PROTECTIVE ROLE OF VITAMIN C (ASCORBIC ACID) AND E (~-TOCOPHEROL) AGAINST CADMIUM INDUCED HEPATOTOXICITY IN RATS

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Asian Journal of Environmental Science, Vol. 3 No. 1 : 23-28 (June, 2008)

SUMMARY

The study was carried out to evaluate the effect of dietary vitamins C (ascorbic acid) and E (α-tocopherol) on serum hepatic marker enzymes, antioxidant and lipid peroxidation status in liver of cadmium intoxicated rats. Ral administration of cadmium chloride (5 mg/kg body wt./day) for 30 days resulted in a significant elevation of AST, ALT, ALP, LDH and the levels of lipid peroxidation markers (TBARS) in liver. Cadmium also caused a significant reduction in the activities of SOD, CAT, GPx and GSH level in liver. Prior oral administration of vitamins C and E (50 mg/kg body wt./day) along with cadmium significantly decreased the activities of serum, AST, ALT, ALP and LDH along with significant decrease in the level of lipid peroxidation in the liver. In addition to that, combined treatment of vitamins C and E significantly increased the glutathione level together with other hepatic antioxidant enzymes. The results suggest that combined treatment of vitamins C and E exhibited better antioxidant property and decrease the level of lipid peroxidation against cadmium induced oxidative stress in liver.

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Accepted : March, 2008

Key words : Cadmium, Lipid peroxidation, Antioxidant, vitamins, Rats.

Exposure of toxic heavy metals has become an increasingly recognized source of illness worldwide. Pollution due to heavy metals is of serious concern and among them cadmium merits the special attention. Cadmium is very toxic heavy metal and an important environmental pollutant that can be injested or inhaled from a variety of industrial and dietary sources. It causes severe damage to various tissues of humans and experimental animals, when exposed chronically (Yadav and Khandelwal, 2005).

Cadmium has a significant effect on hepatic and renal function and as a result, it alters bone mineralization, leading to osteoporosis and osteomalacia. Cadmium induced genotoxicity can also increase the risk of several cancers. The mechanism of cadmium induced damage include the production of free radicals that alter the mitochondrial activity and genetic information (Partrick, 2003).

Although the biochemical mechanisms involved in cadmium hepatotoxicity remain to be elucidated. One of the major concepts regarding the toxicity of cadmiums is attributed to its ability to generate reactive oxygen species which cause oxidative stress. Excessive production of oxygen radicals leads to altered enzyme activity, decreased DNA repair, impaired utilization of oxygen, lipid peroxidation (LPO) and protein oxidation. Some of these alterations induced by oxidative stress have been recognized to be characteristic features of necrosis and subsequently leads to organ damage by cadmium (Pande *et al.,* 2001).

Various studies connects cadmium with oxidative stress since this metal can alter the antioxidant defense system in several tissues of experimental animals, causing depletion in the levels of cellular reduced glutathione, as well as changes in the permeability of the cellular membrane through a process of lipid peroxidation (Bagchi *et al.,* 1997; Milton Prabu *et al.,* 2007b). Studies on mammals showed that cadmium stimulated the formation of ROS including superoxide, anion radical (Amoruso *et al.,* 1982), hydrogen peroxide (Wong *et al.,* 1990) and most probably hydroxyl radical (Ochi *et al.,* 1988).

Lipid peroxidation is essential to understand the extend of metal toxicity in animals. Increased lipid peroxidation is generally believed to be an important underlying cause of initiation of oxidative stress related to various tissue injury, cell death and further progression to many acute and chronic diseases (Basu, 2003). Oxygen free radicals can elicit wide spread damage to cells by the peroxidation of polyunsaturated membrane lipids (Pereira *et al.,* 1998). Manca *et al.* (1991) have also reported a significant increase of lipid peroxidation in the liver and kidney of cadmium intoxicated rats.

Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) are the enzymes that provide cellular protection against the damage caused by free radicals and reactive oxygen species (ROS). Impairment in the function of these antioxidative enzymes leads to the accumulation of toxic oxidative free radicals and consequent degenerative changes in tissues

(Nishiyama *et al.,* 1998).

