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### RESEARCH ARTICLE

#### AMELIORATING EFFECT OF VITAMIN-E AGAINST CADMIUM (CD) INDUCED BRAIN OXIDATIVE DAMAGE IN ALBINO RATS.

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#### Abstract

Acute exposure to moderately high concentrations of freshly generated cadmium oxide fumes (200-500 µg cadmium/m<sup>3</sup>) may cause symptoms like the metal fume fever. The present study addressed the effect of Cadmium (Cd) on Oxidative Stress in rat brain. The three months rats were exposed to Cd intraperitoneally at concentrations of low dose (1.5mg/kg bw) and high dose (3mg/kg bw) for a period of three weeks. A separate batch of low dose and high dose of Cd exposed rats received Vitamin-E intraperitoneally for a week. In this study, we have examined the Glutathione peroxidase (GPX) activity, Glutathione reductase (GR) activity, Glutathione-s-transferase (GST) activity, Xanthine oxidase (XO) activity levels and Lipid peroxidation (LP) in three brain regions, Cerebral Cortex, Cerebellum, and Hippocampus. We assessed the biochemical end points indicative of oxidative Stress in these three brain regions. The GPX, GR, and XO activity levels showed significant decrease and the GST activity, LP levels showed significant increase over control rats in dose dependent manner. The exposure to Vitamin-E however to low and high dose of Cd showed recovery, the GPX, GR, and XO activity levels were increased and decreased GST activity, Lipid Peroxidation were observed. The results may be attributed to the reason that the Cd exposure disrupts the functioning of brain which resulted in oxidative deficits. The Vitamin-E lessened the Cd burden in the brain as effective chelating agent decreasing the oxidative stress.

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#### Introduction:-

Cadmium (Cd) is a highly toxic element that is naturally present in all parts of the environment, including food, water, and soil (Sherlock, 1984). It is also present in cigarette smoke, representing a significant source of exposure (Stohs, 1997). Cadmium accumulates and proves to cause severe damage to a variety of organs such as lung, brain, testis, kidney, liver, blood system and bone (Manca, 1991). Pneumonitis, pulmonary emphysema, interstitial fibrosis together with cancer is a frequent response to cadmium exposure (Brandao *et al.*, 2007). Because of its characteristics as a lung carcinogen, cadmium has been classified as a category carcinogen (human carcinogen) by the International Agency for Research on Cancer and the National Toxicology Program of the USA (Waisberg *et al.*,

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2003). Acute inhalation of sufficient exposure of cadmium can cause both a chemical pneumonitis and pulmonary edema from the toxic effect to the alveolar epithelium and endothelium (Newman *et al.*, 1996). Within 24h of exposure, workers develop shortness of breath, fever, and fatigue, which can progress to pulmonary edema and death (Fernandez, 1996). Chronic exposure to cadmium dusts and fumes has been suspected as a cause of emphysema, obstructive lung disease, pulmonary fibrosis, and lung cancer. Exposure of adult rats to low or moderate doses of Cd induced lipid peroxidation (LP) in all tissues, mainly lung and brain (Manca, 1991). Monroe and Halvorsen, 2006 provided evidence that CdCl<sub>2</sub> increases oxidative stress in nervous cells. In experimental studies with animals, cadmium can induce neurotoxicity with a wide spectrum of clinical entities including neurological disturbance (Viaene *et al.*, 2000). Changes in the normal neurochemistry of the brain (Gutierrez-Reyes *et al.*, 1998), several authors have shown that antioxidants should be one of the important components of an effective treatment of cadmium poisoning (Valko *et al.*, 2006). Accordingly, to prevent undesired oxidative damage induced by reactive species, organisms are equipped with several lines of antioxidant defence. Vitamin C is an aqueous phase antioxidant has been established for many decades and has several important roles. It modulates intracellular redox status through maintaining sulfhydryl compounds, including glutathione, in their reduced state. It has been suggested to repair protein hydroperoxides, through regeneration of parent amino acids by reduction. Also, it serves to maintain membrane alpha tocopherol and enzyme activities including hepatic mixed function oxidase activity (Suberlich *et al.*, 1994). Gupta and Kar, (1998) reported that vitamin C can prevent increased lipid peroxidation levels resulting from cadmium toxicity. Vitamin C could serve as an effective antioxidant against restraint stress induced pro-oxidant status and increase the antioxidant enzyme activity in rat brain (Zaidi *et al.*, 2004). In view of the above studies mentioned the present study has implicated the importance of antioxidants such as vitamin E in protecting living organism against the toxic effect of cadmium exposure in the brain regions of young Albino rats.

