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**ORIGINAL ARTICLE**

# Lead induced impairments in brain mitochondrial metabolic and oxidative enzymes in pregnant and non-pregnant rats: protective effect of α-tocopherol

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**ABSTRACT**
Our earlier studies showed that exposure to lead (Pb) caused irreversible perturbations in the cholinergic system and neurobehavioral functions of female rats. In this study, we extended our studies to investigate the role of mitochondrial metabolic and oxidative enzymes in response to Pb exposure in pregnant and non-pregnant rats. Further, we evaluated the protective effect of α-tocopherol against Pb-induced mitochondrial dysfunction in female rats. Pregnant (GD 1 to PND 21) and non-pregnant rats were exposed to 0.2% Pb for the period of 42 days (6 weeks) while alpha (α)-tocopherol (100 mg/kg) was given orally through gavage for a period of 21 days (last 3 weeks) to Pb exposed pregnant and non-pregnant rats. Rats were decapitated on 7$^{th}$ day and 30$^{th}$-day after the 42 days Pb exposure. Pb exposure decreased the activities of mitochondrial succinate dehydrogenase (SDH), isocitrate dehydrogenase (ICDH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) enzymes whereas the glucose-6-phosphate dehydrogenase (G6PDH), and malondialdehyde (MDA) levels increased in the cortex, cerebellum, and hippocampus at both 7$^{th}$ and 30$^{th}$ day points of pregnant and non-pregnant rats. Pb-induced alterations were greater in cortex coinciding with higher Pb levels observed in the cortex than hippocampus and cerebellum. Although the supplementation of α-tocopherol significantly reversed the Pb-induced alterations in the mitochondrial metabolic and oxidative enzymes, the reversal effect on Pb levels in different brain regions was marginal in both pregnant and non-pregnant rats. In conclusion, our data demonstrate that exposure to Pb significantly alters the mitochondrial enzymes in brain region-dependent manner and the effect of Pb was greater in non-pregnant female rats than pregnant rats. Further, the data provide evidence for the protective efficacy of α -tocopherol against Pb-toxicity.

**KEY WORDS:** lead toxicity; vitamin E; pregnancy; rats; mitochondrial enzymes

## Introduction

Lead (Pb) is a ubiquitous environmental and industrial pollutant that has been detected in almost all phases of biological systems (EFSA, 2012). Pb is known to induce a broad range of physiological, biochemical and behavioral dysfunctions in animals and humans (Basha & Reddy, 2015; Gao *et al.,* 2010). Exposure to Pb can result in significant alterations in various organs, the nervous system being an important target (Flora *et al*., 2012). The mean half-life of Pb in blood is approximately 21–28 days, whereas Pb accumulates in bones with a mean half-life of 5–19 days and this burden may be mobilized during pregnancy and readily crosses the placenta into the blood of the developing fetus (Beier *et al.,* 2015; ATSDR, 2007). Neuro-behavioral deficits in children such as impaired cognition, impulsivity, hyperactivity and decreased motor functions, due to exposure to low-level Pb, have been well documented (Liu *et al.,* 2013). Our previous studies also clearly stated that early life exposure to Pb-induced the broad range of neurological problems and these Pb-induced neurobehavioral dysfunctions in young rats persisted in later life (Gottipolu *et al.,* 2014; Basha *et al.,* 2012). However, compared with children, the impact of Pb exposure on women health has been neglected.

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One of the possible mechanisms underlying Pb-induced neurotoxicity is its capacity to produce reactive oxygen species (ROS) and oxidative stress, which contributes to the pathogenesis of poisoning by disrupting the pro-oxidant/antioxidant balance in cells (Jia *et al.,*2012). Neurons are particularly vulnerable to free radical attack since impaired defences or exposure to excess free radicals may lead to neuronal death (Gutowicz, 2011; Akitane *et al.,* 1994). Mitochondria are important intracellular target site for Pb toxicity (Sousa & Soares, 2014). Experimental studies confirmed the possible involvement of reactive oxygen species (ROS) in Pb-induced toxicity (Lopes *et al.,* 2016) by increased lipid peroxidation (TBARS) and decreased activities of mitochondrial enzymes, succinate dehydrogenase (SDH), isocitrate dehydrogenase (ICDH), catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) in rat brains (Basha *et al.,* 2012; Pande & Flora 2002; Kuhad & Chopra, 2007).

Vitamin E (vit-E) has been used as antioxidant to prevent the Pb-induced oxidative damage in nervous system (Salehi *et al.,* 2015). Few studies reported that supplementation of vit-E lowered ROS that generate cellular damage in different cell types, prevented the Pb-induced perturbations in aminolevulinic acid dehydratase (ALAD) activity in blood (Rendón-Ramirez *et al.,* 2007), increased the total antioxidant capacity as well as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities (Prasanthi *et al.,* 2010, Machartová *et al.,* 2000). Our previous study also reported the beneficial effects of vit-E against Pb-induced neurobehavioral dysfunctions in female rats (Sreenivasulu *et al.,* 2015). The present study is designed to investigate the adverse effects of Pb on mitochondrial metabolic and oxidative enzymes in different brain regions of pregnant and non-pregnant rats and further examine the beneficial effect of the administration of vit-E on Pb-induced mitochondrial dysfunction in rats.

## Materials and methods

### Chemicals

Chemicals (lead acetate and α-tocopherol acetate) used in this study were purchased from Sigma Chemical Co. St Louis., USA and all other chemicals were purchased from Merk, India.

### Animal exposure

For the present study, non-pregnant (adult) and pregnant female (adult) rats were selected. Pregnant (GD 1 to PND 21) and non-pregnant rats were exposed to 0.2% Pb for the period of 42 days (6 weeks) while alpha (α)-tocopherol (100 mg/kg) was given orally through gavage for a period of 21 days (last 3 weeks) to Pb exposed pregnant and non-pregnant rats (Figure 1). Control rats received only deionized water without Pb. Rats were decapitated on 7$^{th}$ day and 30$^{th}$-day after the 42 days Pb exposure (*i.e.,* 6 animals of each group were sacrificed for each time point (7$^{th}$ and 30$^{th}$ days). Different brain regions such as cerebral cortex, hippocampus and cerebellum were isolated on ice and used for estimating biochemical analysis and Pb levels. The experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Government of India, and Animal Ethical Committee of S.V. University.

### Preparation of brain mitochondrial fraction

Mitochondrial fractions were prepared by following the method of Lai and Clark (1979). The tissues were homogenized in 10 volumes (w/v) of SET buffer (0.25 M sucrose, 10 mM Tris-Hcl, and 1 mM EDTA, pH 7.4). The homogenate was first centrifuged at 800 × *g* for 10 min at 4 °C, and then supernatant was centrifuged at 10,000 × *g* for 20 min. Then the pellet of mitochondrial fraction was suspended in SET buffer.