Vitamin E is a lipid soluble free radical scavenger that protects the membrane from lipid peroxyl radical (Buttner and Burns, 1996). Similarly, vitamin C is the water soluble antioxidant that reacts with peroxyl radicals formed in the cytoplasm before they reach the membrane (Khoja and Marzouki, 1994) and serves to regenerate the reduced vitamin E (Tanaka *et al.,* 1997). A good number of studies have established the effectiveness of antioxidant vitamins against oxidative stress (Farris, 1991; Verma and Nair, 2001; Ognjanovic *et al.,* 2003). The aim of this paper is to establish a possible protective role of vitamins C and E against cadmium induced oxidative stress in the liver of rats.

Materials and Methods

Adult male Wistar rats weighing 120-150 g were collected from Central Animal House, RMMC & H, Annamalai University. The animals were housed in polypropylene cages (47 ´ 34 ´ 18 cm) in an air-conditioned room with controlled temperature (25 ± 2^0C) and automatic lighting (alternating 12 hr periods of light and dark). The animals were fed with standard pellet diet and water *ad libitum*. The standard pellet diet comprised of 21% protein, 5% lipids, 4% crude fibre, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract (carbohydrates).

The animals were categorized into five groups of six animals in each (n = 6). To the first group 0.5 ml of normal saline (0.9%) was given for 30 days and served as control group. The second group received cadmium chloride alone (5 mg/kg body wt./day). The third group received vitamin C alone (50 mg/kg body wt./day) and the fourth group received vitamin E mixed with olive oil alone (50 mg/kg body wt./day) and the fifth group received cadmium (5 mg/kg body wt./day) along with vitamin C (50 mg/kg body wt./day) and vitamin E (50 mg/kg body wt./day). Vitamins were administered one hour prior to the administration of cadmium. The experimental design has got the ethical clearance from the Animal Ethical Committee, Annamalai University (Reg. No. 160/1994, CPSEA).

At the end of the experimental period, blood samples were collected with great care by sino-occular puncture and were sacrificed by cervical decapitation with mild ether anesthesia. Blood samples were centrifuged (1000 ´g for 15 min) for serum separation. Liver was dissected out, weighed and washed in chilled normal saline. Known weight of the liver tissue was then minced and homogenized (10% w/v) in ice cold phosphate buffer (0.1 M, pH 7.4) using Potter- Elvehjem Teflon homogenizer. The homogenate was centrifuged (5000 ´ 5 for 30 min). The clear supernatant obtained was used for the assay of various enzymes in liver. The levels of serum AST, ALT, ALP, LDH were assayed spectrophotometrically according to the standard procedures using commercially available diagnostic kits (Sigma Diagnostics (I) Pvt. Ltd., Baroda, India).

The level of lipid peroxidation (as TBARS) was measured by Hogberg *et al*. (1974). The reduced glutathione content was determined by the method of Moron *et al.,* (1979). 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. Protein content in the liver homogenate was measured as per Lowry *et al*. (1951) method using BSA as a standard. Statistical analysis was performed by one way analysis of variance (ANOVA) by using SPSS version 9.0 and Duncan's multiple range test (DMRT) obtained from the individual comparison. A value of $p < 0.05$ was considered to indicate a significant differences between the groups. Results were expressed as mean ± SD for 6 observations in each group.

Results and Discussion

The activities of serum hepatic marker enzymes such as AST, ALT, ALP and LDH were significantly increased ($p < 0.05$) in cadmium treated rats when compared with control group. Co-administration of vitamins C and E along with cadmium which significantly ($p < 0.05$) decreased the level of serum hepatic marker enzymes than the individual treatments with vitamin C and E when compared with cadmium treated rats (Table 1).

A significant increase ($p < 0.05$) was observed in the levels of TBARS in liver as compared to control group and this was significantly decreased by the combined administration of vitamins C and E along with cadmium than the individual treatments with vitamins C and E compared with cadmium treated rats. The activities of SOD, CAT, GPx and GSH were significantly decreased in cadmium treated group as compared with normal control and this values were reverted to their normal levels by the combined administration of vitamins C and E along with cadmium than the individual treatments in comparison with that of the cadmium treated rats.