## Materials And Methods:-

### Chemicals:-

Cadmium (Cd) and Vitamin E were selected as test chemicals. The chemicals used in this study namely Thiobarbitric acid, Glutathione oxidized, NADPH, DTNB, Reduced glutathione, Epinephrine were obtained from Sigma, USA. The remaining chemicals obtained from Qualigens and Loba Chemie, India.

### Procurement and maintenance of experimental animals:-

The young and adult albino rats (1 month and 3 months old) (Wistar) were purchased from Sri Venkateswara traders, Bangalore and maintained in the animal house of Watson Life Sciences, Tirupati. The animals were housed in transparent plastic cages with hardwood bedding in a room maintained at  $28^{\circ}\pm 2^{\circ}$  C and relative humidity  $60\pm 10\%$  with a 12 hour light/day cycle. The animals were fed in the laboratory with standard pellet diet supplied by Sri Venkateswara traders, Bangalore and water *ad libitum*. The protocol and animal use were approved by Y.V. University, India.

### Animal exposure to Cd and Vitamin E:-

The young albino rats (3 months) were exposed to Cd (Low dose: 1.5 mg/kg/body weight and High dose: 3mg/kg/body weight). A separate batch of low dose and high dose of Cd exposed rats received vitamin-E (5mg/kg/body weight) intraperitoneally for a week. The control animals received only deionized water without Cd. After the period of dosage, the animals were sacrificed through cervical dislocation and the tissues were stored at  $-80^{\circ}$ C for the further biochemical analysis.

## Biochemical Studies:-

### Preparation of Brain Mitochondrial Fraction:-

Brain mitochondrial fractions were prepared following Lai and Clark, 1979. Briefly, the tissue was homogenized in 5 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 centrifuge at 800 g for 10 min at 4°C, and then the supernatant was centrifuged at 10,000 g for 20 min at 4°C. Then the pellet of mitochondrial fraction was suspended in SET buffer.

### Glutathione peroxidase (GPx) activity:-

GPx activity in the mitochondrial fraction of rat kidney was assayed as described by Rotruck *et al.*, (1973). The reaction mixture contained 0.2 ml of EDTA, 0.2ml of 4mM sodium azide, 0.2ml of glutathione reduced, 0.2ml of H<sub>2</sub>O<sub>2</sub>, 0.4ml of 0.32M Sodium pyrophosphate buffer (pH-7.0), 0.1ml 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 centrifuged at 2000 rpm for 10 min. To 0.5 ml of supernatant, 3.0 ml of 0.3M disodium hydrogen phosphate and 1.0 ml of DTNB were added and the reaction was read at 412 nm in spectrophotometer. The enzyme activity was expressed as  $\mu$  mole/min/mg protein.

#### **Glutathione reductase (GR):**

GR activity in the mitochondrial fraction of rat brain was assayed as described by Staal *et al.*, (1969). The reaction mixture in a final volume of 3.0ml contained, 1.0ml of 0.3 M Sodium phosphate buffer (pH-6.8), 0.5ml of 250 mM EDTA, 0.5ml of 12.5 mM GSSG, 0.7ml of distilled water, 0.2ml of 30mM NADPH and 0.1ml of enzyme extract. Changes in absorbance were recorded at 340 nm in a spectrofluorometer. The enzyme activity was expressed as  $\mu$  moles of NADPH oxidised/mg protein/min.

#### **Xanthine oxidase (XO) activity:-**

Xanthine oxidase activity was estimated by the method given in Worthington Manual (2004). The assay mixture contained 1.9ml of phosphate buffer (pH 7.5), 1.0 ml of hypoxanthine and 0.1 ml of enzyme source. Increase in absorbance was recorded and absorbance was recorded at 290 nm from the linear curve. The rate is proportional to enzyme concentration within limits of 0.01-0.02 units per test. The activity was expressed as moles of  $\mu$  urate formed /mg protein/min.

#### **Glutathione-S-transferase (GST) activity:-**

GST activity in the mitochondrial fraction of rat brain was assayed by using 1-chloro-2, 4-dinitro benzene (CDNB) (at 340 nm) substrate as described by Habig *et al.*, (1974).

The reaction mixture in a final volume of 3.0 ml contained: 150 mM phosphate buffer (pH 7.5), 1 mM CDNB, 5 mM glutathione (GSH) and an appropriate amount of enzyme protein. The reaction was initiated by the addition of GSH and incubated at 37°C. The formation of a thioether by the conjugation of CDNB to GSH was monitored at 340 nm in a spectrofluorometer. Thioether concentration was determined from the slopes of initial reaction rates. The activity was expressed as  $\mu$  moles of thioether formed/mg protein/min, where one unit of enzyme activity is defined as one  $\mu$  moles of thioether formed/mg protein/min.