### Estimation of mitochondrial metabolic enzymes

*Succinate dehydrogenase (SDH)*

SDH activity was estimated by the method of Nachlas *et al.* (1960) as modified by Prameelamma and Swami (1975) with slight modifications. The total reaction mixture contained 0.5 ml of phosphate buffer (pH 7.2), 0.4 ml of sodium succinate, 0.4 ml of INT and 0.2 ml of water in a final volume of 2 ml. The reaction was initiated by addition of 0.2 ml of mitochondrial fraction. The contents

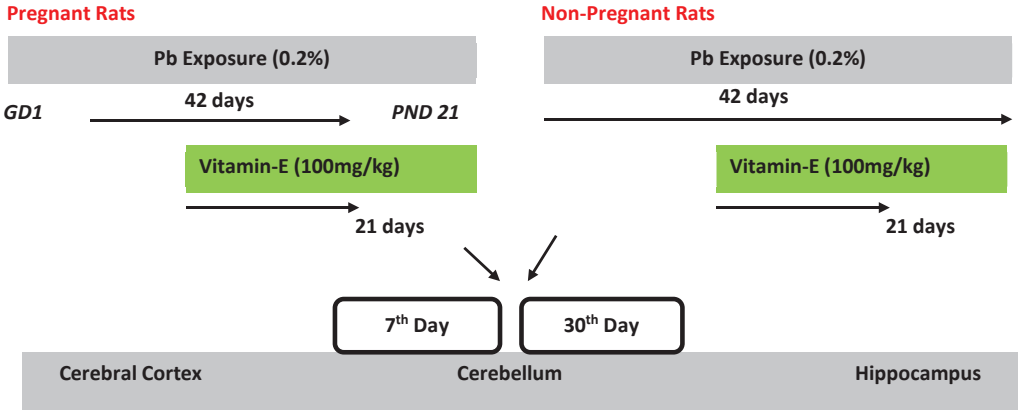


**Figure 1.** Study design: A schematic representation of exposure.

were incubated for 30 min at 37 °C. After incubation, the reaction was stopped by addition of 5 ml of glacial acetic acid. Later 5 ml of Toluene was added and kept for overnight. The formazan formed was extracted into 5.0 ml of toluene and the colour was measured at 495 nm in spectrophotometer (Hitachi model, U-2001) against toluene blank.

*Isocitrate dehydrogenase (ICDH)*

Isocitrate Dehydrogenase activity was estimated by the method of Lee and Lardy (1965). The final volume of 2 ml assay mixture contains 0.5 ml of phosphate buffer (pH 7.2), 0.4 ml of Isocitrate, 0.4 ml of INT, 0.1 ml of $MgCl_2$, 0.1ml of ADP and 0.2 ml of NAD. The reaction was initiated by addition of 0.3 ml of mitochondrial fraction. The contents were incubated for 30 min at 37 °C. After incubation, the reaction was stopped by addition of 5 ml of glacial acetic acid. Later 5 ml of Toluene was added and kept for overnight. The formazan formed was extracted into 5.0 ml of toluene and the colour was measured at 545 nm in spectrophotometer (Hitachi model, U-2001) against toluene blank.

*Glucose-6-phosphate dehydrogenase (G6PDH)*

G6PDH activity was estimated by the method of Bergmeyer and Bruns (1965). The final volume of 2 ml assay mixture contains 0.5 ml of phosphate buffer (pH 7.2), 0.4 ml of Glucose-6-Phosphate, 0.4 ml of INT, 0.1 ml of NADP and 0.2 ml of water. The reaction was initiated by addition of enzyme source. The contents were incubated for 30 min at 37 °C. After incubation, the reaction was stopped by addition of 5 ml of glacial acetic acid. Later 5 ml of Toluene was added and kept for overnight. The formazon formed was extracted into 5.0 ml of toluene and the colour was measured at 545 nm in spectrophotometer (Hitachi model, U-2001) against toluene blank.

**Estimation of mitochodrial oxidative marker enzymes**

*Superoxide dismutase (SOD)*

SOD activity was determined by using the epinephrine assay of Misra and Fridovich (1972). At alkaline pH, superoxide anion $O_2^-$ causes the autooxidation of epinephrine to adenochrome; while completing this reaction, SOD decreased the adenochrome formation. One unit of SOD is defined as the amount of extract that inhibits the rate of adenochrome formation by 50%. The reaction mixture in a final volume of 2.0 ml contained 0.05 M carbonate buffer (pH 10.2), 30 mM epinephrine (freshly prepared) and the enzyme extract. The changes in absorbance were recorded at 480 nm, measured at 10 sec intervals for 1 min in a spectrophotometer (Hitachi model, U-2001).

*Catalase (CAT)*

Catalase activity in the brain mitochondrial fraction was assayed by following the method of Chance and Maehly (1955). The reaction mixture in a final volume of 2.5 ml contained: 0.05 M phosphate buffer (pH 7.0) and appropriate amount of enzyme protein. The reaction was initiated by the addition of 19 mM hydrogen peroxide ($H_2O_2$). The decomposition of $H_2O_2$ was followed directly by measuring the decrease in absorbance at 240 nm, at 10 sec intervals for 1 min in a spectrophotometer (Hitachi model, U-2001).

*Glutathione peroxidase (GPx)*

$GP_X$ activity in the mitochondrial fraction of brain was assayed as described by Rotruck *et al.* (1973). The reaction mixture contained 0.2 ml of EDTA, 0.2 ml of 4 mM sodium azide, 0.2 ml of glutathione reduced, 0.2 ml of $H_2O_2$, 0.4 ml of 0.32 M sodium pyrophosphate buffer (pH 7.0) and 0.1 ml of enzyme source. Then the reaction mixture was incubated at 37 °C for 10 min. Then the reaction was arrested by adding of 0.5 ml 10% TCA. Then, samples were centrifuged at 2000 rpm for 10 min. To 0.5 ml of supernatant, 3.0 ml of 0.3 M disodium hydrogen phosphate and 1.0 ml of DTNB were added and the reaction was read at 412 nm in spectrophotometer (Hitachi model, U-2001).

*Lipid peroxidation*

The lipid peroxides were determined by the TBA method of Ohkawa and Yagik (1979). The tissues were homogenized in 1.5%KCl (20% W/V). To 1 ml of tissue homogenate 2.5 ml of 20%TCA was added and the contents were centrifuged at 3,500 g for 10 minutes and the precipitate was dissolved in 2.5 ml of 0.05 M sulphuric acid. To this, 3 ml of thiobarbituric acid (TBA) was added and the samples were kept in a hot water bath for 30 minutes. The samples were cooled and malonaldehyde (MDA) was extracted with 4 ml of n-butanol and the color was read at 530 nm in a spectrophotometer (Hitachi model, U-2001) against the reagent blank. Trimethoxy pentane (TMP) was used as external standard.

