Heavy metal toxicities have received widespread attention because of increasing amounts being released into the environment and their extended persistence and toxicity to a wide variety of organisms. Cadmium is perhaps one of the most toxic industrial and environmental pollutant and it poses a continuing health hazard. Cadmium is able to induce neurotoxicity with a wide spectrum of clinical entities including neurological disturbances (Viaene et al., 2000) and changes the normal neurochemistry of the brain tissue (Gutierrez-Reyes et al., 1998).

The brain is highly vulnerable to lipid peroxidation (LPO) because of its high rate of oxygen utilization, an abundant supply of polyunsaturated fatty acids, a deficient antioxidant defense and a high content of transition metals like copper and iron in several regions (Calabrese et al., 2000). The enhanced susceptibility of membrane to LPO can lead to the loss of adenosine triphosphatases (ATPases) activity and depletion of thiols in brain (Bonting, 1970). ATPases are lipid dependent membrane bound enzymes which are involved in active transport, maintenance of cellular homeostasis and also involved in neurotransmission process (Ohinishi et al., 1982).

Cadmium enhances the production of free radicals in the brain and interferes with the antioxidant defense system which in turn leads to cadmium induced alterations of the structural integrity of lipids and secondarily affects the membrane bound enzymes (Acan and Tezcan, 1995; Shukla et al., 1996). In adult rats chronic cadmium exposure leads to the increase of LPO in the corpus striatum and cerebral cortex (Pal et al., 1993) and it also inhibits the choline transport in synaptosomes (Chandra et al., 1994). Several studies have reported that cadmium induces the LPO and it has an inhibitory action on the antioxidant enzymes and membrane bound ATPases is brain (Gutierrez-Reyes et al., 1998; Garcia and Corredor, 2003; Carageorgiou et al., 2004).

Antioxidants are becoming increasingly popular in oxidative stress related disorders and hold promise as therapeutic agents. The antioxidant compounds can counteract the decrease in ATPase activity and the increase in oxidative stress that are induced by cadmium (El-Missiry and Shalaby, 2000). Vitamin E is a major antioxidant in biological systems and acting as a powerful chain breaking agent through its scavenging action on peroxyl radicals (Beyer, 1994). Vitamin E terminates the chain reaction of lipid peroxidation in...
membranes and lipoproteins (Ibrahim et al., 2000). Similarly, vitamin C is the water soluble antioxidant that reacts with peroxyl radicals formed in the cytoplasm before they reaches to the membrane (Khoja and Marzouki, 1994) and serves to regenerates the reduced vitamin E (Tanaka et al., 1997). Currently there is a considerable interest on the role of vitamins C and E in the protection of membrane lipids against metal induced oxidative stress (Buttner and Burns, 1996). Therefore, the objective of this study was to evaluate the protective effect of vitamin C and E on cadmium induced neurotoxicity in the brain of Wistar rats.

MATERIALS AND METHODS

Animals:

Male Wistar rats of initial body weight 120-150 g were used for the present study. They were maintained under standard laboratory conditions (temperature 24 ± 2°C, natural light-dark cycle). The laboratory animal protocol used in this study was approved (Approval No. 157, 2007) by the Institutional Animal Ethical Committee (IAEC) at Annamalai University, India. All the animals were fed on a standard rat feed and water ad libitum.

Reagents and chemicals:

All chemicals were of analytical grade and chemicals required for sensitive biochemical assays were obtained from Sigma Chemical Co., USA. Double distilled water was used in all biochemical assays.

Dosage and treatments:

All treatments were given orally through intragastric tubes for the period of 28 days. Cadmium were given to the rats at an oral dose of (5 mg/kg body weight/day). Vitamin C and E were given at a oral dose of 50 mg/kg body weight/day.

Experimental procedure:

The rats were divided into five groups of six animals in each group –

- Group I - Normal control (0.5 ml of normal saline (0.9%)/animal/day)
- Group II - Rats received cadmium chloride alone (5 mg/kg body weight/day)
- Group III - Rats received vitamin C (50 mg/kg body weight/day) and cadmium chloride (5 mg/kg body weight/day)
- Group IV - Rats received vitamin E (50 mg/kg body weight/day) and cadmium chloride (5 mg/kg body weight/day)
- Group V - Rats received cadmium chloride (5 mg/kg body weight/day) along with vitamin C (50 mg/kg body weight/day) and vitamin E (50 mg/kg body weight/day)

Doses of vitamins were given to rats one hour before to the administration of cadmium. All the treatments were carried out for the period of 4 weeks (28 days)

Tissue decapsulation:

At the end of the experimental period, the rats were fasted overnight and sacrificed by cervical decapitation with mild ether anesthesia. Whole brain was immediately dissected out, washed in an ice-cold saline to remove the blood. The brain tissue was homogenized (10% w/v) in appropriate buffer (pH 7.4) and centrifuged (1000 X g for 10 min) and the clear supernatant was used for various biochemical assays.