#### **Lipid peroxidation:-**

The level of lipid peroxidation in the tissues was measured in terms of malondialdehyde (MDA; a product of lipid peroxidation) content and determined by using the thiobarbituric acid (TBA) reagent. The reactivity of TBA is determined with minor modifications of the method adopted by Hiroshi *et al.*, (1979).

To 2.5 ml of homogenate, 0.5 ml of saline (0.9% sodium chloride), 1.0 ml of (20% w/v) trichloroacetic acid (TCA) were added. The contents were centrifuged for 20 minutes on a refrigerated centrifuge at 4000 x g. To 1.0 ml of supernatant, 0.25 ml of TBA reagent was added and the contents were incubated at 95°C for 1hr. 1ml of n-butanol was added to it. After thorough mixing, the contents were centrifuged for 15 minutes at 4000 g in a refrigerated centrifuge. The organic layer was transferred into a clear tube and its absorbance was measured at 532 nm. The rate of lipid peroxidation was expressed as  $\mu$  moles of malondialdehyde formed/g wet wt. of tissue.

#### **Estimation of protein content:-**

Protein content of the kidney 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, 1ml 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 1N NaOH. And 4 ml of alkaline copper reagent was added followed by 0.4 ml of folin-phenol reagent (1:1 folin: H<sub>2</sub>O). The color was measured at 600 nm in a UV- Vis spectrophotometer (Hitachi model U-2000) against blank. The protein standard graph was prepared using Bovine serum albumin. The protein content of the tissues was calculated using the standard graph.

#### **Statistical treatment of the data:-**

The mean and standard deviation (SD), analysis of variance (ANOVA) was calculated using standard statistical software package.

**Results:-****Glutathione peroxidase activity:-**

From Fig.1, it was observed that the increased mitochondrial GPx activity observed in Cerebral Cortex (CC), Cerebellum (CB) and Hippocampus (HP). The GPx activity in Cd-exposed (both low and high dose) rats was decreased when compared to control. However, the effect was highly pronounced in high dose exposed animals compared to the low dose exposed animals. GPx activity was found to be increased in the animals supplemented with Vitamin E along with low and high dose of Cd-exposed animals. Among the brain regions, CC was found to be more susceptible compared to the other brain regions.

**Glutathione Reductase activity:-**

From Fig.2, it was observed that the mitochondrial GR activity was found high in CC followed by CB, and HP. The GR activity in mitochondrial fraction of Cd-exposed (both low and high dose) rats was decreased when compared to control. However, the effect was highly pronounced in high dose exposed animals compared to the low dose exposed animals. The GR activity increased with vitamin E supplementation in low and high dose Cd-exposed animals in all three regions of brain CC, CB and HP.

**Xanthine oxidase activity:-**

From Fig.3, it was observed that the XO mitochondrial fraction of Cd-exposed (both low and high dose) rats was decreased when compared to control. However, the effect was highly pronounced in high dose exposed animals compared to the low dose exposed animals. XO was found to be increased in the animals supplemented with Vit-E along with low and high dose Cd-exposed animals. Among the brain regions, CC was found to be more susceptible compared to the other brain regions.