**Estimation of brain regional Pb levels**

Pb levels in different brain regions of control and experimental rats were estimated with atomic absorption spectrophotometer (AAS- Shimadzu-AA 6300) with graphite furnace (GFA-EX7i). After digestion with concentrated nitric acid using a microwave digestion system followed by addition of 30% hydrogen peroxide, samples were brought to a constant volume. The aliquots were used for estimation of Pb levels.

**Protein content**

Protein content of the tissues was estimated by the method of Lowry *et al.* (1951). 1% (W/V) homogenate was prepared in 0.25 M ice cold sucrose solution. To 0.5 ml of crude homogenate, 1 ml of 10% TCA was added and the samples were centrifuged at 1000 × g for 15 min. The residue was resuspended in 0.5 ml of 1 N NaOH. And 4 ml of alkaline copper sulphate reagent was added followed by 0.4 ml of folin-phenol reagent (1:1 folin: $H_2O$). The color was measured at 600 nm in a UV–vis spectrophotometer (Hitachi model U-2001) against blank. The protein standard graph was prepared using Bovine serum albumin.

### Statistical analysis of the data

The data were subjected to one way analysis of variance (ANOVA) followed by student Newman-Keuls (SNK) *post hoc* test. The 0.05 level of probability was used as the criterion for significance.

## Results

The specific activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), succinic dehydrogenase (SDH), isocitrate dehydrogenase (ICDH) and glucose-6-phosphate dehydrogenase (G6PDH) enzymes were estimated in the mitochondrial fractions of the cerebral cortex, cerebellum and hippocampus of Pb exposed pregnant and non-pregnant rats (Figures 2 to 7). Exposure to Pb exhibited significant decrease in the specific activities of SOD, GPx, CAT, SDH and ICDH enzymes in all selected brain regions of pregnant and non-pregnant rats at $7^{th}$ ($p<0.001$) and $30^{th}$ ($p<0.01$) day time intervals, where as G6PDH showed increased levels (45.01%, 19.33% in cortex; 42.71%, 16.65% in hippocampus; 36.32%, 15.27% in cerebellum at $7^{th}$ ($p<0.001$) and $30^{th}$ ($p<0.01$) day time intervals respectively in non-pregnant rats; 32.30%, 27.32% in cortex; 21.79%, 20.48% in hippocampus; 18.52%, 15.70% in cerebellum at $7^{th}$ ($p<0.001$) and $30^{th}$ ($p<0.01$) day time intervals respectively in pregnant rats) (Figure 7). The MDA formation was observed as a level of lipid peroxidation in control and experimental rats of different brain regions. Pb-exposure increased the MDA levels in the cortex, cerebellum, and hippocampus compared to controls and the increase was found to be greater in non-pregnant rats (55.66%, 34.44% in cortex; 46.18%, 32.50% in hippocampus; 39.22%, 30.6% in cerebellum at $7^{th}$ and $30^{th}$ ($p<0.01$) time intervals respectively) than pregnant rats (44.10%, 39.18% in cortex; 43.94%, 31.92% in hippocampus; 40.28%, 29.80% in cerebellum at $7^{th}$ ($p<0.001$) and $30^{th}$ ($p<0.01$) day time intervals respectively) (Figure 8). However, Pb-induced impairments in the specific activities of mitochondrial enzymes and MDA levels were more pronounced in cortex compared to hippocampus and cerebellum. Further, these results clearly show that Pb-induced effect was more pronounced in non-pregnant rats than pregnant rats following exposure to Pb. The concentrations of Pb in different brain regions of non-pregnant and pregnant rats at $7^{th}$ ($p<0.001$) and $30^{th}$ ($p<0.01$) day after exposure are shown in Table 1. A significant increase was observed in the concentration of Pb levels in all three brain regions of both female rat groups. Among the brain regions, the cortex showed the highest content of Pb compared to cerebellum and hippocampus. Further greater Pb content was observed in non-pregnant rats compared to pregnant rats (Table 1). In addition, we investigated the protective effect of vit-E against Pb induced alterations in brain mitochondrial dysfunction in pregnant and non-pregnant