Biochemical analysis:

The level of lipid peroxidation (TBARS) and lipid hydroperoxides (LOOH) were measured by the method of Niehaus and Samuelsson (1968) and Jiang et al. (1992), respectively. Acetylcholinesterase (AChE) activity of the brain was determined by the method of Ellman et al. (1961). Total ATPases activity in brain homogenate was measured by the method of Evans (1969). The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were assayed by the method of Kakkar et al. (1984), Sinha (1972) and Rotruck et al. (1973), respectively. The reduced glutathione content was determined by the method of Ellman (1959). The protein content in the brain homogenate was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Statistical analysis:

All the data were expressed as mean ± SD of six individual observations (n = 6). The statistical significance was evaluated by one way analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT). Values were considered statistically significant when p < 0.05.

RESULTS AND DISCUSSION

The levels of LPO products (TBARS and LOOH) were significantly (p < 0.05) increased in brain of cadmium intoxicated rats when compared with control rats. Administration of vitamins C and E along with cadmium which significantly (p < 0.05) decreased the elevation of TBARS, LOOH than the individual treatments with vitamin C and E in comparison with that of the cadmium
A significant decrease (p < 0.05) was observed in the levels of AChE and total ATPases in brain of cadmium treated rats as compared to control rats and these values were significantly increased (p < 0.05) by the combined administration of vitamin C and E along with cadmium than the individual treatments when compared with cadmium treated rats (Fig. 1 and 2).

The activities of SOD, CAT, GPx and GSH were significantly (p < 0.05) decreased in cadmium treated group as compared with normal control and these values were reverted to their normal levels by the combined administration of vitamin C and E along with cadmium than the individual treatments with vitamin C and E in comparison with the cadmium treated group (Table 1).

Oxidative tissue injury induced by cadmium can be monitored in experimental animals by detecting the lipid peroxidative products such as thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides. Cadmium administration resulted in an excessive generation of free radicals such as, hydroxyl radical, superoxide radical, peroxyl radical and hydrogen peroxide. All these radicals have a great potential to react rapidly with lipids which in turn leads to lipid peroxidation (El-Maraghy et al., 2001).

Increasing evidences suggested that the excessive production of free radicals in brain and the imbalance between oxidative species and antioxidant defenses is related to the pathogenesis and neurodegenerative diseases (Schulz et al., 2000; Zhu et al., 2006). Cadmium can penetrate the blood brain barrier and accumulate into the brain which is easily susceptible to Cd induced lipid peroxidation (Manca et al., 1991). In the present study the elevation of LPO and lipid hydroperoxides might be due to the over production of free radicals by Cd ions, which in turn leads to oxidative modifications of proteins.

The study of brain enzymatic activities such as AChE is essential in detecting the neurotoxic effects of certain

### Table 1: The levels of brain lipid peroxidation and the antioxidant status of control and experimental groups

<table>
<thead>
<tr>
<th>Treatments</th>
<th>TBARS</th>
<th>LOOH</th>
<th>GSH</th>
<th>SOD</th>
<th>CAT</th>
<th>GPx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.35 ± 0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.07 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.97 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.17 ± 0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.04 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.31 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cadmium</td>
<td>19.50 ± 1.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.67 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.34 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.18 ± 0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.67 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.49 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cd + Vit C</td>
<td>16.10 ± 1.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.42 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.68 ± 0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.11 ± 0.64&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.49 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.79 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cd + Vit E</td>
<td>14.18 ± 0.94&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.37 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.73 ± 0.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.41 ± 0.59&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.72 ± 0.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.05 ± 0.15&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cd + Vit C + Vit E</td>
<td>13.45 ± 0.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.23 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.12 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.24 ± 0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.98 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.17 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. for 6 rats in each group
Values bearing different superscripts in the same column are significantly different at p < 0.05 (DMRT)

TBARS – mmoles/g tissue, LOOH – mmole/g tissue, GSH – μg/mg protein, SOD – Units/mg protein, CAT – μmoles of H₂O₂ utilized/min/mg protein, GPx – μg of GSH consumed/min/mg protein

**Fig. 1:** Activity of acetylcholinesterase (AChE) in the brain of control and experimental rats

**Fig. 2:** Activity of total ATPases in the brain of control and experimental rats
heavy metals. Various studies observed that the free radical production could at least or partly be associated with the decrease in the activity of AChE in brain (Tsakiris et al., 2000). It has been shown that decreased AChE activity leads to the accumulation of acetylcholine, which causes cholinergic hyperactivity, convulsion and status epileptics (Olney et al., 1986). Since brain AChE activity is an important regulator of the behavioural processes, the decreased level of AChE in brain might be one of the indicators for Cd induced hurdle in brain (Pari and Murugavel, 2007).