**Glutathione-S-transferase activity:-**

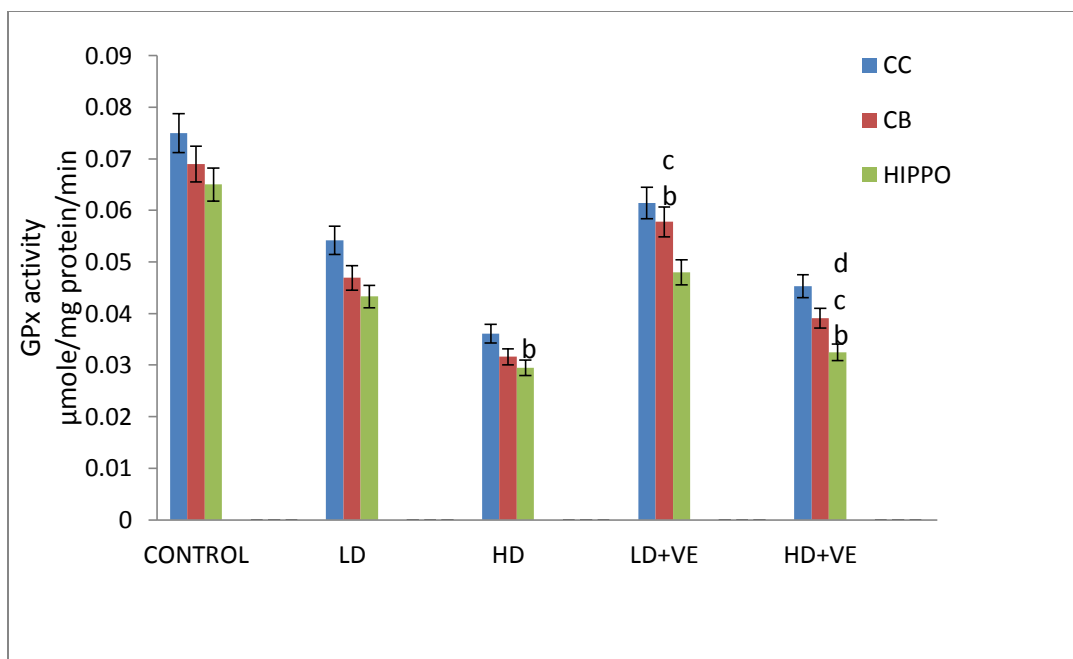
From Fig.4, it was observed that the activity of GST was found highest in CC followed by CB and HP. The GST activity in Cd-exposed (both low and high dose) rats was decreased when compared to control. However, the supplementation with vitamin E decreased the GST activity in low and high dose Cd-exposed animals. The low dosed animals showed more recovery.

**Lipid peroxidation:-**

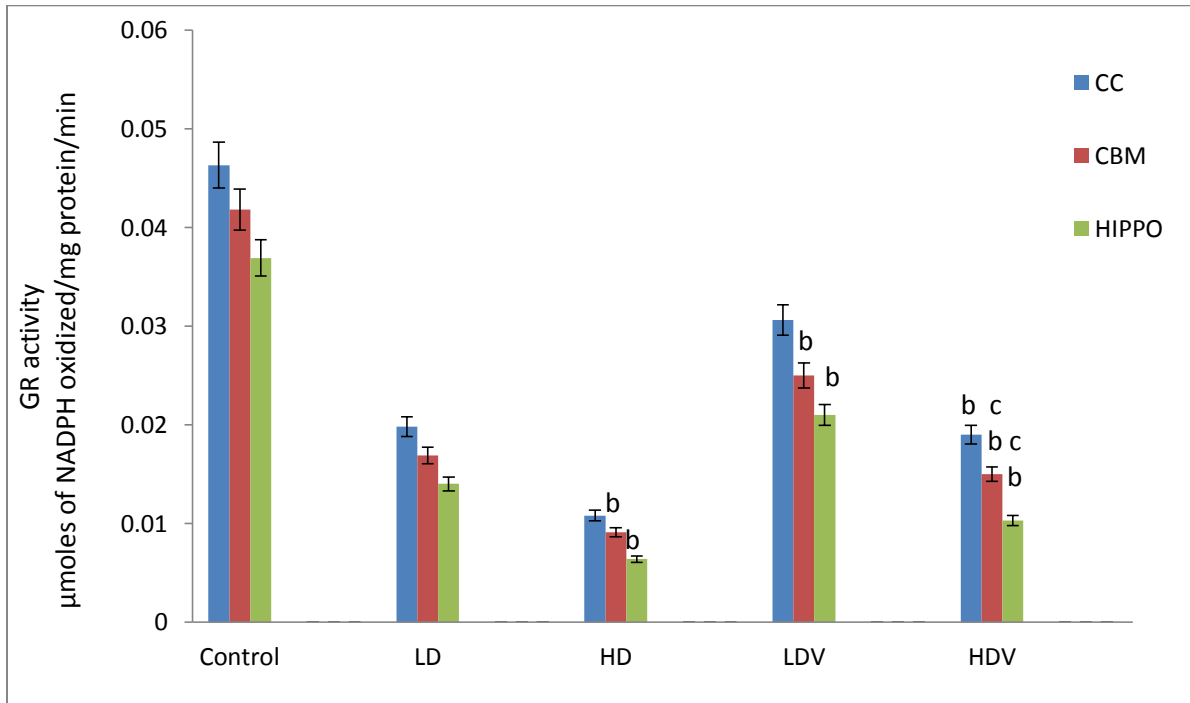
The mitochondrial lipid peroxidation was calculated in terms of MDA levels. From Fig.5, it was observed that the MDA levels in mitochondrial fraction of Cd-exposed (both low and high dose) rats were recorded high when compared to controls. The effect was highly pronounced in high dose exposed animals compared to the low dose exposed animals. However, the vitamin E reversed the Cd effect and MDA levels were found decreased in low and high dose (1.5mg/kg body weight and 3mg/kg body weight) Cd-exposed animals in all the three brain regions.