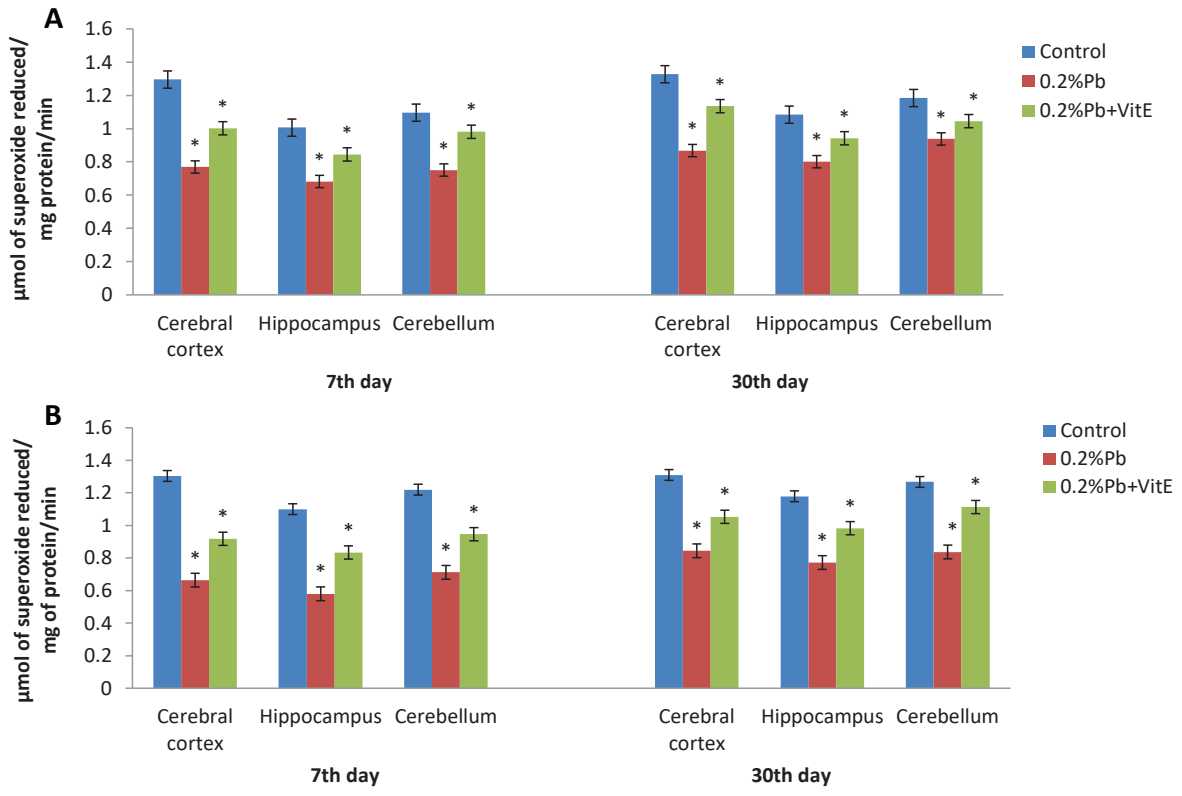


**Figure 2.** Effect of Pb (0.2%) exposure on superoxide dismutase (SOD) activity (µ moles of superoxide reduced/mg of protein/min) in mitochondrial fractions of cerebral cortex, hippocampus and cerebellum of rats and protective effects of α-tocopherol acetate (vitamin E) (100 mg/kg body wt). A: non-pregnant rats, B: pregnant rats; *$p \leq 0.05$.

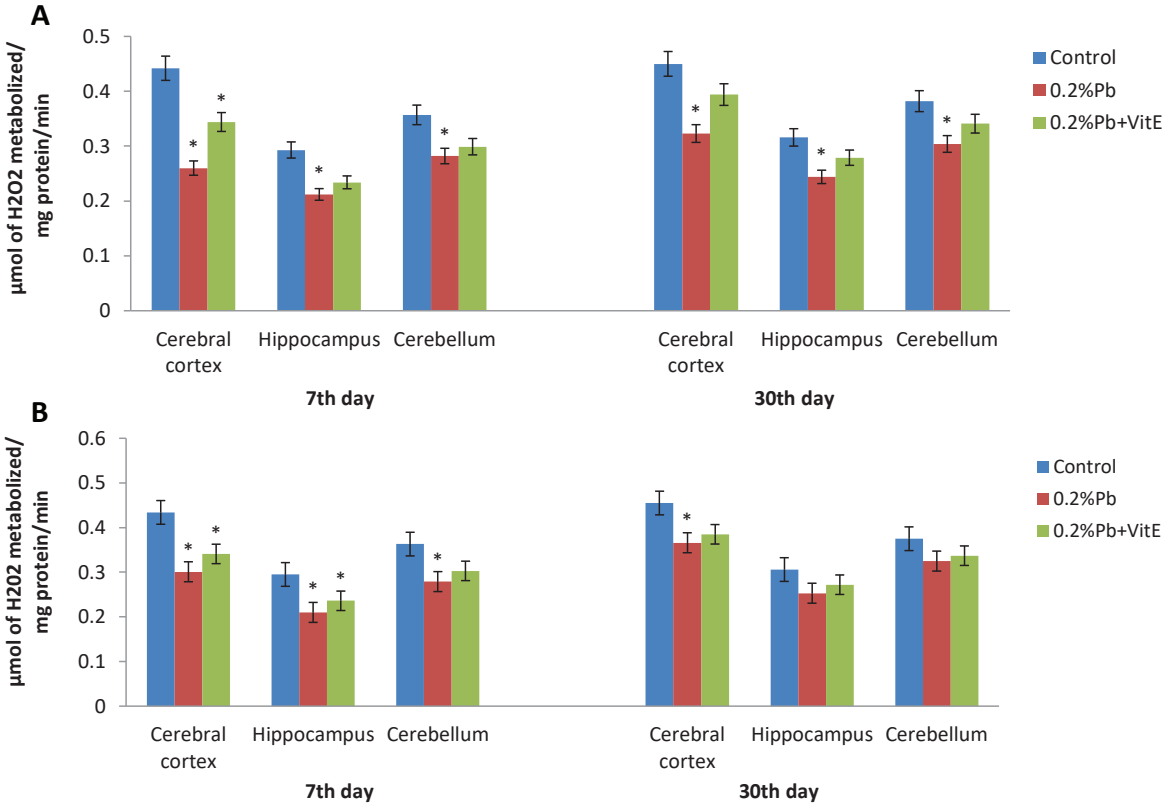


**Figure 3.** Effect of Pb (0.2%) exposure on catalase (CAT) activity (µ moles of $H_2O_2$ decomposed/mg protein/hr) in mitochondrial fractions of cerebral cortex, hippocampus and cerebellum of rats and protective effects of α-tocopherol acetate (vitamin E) (100 mg/kg body wt). A: non-pregnant rats, B: pregnant rats; *$p \leq 0.05$.

