing effects were partially reversed by FA treatment group.

11. In general, the protective effects of FA supplements among Pb intoxicated rats of LPD group were similar when compared to those of NPD group.
12. FA supplements *per se* significantly increased the antioxidant enzyme activities. FA supplement completely normalized the MDA and HP levels in Ct, and partially reduced the levels in Cb.

13. FA supplement restored the ROS levels to normalcy in Hc and further decreased the levels in St.

14. FA supplements normalized the Pb-induced increase in the activity levels of SOD and GPx in Ct/ St and TRR activity in both Ct and Cb.

15. These findings emphasize the need for further studies to understand the molecular events and the protective pathways which are responsible for the neuroprotective action of FA supplements under conditions of Pb-intoxication.