**Protein content:-**

From Fig.6, it was observed that the protein content in mitochondrial fraction of Cd-exposed (both low and high dose) rats was decreased when compared to control. However, the effect was highly pronounced in high dose exposed animals compared to the low dose exposed animals. Protein content was found increased in the animals supplemented with Vitamin E along with low and high dose Cd-exposed animals.

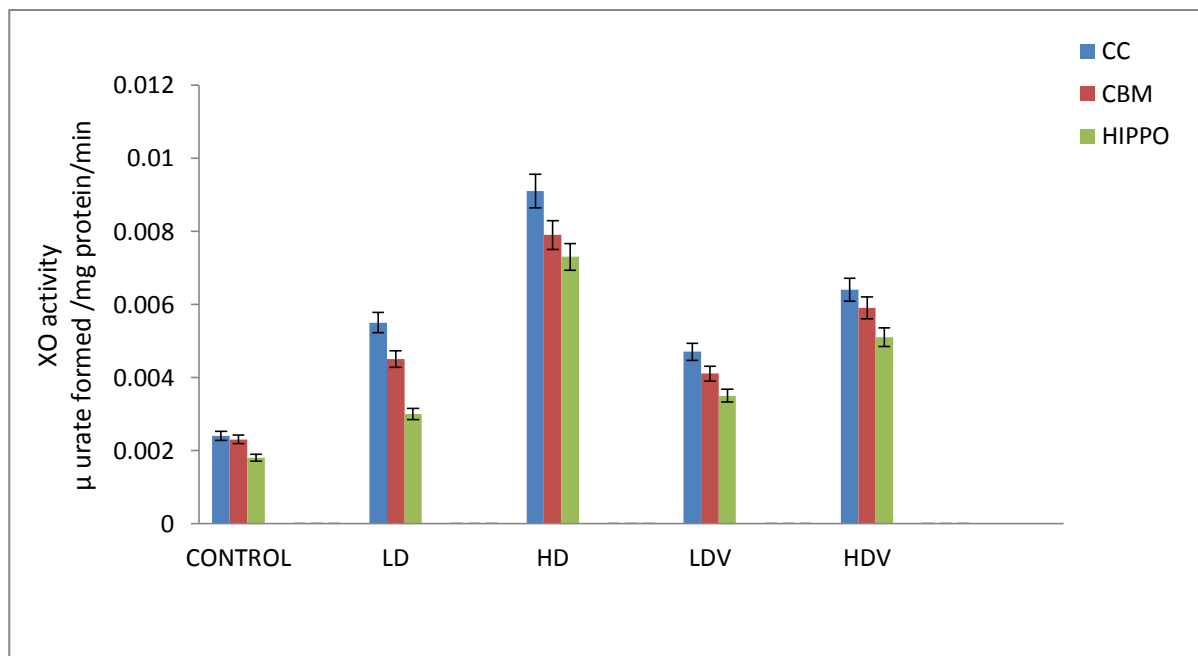


**Fig. 1:-** Protective effect of Vitamin E against low dose(LD) (1.5mg/kg bw) and high dose (HD) (3mg/kg body weight) of Cd induced alteration in GPx levels in brain specific regions i.e. CC (Cerebral Cortex), CBM (Cerebellum) and HIPPO (Hippocampus). The rats treated with Cd through intraperitoneally daily for a period of three weeks and with Vitamin E for a week. Each bar represents mean±SD (n=6). All values are extremely significant at  $p < 0.0001$ , except the values (bars) marked with a; compared with control, b; compared with LD, c; compared with HD, d; compared with LDV.