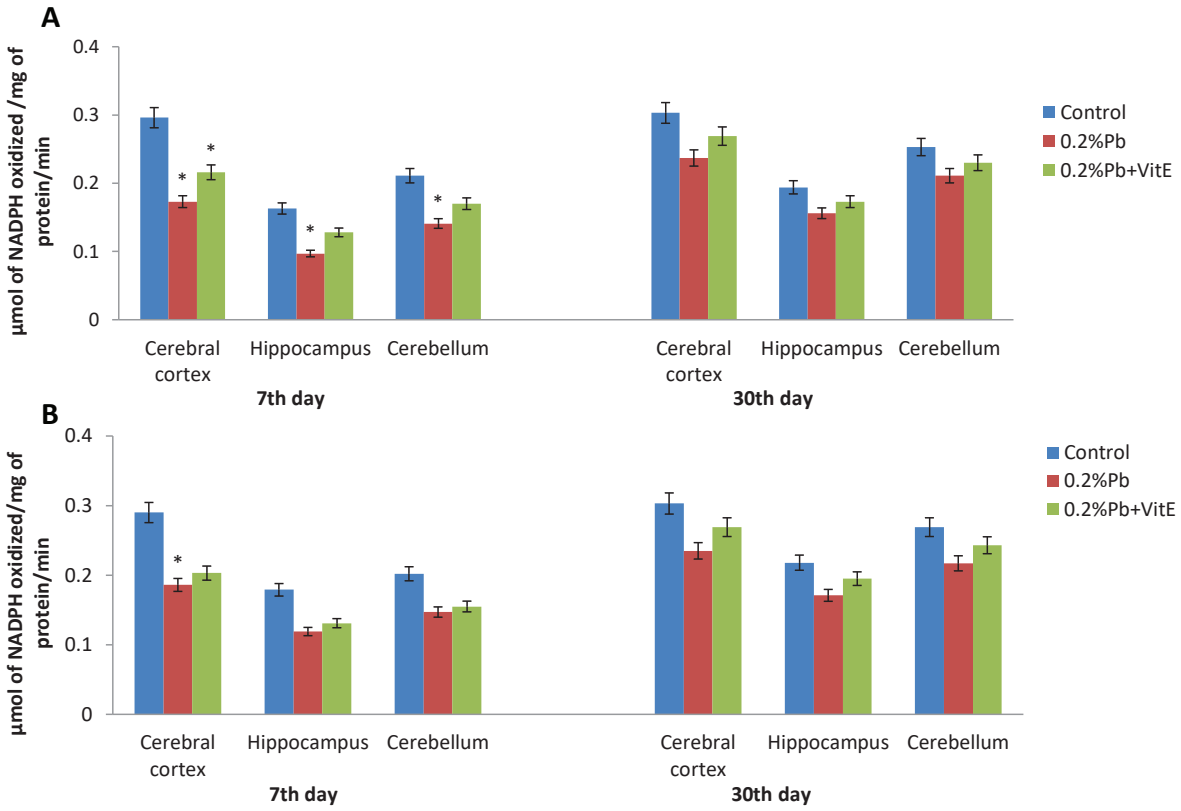


**Figure 4.** Effect of Pb (0.2%) exposure on glutathione peroxidase (GPx) activity (µ moles of NADPH oxidized/mg protein/min) in mitochondrial fractions of cerebral cortex, hippocampus and cerebellum of rats and protective effects of α-tocopherol acetate (vitamin E) (100 mg/kg body wt). A: non-pregnant rats, B: pregnant rats; *$p \leq 0.05$.

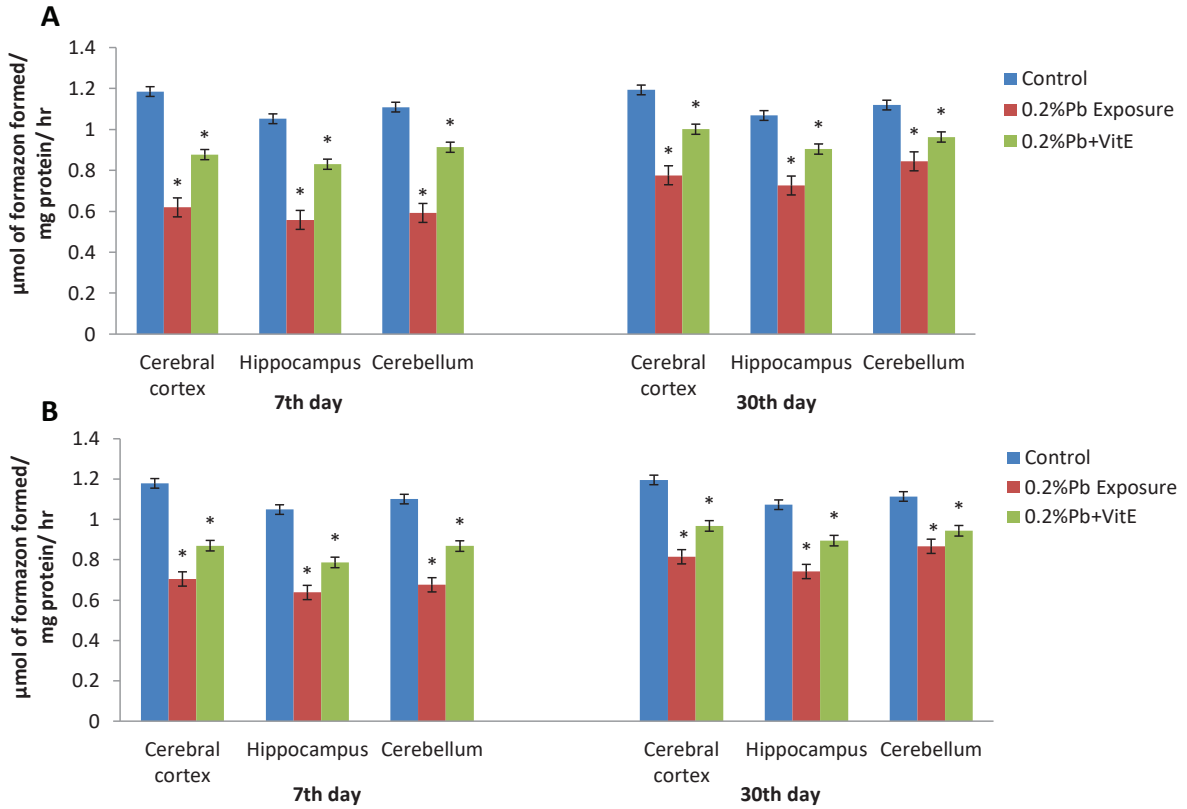


**Figure 5.** Effect of Pb (0.2%) exposure on succinate dehydrogenase (SDH) activity (µmoles of formazan formed/mg protein/hr) in mitochondrial fractions of cerebral cortex, hippocampus and cerebellum of rats and protective effects of α-tocopherol acetate (vitamin E) (100 mg/kg body wt). A: non-pregnant rats, B: pregnant rats; *$p \leq 0.05$.