Various studies connected the activity of antioxidant defense systems are inhibited or diminished in rats exposed to cadmium (Milton Prabu and Rameshkumar, 2007). SOD is considered as the first line of defense against the deleterious effects of oxygen radicals in the cells and it scavenges ROS (reactive oxygen species) by catalyzing the dismutation of superoxide anions into H₂O₂. There is an evidence to indicate that cadmium significantly depressed the SOD activity (Casalino et al., 2001). The ability of cadmium to induce oxidative stress in brain cells has been reported as the induction of ROS, after the interaction of Cd²⁺ with mitochondrial sites, leading to the breakdown of mitochondrial potentials, as a consequence there is a reduction in the level of intracellular GSH and decrease in catalase and SOD activities (Lopez et al., 2006).

Catalase (CAT) and glutathione peroxidase (GPx) are the preventive antioxidant enzymes and plays a crucial role in the protection against the deleterious effects of lipid peroxidation. Reports have been shown that the low doses of cadmium exposure could induce LPO in brain and the inhibition of glutathione peroxidase should be considered as a potentially significant event in the generation of free radicals by cadmium (Manca et al., 1991; Ogungbe and Lawal, 2008).

GSH is the most abundant non-protein thiols that maintains the cellular redox status and providing first line of antioxidant protection against oxidative stress in brain tissue (Dringen et al., 2000). The decrease in the activities of antioxidant enzymes might be due to the binding of cadmium with sulphydryl groups of enzymes and oxidative modifications of amino acid chains, which alters the enzyme structure and leads to the inactivation or decreased activity of antioxidant enzymes. Thus the decreased level of reduced glutathione and the activities of brain antioxidant systems indicates the accumulation of free radicals and increased level of LPO, that increases the oxidative tissue damage in the brain of cadmium intoxicated rats. Depleted level of brain GSH has also been reported by Pari and Murugavel (2007) in rats intoxicated with cadmium.

ATPases are a key enzymes implicated in neural excitability, metabolic energy production, as well as in the uptake and release of catecholamines (Mata et al., 1980) and serotonin (Hernandez, 1989). Mg²⁺ ATPase is to maintain the high intracellular Mg²⁺ level in brain and control the rate of protein synthesis and cell growth (Sanui and Rubin, 1982). Ca²⁺ ATPase functions as second messenger in the central nervous system. The alterations in Ca²⁺ level also leads to several pathological lesions in brain (Repetto, 1997). The Ca²⁺ overload mediated by Cd also inhibit the Ca²⁺ ATPase activity in cell membrane and eventually potentiates the irreversible cell destruction (Joseph et al., 2001). In the present study the activity of total ATPases are affected by the exposure of Cd (Antonio et al., 2003) which indicates the alterations in membrane and neurotransmitter functions of brain tissue in Cd intoxication. The decreased activity of ATPases in cadmium intoxication could be due to the formation of Cd-ATPase, complex through SH group of ATPases that binds with free Cd ions (Rajanna et al., 1990; El-Missiry and Shalaby, 2000).

In the present study, the combined treatment with vitamins C and E than the individual treatment with vitamins significantly reduced the cadmium induced neurotoxicity and oxidative stress in brain. Vitamins play an important role in the maintenance of antioxidant defense and, therefore, have received much attention today. Vitamin C and E are among the most widely studied dietary antioxidants. Vitamin C is considered to be the most important water soluble antioxidant in extracellular fluids. Being a free radical scavenger it effectively scavenges superoxide anion (O₂⁻) and other reactive oxygen species and protects against lipid peroxidation (Beuttnner, 1993; Restky et al., 1993). It is capable of neutralizing ROS in the aqueous phase before peroxidation is initiated (Bradford, 2003).

Vitamin E is a major lipid soluble and most effective chain breaking antioxidant within the cell membrane where it protects, membrane fatty acids from the deleterious effects of lipid peroxidation. Vitamin C has been cited as being capable of regenerating vitamin E (Valko et al., 2006). Vitamin E inhibits the peroxidation of membrane lipids by scavenging lipid peroxyl radicals and is converted into tocopherol radical (Valko et al., 2007). Vitamin E has been demonstrated to act as a direct antioxidant that scavenges oxygen free radicals, thereby inhibiting lipid peroxidation and also acts as an indirect antioxidant that controls the increase in membrane permeability resulting from oxidative stress in brain (Chen and Tappel, 1995). The protective role of these vitamins
is greater when vitamin C and E were administered simultaneously in Cd intoxicated rats. However, vitamin E was more effective than vitamin C possibly because vitamin E protects the polyunsaturated fatty acids from peroxidation, whereas vitamin C acts in the water soluble compartment and has a sparing effect on vitamin E by regenerating the reduced form of vitamin E (Khoja and Marzouki, 1994; Tanaka et al., 1997). Vitamin C and E were reported to act synergistically inhibits the oxidation of cellular antioxidants and prevents the brain tissue from the oxidative stress induced by cadmium (Chen and Tappel, 1995; Lass and Sohal 2000).

In summary, present results concluded that vitamin C and E have beneficial actions against Cd-mediated toxic manifestations, in brain tissue via inhibiting lipid peroxidation and subsequently restoring AChE, membrane bound ATPases and antioxidants. Therefore, vitamin C and vitamin E, have been suggested to be the potential therapeutic agents against cadmium induced neurotoxicity in rats.

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