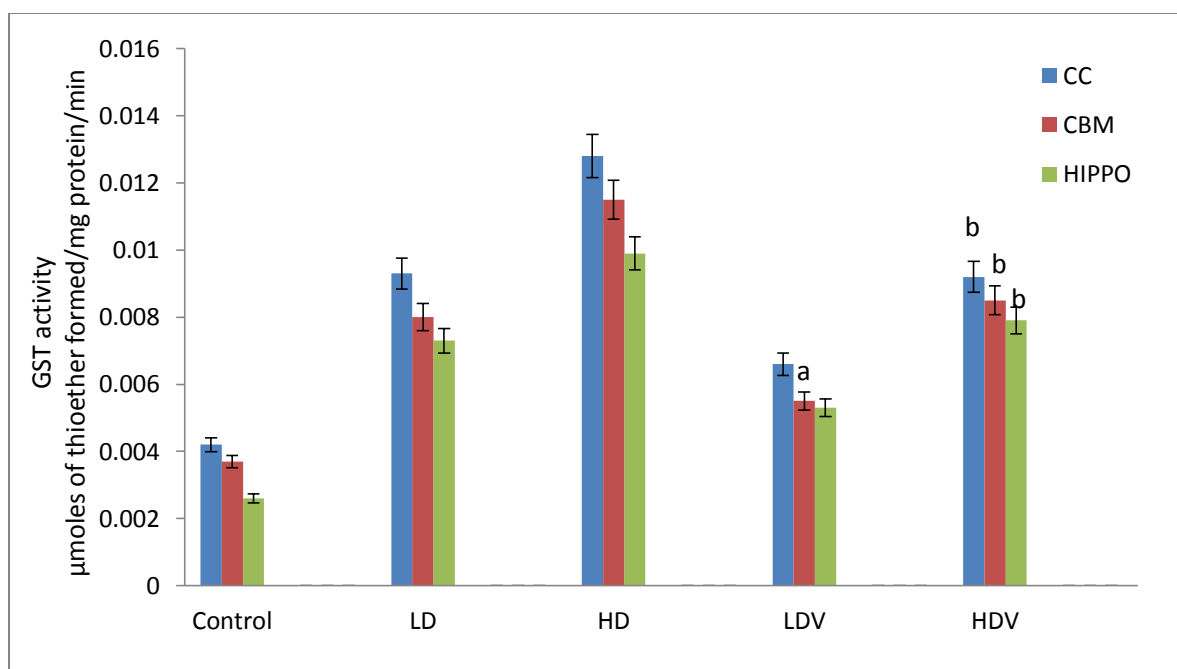


**Fig. 2:-** Protective effect of Vitamin E against low dose(LD) (1.5mg/kg bw) and high dose (HD) (3mg/kg body weight) of Cd induced alteration in GR levels in brain specific regions i.e. CC (Cerebral Cortex), CBM (Cerebellum) and HIPPO (Hippocampus). The rats treated with Cd through intraperitoneally daily for a period of three weeks and with Vitamin E for a week. Each bar represents mean±SD (n=6). All values are extremely

significant at  $p < 0.0001$ , except the values (bars) marked with a; compared with control, b; compared with LD, c; compared with HD, d; compared with LDV.