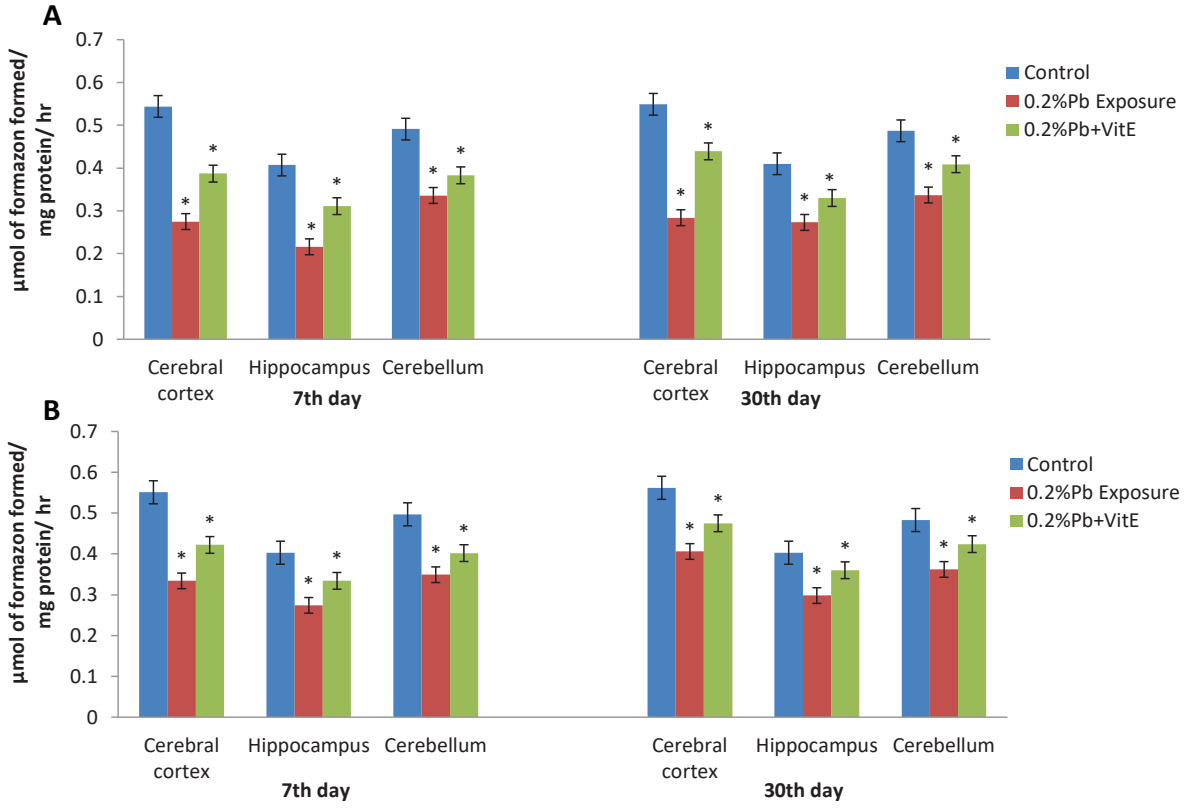


**Figure 6.** Effect of Pb (0.2%) exposure on isocitrate dehydrogenase (ICDH) activity (µmoles of formazan formed/mg protein/hr) in mitochondrial fractions of cerebral cortex, hippocampus and cerebellum of rats and protective effects of α-tocopherol acetate (vitamin E) (100 mg/kg body wt). A: non-pregnant rats, B: pregnant rats; *$p \leq 0.05$.

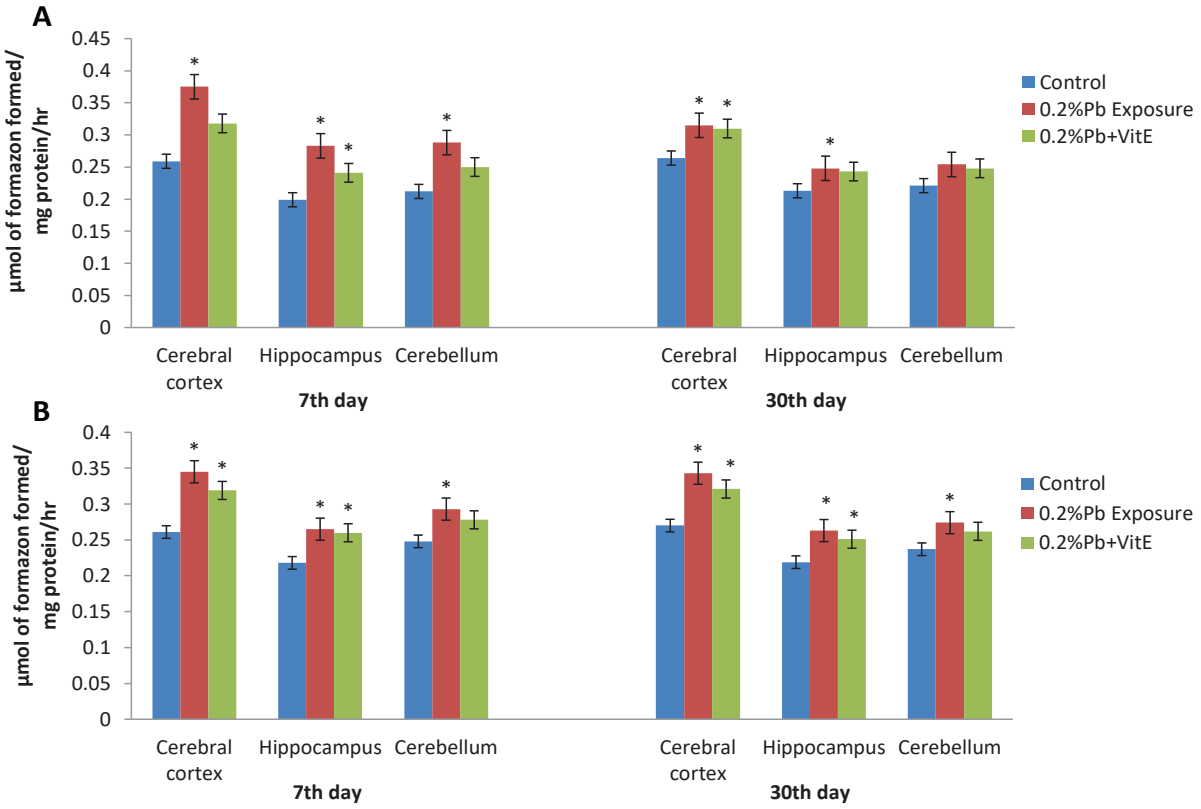


**Figure 7.** Effect of Pb (0.2%) exposure on glucose-6-phosphate dehydrogenase (G-6PD) activity (µmoles of formazan formed/mg protein/hr) in mitochondrial fractions of cerebral cortex, hippocampus and cerebellum of rats and protective effects of α-tocopherol acetate (vitamin E) (100 mg/kg body wt). A: non-pregnant rats, B: pregnant rats; *$p \leq 0.05$.

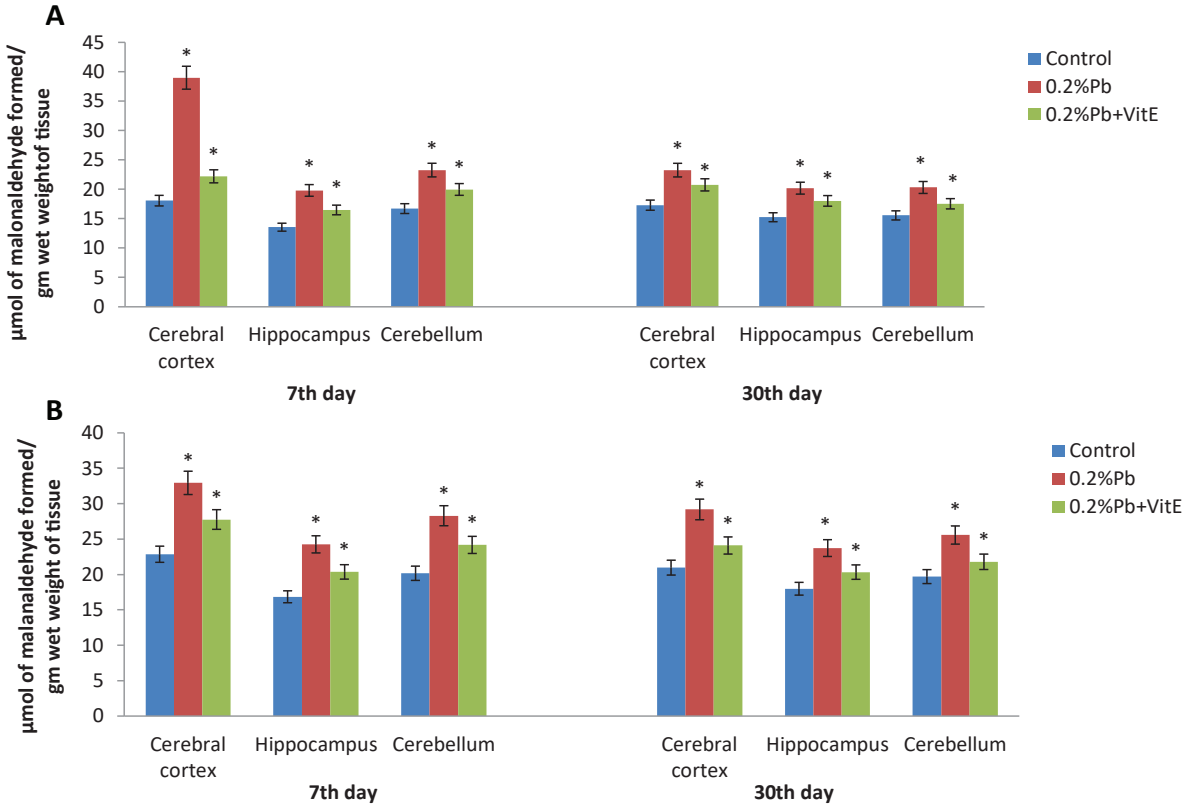


**Figure 8.** Effect of Pb (0.2%) exposure on malondialdehyde content (MDA) (µ moles of malondialdehyde/gm wet wt. of tissue) in mitochondrial fractions of cerebral cortex, hippocampus and cerebellum of rats and protective effects of α-tocopherol acetate (vitamin E) (100 mg/kg body wt). A: non-pregnant rats, B: pregnant rats; *$p \leq 0.05$.

**Table 1.** Pb levels (µg/g) estimated in different brain regions in control, Pb exposed and vit-E supplemented rats.

| | 7th day | | | 30th day | | |
|---|---|---|---|---|---|---|
| | **Cortex** | **Hippocampus** | **Cerebellum** | **Cortex** | **Hippocampus** | **Cerebellum** |
| **Non-Pregnant Rats** | | | | | | |
| Control | 0.014±0.007 | 0.020±0.001 | 0.017±0.008 | 0.007±0.003 | 0.016±0.007 | 0.012±0.005 |
| 0.2% Pb | 0.36±0.008* | 0.19±0.006* | 0.21±0.007* | 0.28±0.006* | 0.14±0.005* | 0.18±0.007* |
| 0.2% Pb +Vit-E | 0.31±0.004* | 0.17±0.003* | 0.18±0.004* | 0.24±0.003* | 0.10±0.003* | 0.12±0.002* |
| **Pregnant Rats** | | | | | | |
| Control | 0.011±0.005 | 0.018±0.007 | 0.013±0.006 | 0.009±0.0004 | 0.017±0.0007 | 0.012±0.005 |
| 0.2% Pb | 0.29±0.007* | 0.17±0.006* | 0.18±0.007* | 0.19±0.006* | 0.12±0.005* | 0.14±0.005* |
| 0.2% Pb +Vit-E | 0.23±0.003* | 0.14±0.002* | 0.16±0.003* | 0.18±0.003* | 0.11±0.003* | 0.10±0.003* |

The values are mean ± S.D of six separate experiments. Differences between values marked with asterisk (*) within each row are statistically significant at $p<0.05$ level of probability

rats ($p<0.005$). The supplementation of vit-E along with Pb exposure was found to be effective in reducing lipid peroxidation and the activities of mitochondrial enzymes but the reversal effect of vit-E on Pb levels was marginal in both non-pregnant and pregnant rats ($p<0.005$) (Figures 2 to 8 and Table 1).

## Discussion

The findings of the present study demonstrate that exposure to Pb causes significant alterations in mitochondrial metabolic and antioxidant enzymes and MDA levels in different brain regions of pregnant and non-pregnant rats. Further, supplementation of vit-E provided a significant protection from Pb-induced oxidative damage in the brain. Mitochondria are the major intracellular target site for Pb-induced ROS and oxidative damage in the brain cells (Ma *et al.,* 2017). Previous studies demonstrated that the deleterious effect of Pb on mitochondrial membranes is associated with changes in fatty acid composition. The damage of the fatty acids alters the function of the enzymes (Armstrong, 2006), particularly mitochondrial dehydrogenases (Ramanathan *et al.,* 2003). In this study, we observed that Pb exposure decreased the mitochondrial SDH and ICDH activities whereas the activity of G6PDH increased in the cortex, hippocampus and cerebellum of pregnant and non-pregnant rats. Similar results were observed by Kilikdar *et al.* (2011) and Jarrar *et al.* (2002) in rats following exposure to Pb. Recently, Ma *et al.* (2017) reported that the addition of Pb into isolated liver mitochondria lead to rapid increase in ROS ($H_2O_2$) formation suggesting that Pb may impair complex III resulting in increased leakage of electrons and generating more ROS, which ultimately lead to impairments in enzymes and mitochondrial dysfunction. According to Basha *et al.* (2012), exposure to Pb during development decreased heart mitochondrial SDH and ICDH enzyme activities and these alterations persist in the later life of rats. However, a significant decrease observed in both SDH and ICDH activities might also be due to the direct interaction of Pb with -SH groups of these enzymes. The G6PDH enzyme plays an important role in the regulation of sugar metabolism and determines whether glucose shall undergo glycolysis or be utilized via the pentose phosphate pathway (Stanton, 2012). The results of the present investigation have shown an increase in the activity of G6PDH in different brain regions of Pb exposed female rats. The increase in the activity of G6PDH, due to Pb intoxication, might indicate an increased demand to generate reducing power in the form of NADPH (Patra *et al.,* 2011). Gurer and Eracal (2000) reported that Pb-induced perturbations in G6PDH activity depend on the concentration and duration of Pb exposure, and magnitude of oxidative stress inside the cell.