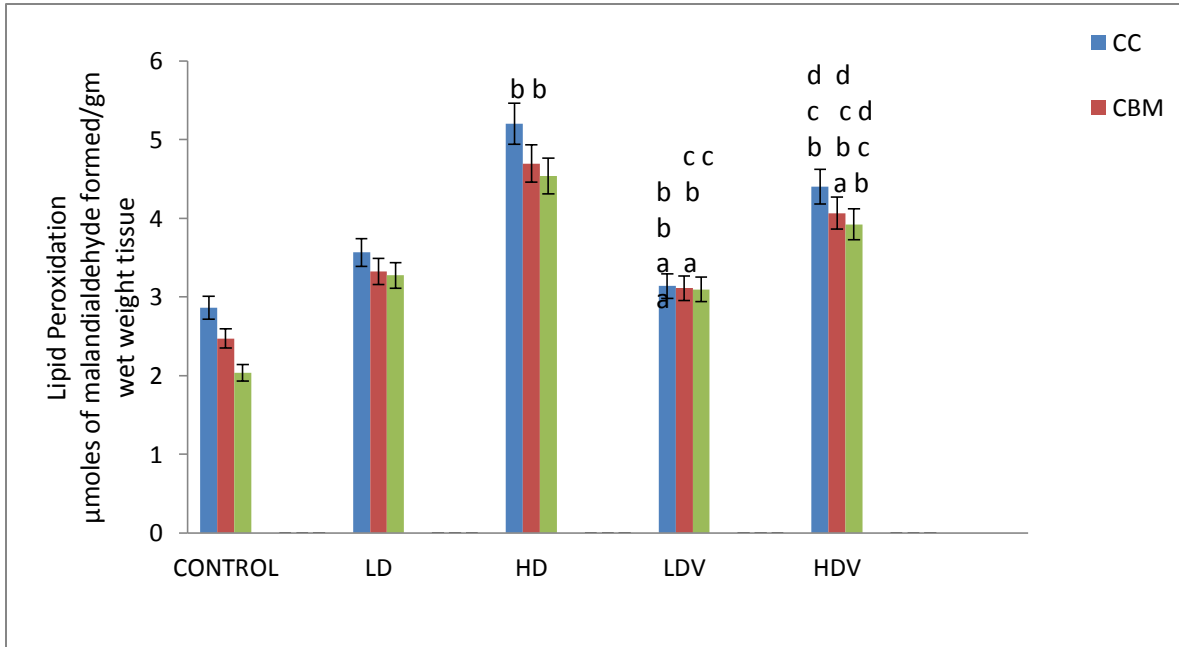


**Fig. 3:-** Protective effect of Vitamin E against low dose(LD) (1.5mg/kg bw) and high dose (HD) (3mg/kg body weight) of Cd induced alteration in XO levels in brain specific regions i.e. CC (Cerebral Cortex), CBM (Cerebellum) and HIPPO (Hippocampus). The rats treated with Cd through intraperitoneally daily for a period of three weeks and with Vitamin E for a week. Each bar represents mean $\pm$ SD (n=6). All values are extremely significant at  $p < 0.0001$ ,

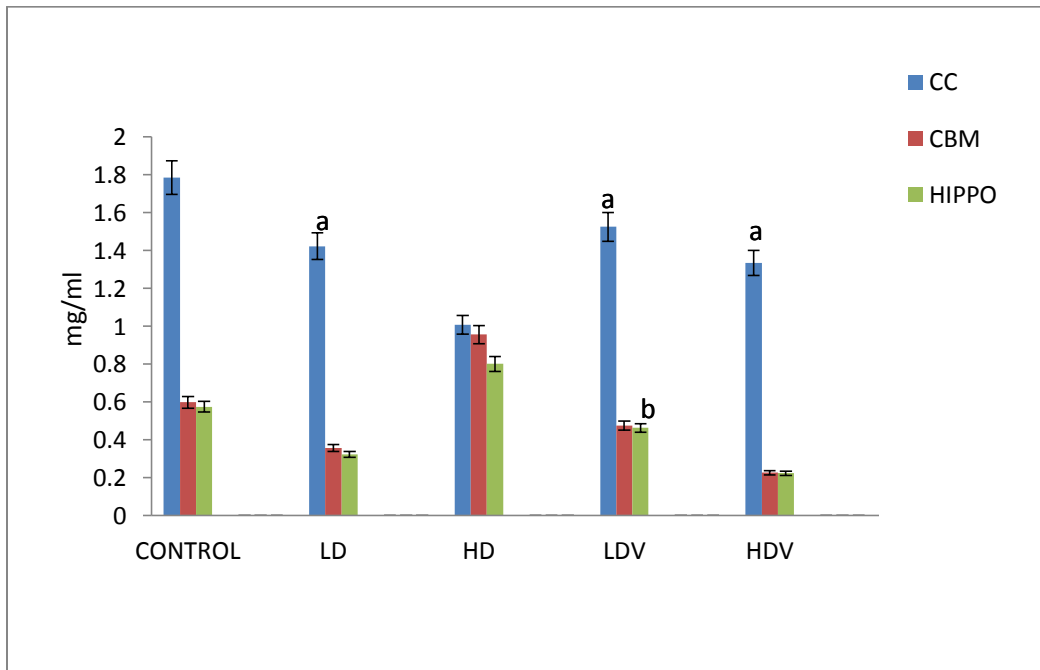


**Fig.4:-** Protective effect of Vitamin E against low dose(LD) (1.5mg/kg bw) and high dose (HD) (3mg/kg body weight) of Cd induced alteration in GST levels in brain specific regions i.e. CC (Cerebral Cortex), CBM (Cerebellum) and HIPPO (Hippocampus). The rats treated with Cd through intraperitoneally daily for a period of three weeks and with Vitamin E for a week. Each bar represents mean $\pm$ SD (n=6). All values are extremely

significant at  $p < 0.0001$ , except the values (bars) marked with a; compared with control, b; compared with LD, c; compared with HD, d; compared with LDV.



**Fig. 5:-** Protective effect of Vitamin E against low dose(LD) (1.5mg/kg bw) and high dose (HD) (3mg/kg body weight) of Cd induced alteration in LP levels in brain specific regions i.e. CC (Cerebral Cortex), CBM (Cerebellum) and HIPPO (Hippocampus). The rats treated with Cd through intraperitonally daily for a period of three weeks and with Vitamin E for a week. Each bar represents mean±SD (n=6). All values are extremely significant at  $p < 0.0001$ , except the values (bars) marked with a; compared with control, b; compared with LD, c; compared with HD, d; compared with LDV.