The toxicity of Pb may be mainly due to its high rate of free radical production and decrease in free radical scavenging enzymes (Sandhir *et al.,* 1994). In the present study, we found that exposure to Pb decreased the mitochondrial SOD, CAT and GPx, activities with a concomitant increase in lipid peroxidation in the cortex, cerebellum and hippocampus of pregnant and non-pregnant rats. SOD is the most important enzyme involved in the antioxidant defence and responsible for scavenging the superoxide ions to yield hydrogen peroxide ($H_2O_2$) and oxygen (Pushpakiran *et al.,* 2004). Previous studies reported that the decreased SOD activity can lead to adverse effects since superoxide anions are extremely toxic to lipids, proteins and DNA (Dobrakowski *et al.,* 2017; Adanylo & Otieza, 1999). Further, it is suggested that Pb-induced deficiency in SOD activity may be due to deficiency of copper and zinc as Pb competes and replaces copper and zinc in their binding sites (Kluska *et al.,* 2018; Prasanthi *et al.,* 2010). GPx is selenium containing metalloenzyme that needs GSH to decompose hydrogen peroxide with the simultaneous oxidation of GSH to GSSG (Sara *et al.,* 2008). We found Pb-induced significant alteration in GPx enzyme activity in the cortex whereas the cerebellum and hippocampus showed marginal alterations. Many studies, either experimental or clinical, have shown much divergence in the influence of Pb on the activity of GPx. Sandhir *et al.* (1994) observed that the differential decrease in GPx activity in different brain regions was due to regional differences in accumulation of Pb. Our previous studies also

showed that Pb exposure significantly decreased the GPx activity in cerebellum and hippocampus regions during development of rats (Reddy *et al.,* 2014; Basha *et al.,* 2012; Prasanthi *et al.,* 2010). Our results clearly showed that Pb exposure significantly decreased the CAT activity in all selected brain regions of pregnant and non-pregnant rats. These antioxidant enzymes are the potential target for Pb-toxicity due to the high affinity of Pb for -SH group or metal co-factor in these enzymes (Armstrong, 2006). However, significant decrease in the levels of these enzymes following Pb exposure could lead to increased susceptibility of the brain tissue to free radical damage.

Free radical damage in specific regions of the brain has been recognized in cases like epilepsy, schizophrenia, Parkinson's or Alzheimer's diseases (Wrangler & Richardson, 1991). The most widely used test for oxidative stress is the measurement of MDA, a product of lipid peroxidation. In the current study, Pb-exposure significantly increased the MDA levels in various brain regions of pregnant and non-pregnant rats. Pb is known to promote oxidative damage in the brain by enhancing peroxidation of membrane lipids (Stohs & Bagchi, 1995), generation of ROS and decrease in the activity levels of antioxidant enzymes (Prasanthi *et al., 2*010). Neurons are particularly vulnerable to free radical attack since impaired defences or exposure to excess free radicals may lead to neuronal death (Akitane *et al.,* 1994). Increased lipid peroxidation is also responsible for altered membrane fluidity (Viani *et al.,* 1991) in the affected tissue. Our results clearly showed that an increased MDA level was accompanied by a reduction in the activities of SOD, CAT and GPx enzymes in various regions of female rats. Pb induced damage in the brain appears to be region specific and this might be due to either differential accumulation of Pb in various regions of the brain or because of differential susceptibility of cells to Pb in each region of the brain (Zawia *et al.,*1996). In this study, Pb exposure resulted in a significant increase in Pb levels in all the three brain regions of both female rat groups. Pb-induced alterations were greater in cortex coinciding with higher Pb levels observed in the cortex compared to hippocampus and cerebellum. From our results it is clear that the alterations observed in the enzyme activities and MDA levels were due to regional differences in the accumulation of Pb. Further, the non-pregnant rats were found to be more sensitive to Pb-induced impairments in mitochondrial enzymes than pregnant rats. This could be attributed to the fact that in pregnant rats part of Pb was accumulated in placenta and transferred to pups whereas in non-pregnant rats Pb will be retained in the body. Gardella (2001) and Bhattachary (1983) also reported that there is a strong correlation between maternal and umbilical cord blood Pb levels indicating the transfer of Pb from mother to foetus. Few other studies also reported that Pb exposed pregnant rats transfer the Pb content to foetus and pups through placenta and maternal milk (Kelman & Walter, 1980; Dietrich, 1991; Patriarca *et al.,* 2000) as a result Pb-induced effect was more pronounced in non-pregnant rats than pregnant rats.

We found that co-administration of vit-E with Pb exposure decreased Pb-induced oxidative damage by reducing the levels of MDA and restoring the levels of metabolic and antioxidant enzymes in different brain regions of female rats. Different studies have shown that vit-E can remove superoxide anion and other free radicals (Traber & Stevens, 2011; Patrick, 2006), which might account for the beneficial effects of its supplementation in Pb-exposed female rats. Moreover, different experimental studies showed that vit-E treatments ameliorated brain injury induced by exposure to Pb. Sajitha *et al.* (2010) observed that Pb exposure causes significant alternations in antioxidant enzymes and increases MDA levels in liver and heart of rats. Further, this study also suggested that vit-E may be a useful preventive agent against the Pb-induced oxidative stress. Al-Attar *et al.* (2011) evaluated the antioxidant and protective effect of vit-E on a mixture of heavy metals (Pb, Hg, Cd and Cu) induced oxidative stress in male mice and suggested that vit-E might be a useful preventive agent against the effect of the studied heavy metals due to its antioxidant properties. Vit-E was found to be effective in decreasing the Pb-induced oxidative stress and lipid peroxide levels in different organs of rats. Interestingly, vitamin E supplementation caused no significant reduction in Pb concentration in all selected brain regions of pregnant and non-pregnant female rats. Similar results were also observed in different studies with vit-E supplementation against Pb-induced oxidative damage in liver, kidney, brain, and blood (Patra *et al.,* 2001; Nascimento & Risso, 2016). Few other studies reported that supplementation of vit-E with chelating agents was very effective in reducing metal concentrations in different organs of rats (Flora *et al.,* 2012, 2003; Patra *et al.,* 2001). In this study, we found vit-E supplementation to be efficient to protect the brain from Pb-induced oxidative damage but not effective to reduce metal concentration in brain regions of female rats. However, the mechanisms of dietary supplementation of vit-E remain to be further examined in Pb exposed humans or animals.

## Conclusion

This study demonstrated that the exposure to Pb has a potential to induce significant perturbations in mitochondrial oxidative and antioxidant enzymes in both pregnant and non-pregnant female rats. Further, this study indicated that Pb-induced effect was more pronounced in non-pregnant rats than pregnant rats because of transfer of part of Pb from placenta and milk to pups. Supplementation of vit- E along with Pb might alleviate the brain mitochondrial perturbations from the oxidative stress suggesting that adequate vit-E intake may be useful in treating the Pb induced brain oxidative stress in female rats. However, further studies are required to establish the mechanism actions of vit-E as a therapeutic agent against the neurotoxic influence of the heavy metal Pb.

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