**Fig. 6:-** Protective effect of Vitamin E against low dose(LD) (1.5mg/kg bw) and high dose (HD) (3mg/kg body weight) of Cd induced alteration in Protein levels in brain specific regions i.e. CC (Cerebral Cortex), CBM (Cerebellum) and HIPPO (Hippocampus). The rats treated with Cd through intraperitonally daily for a period of

three weeks and with Vitamin E for a week. Each bar represents mean $\pm$ SD (n=6). All values are extremely significant at  $p < 0.0001$ , except the values (bars) marked with a; compared with control, b; compared with LD, c; compared with HD, d; compared with LD+V.

### Discussion:-

Cadmium has been recognized as one of the most toxic environmental and industrial pollutant. Cadmium is ubiquitous toxic metal that may induce oxidative damage by disturbing the prooxidant-antioxidant balance in tissues was observed in rats treated with cadmium (Kostic *et al.*, 1993; Zikic *et al.*, 1998; Pavlovic *et al.*, 2001). The severity of intoxication depends upon the route, dose, and duration of exposure to the metal (Ognjanovic *et al.*, 1995).

Our data showed that significant decrease in the activities of GPX, GR, XO and increased GST activity in both doses of Cd exposure. However, the effect was more in high dose exposed animals compared to the low dose exposed animals. And moreover, among the brain regions studied, cerebral cortex was found to be more vulnerable to the Cd induced neurotoxicity compared to the other brain regions. And we also observed a significant increase in lipid peroxidation (i.e., increased MDA level) in all the three brain regions of Cd-exposed rats. Further, inhibition of antioxidant enzymes, viz., glutathione peroxidase, glutathione reductase in cadmium-exposed animals suggests disturbed antioxidant/pro-oxidant ratio, resulting in oxidative stress. A number of antioxidant defense systems, both enzymatic and nonenzymatic, operate to control excessive levels of ROS.

The brain is particularly sensitive to oxidative damage because of its high rate of oxidative metabolism (Pajovic *et al.*, 2003). Cd also enhances the production of free radicals in the brain of adult rats and interferes with the antioxidant defense system that in turn leads to a Cd-induced alteration of the structural integrity of lipids (Shukla *et al.*, 1996). In adult rats exposed to Cd, there was an increase of LP in the corpus striatum and cerebral cortex (Pal *et al.*, 1993). (Nishimura *et al.*, 2006) reported that Cd can enter the brain parenchyma and neurons and causes neurological alterations in humans (Rose *et al.*, 1992) and animal models (Lukawski *et al.*, 2005), leading to lower attention, hyper nociception, and olfactory dysfunction and memory deficits.

The GPx activity with Vitamin-E co-administration along with cadmium, this could be due to the supplements enhanced the antioxidative defense system protected against cadmium toxicity (Shaikh *et al.*, 1999).

Cadmium causes significant increases LP concentrations in kidney of rat, since it cause Lipid peroxidation in numerous tissues (Kostic *et al.*, 1993; Ognjanovic *et al.*, 2003; El-Demerdash *et al.*, 2004), superoxide ions, nitric oxide and H<sub>2</sub>O<sub>2</sub> (Waisberg *et al.*, 2003). The activity of LP was significantly lowered in rats co-supplemented with Vit-E supplement. This is due to the antioxidant and antioxidant defense systems protects cells from Cd-induced toxicity (Shaikh *et al.*, 1999). The LP activity was much more lowered in rats supplemented with Vitamin E. This could be due to the Vitamin E was liposoluble antioxidant, has key role in scavenging free radicals and stabilizing the cell membranes thus maintaining their permeability. Moreover, it is known that the antioxidant (Vitamin E) may act synergistically preventing lipid peroxidation and cell destruction (El Demerdash *et al.*, 2004). Since Vitamin E ( $\alpha$ -tocopherol) is primarily located in cell membranes, it is possible that  $\alpha$ -tocopherol modifies the kinetics of distribution of cadmium in cell membranes, such that its delivery to neuron or glia cells may be impeded. However, our observation that cadmium concentration in the nervous system was not reduced by  $\alpha$ -tocopherol supplementation suggests that the benefits of  $\alpha$ -tocopherol treatment were not a result of changes in cadmium distribution in the nervous system. Rather our findings are consistent with the view that protective effects of  $\alpha$ -tocopherol against sub chronic high dose cadmium exposure might be due to its antioxidant properties, and this may have a major impact on improving the quality of life of individuals suffering from neurotoxicity caused by cadmium exposure.

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