In the study, a significant increase was observed in the activities of liver marker enzymes AST, ALT, ALP and LDH during chronic cadmium administration (Table 1). Liver dysfunction is accompanied by elevated levels of serum enzymes which are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Zimmerman and Seeff, 1970). High levels of aspartate

Table 1 : Serum biochemical parameters of control and experimental groups

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	LDH (IU/L)
Control	61.26 ± 3.16^{a}	35.40 ± 1.76^{a}	75.42 ± 4.16^{a}	168.12 ± 10.20^{a}
Cd	98.26 ± 7.33^{b}	64.25 ± 4.82^{b}	125.07 ± 7.23^{b}	210.21 ± 14.02^{b}
Vitamin C	72.21 ± 4.28^{c}	44.81 ± 2.62^{c}	87.28 ± 5.72^{c}	165.16 ± 9.24^{c}
Vitamin E	69.47 ± 3.18^{cde}	40.63 ± 3.11^{cde}	84.78 ± 4.34^{cde}	162.34 ± 8.32^{cde}
Cd + Vit C + Vit E	67.62 ± 4.35^{e}	39.31 ± 1.92^{e}	82.08 ± 5.23^{e}	159.43 ± 8.62^{e}

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

and alanine transaminases are better indicators to detect liver damage (Williamson *et al.*, 1996). Serum ALP is also related to the status and function of hepatic cells. Increase in serum ALP is due to the increased synthesis, in the presence of increased biliary pressure (Moss and Butterworth, 1974). Liver injury induced by cadmium is well shown by the significant elevation of the liver markers *i.e.*, serum AST, ALT, ALP after the administration of $CdCl_2$ to rats. These results are in agreement with El-Maraghy *et al*. (2001) and Milton Prabu and Rameshkumar, 2007a) who found similar increase in the level of serum AST, ALT and ALP after $CdCl_2$ administration in rats. Increase in serum LDH levels in Cd intoxicated rats indicate the hepatocellular damage. Shaikh *et al.* (1999) observed that the increased LDH activity after cadmium treatment in serum indicating hepatic and renal toxicity.

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). Increased level of TBARS in the liver of cadmium treated group. Cadmium administration results 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 turns leads to lipid peroxidation (El-Maraghy *et al.*, 2001). It is generally accepted that the enhanced LPO is one of the toxic manifestations of cadmium toxicity. Furthermore, extensive damage to tissue via free radicals mediated LPO could results in membrane damage and subsequently decreases the membrane fluidity (Bagchi *et al.*, 1996). Combined treatment with vitamins C and E resulted in a significant decrease in the levels of hepatic TBARS. It has been shown that vitamin E might be involved in the free radical scavenging action and decreased the free radicals mediated LPO (Zaidi *and Banu*, 2004). Thus vitamin E has efficiently quenches free radicals, inhibits LPO and protects tissue from further liver damage (Ognjanovik *et al.*, 2003).

Significant depletion of hepatic reduced GSH in cadmium treated rats in the present study corroborates with the findings of Manca *et al.* (1991) and Yadav and Khandelwal (2005) in cadmium treated rats. Cadmium has strong affinity for thiol groups including glutathione (Fotakis *et al.*, 2006). Furthermore cadmium depletes GSH and protein-bound sulfhydryl groups, resulting in an enhanced production of reactive oxygen species thus causing damage consistent with oxidative stress (Stohs *et al.*, 2000). The glutathione is considered to be the major thiol-disulphide redox buffer of the cell and the first line of defense against oxidative stress (Masella *et al.*, 2005). The capacity of glutathione to generate the most important antioxidant is linked to the redox state of glutathione disulphide-glutathione couple (GSSG/2GSH). Some of the experimental data provide strong evidence for two major branches of cellular anti-cadmium defense one via metal responsive transcription factor 1 (MTF1) and its target genes, notably metallothioneins and the other via reduced glutathione (Wimmer *et al.*, 2005).

SOD is considered as the first line of defense against the deleterious effects of oxygen radicals in the cells and it scavenges ROS by catalyzing the dismutation of superoxide to H_2O_2. There is an evidence to indicate that cadmium significantly depressed the SOD activity (Casalino *et al.*, 2001). The inhibition of SOD activity may result in an increased flux of superoxide by free cadmium ions in cellular compartments which may be the reason for the increased lipid peroxidation in the present study. Catalase and glutathione peroxidase are the preventive antioxidant enzymes and plays a crucial role in the protection against the deleterious effects of lipid peroxidation. Reports have shown that there is a significant decrease in the activities of catalase and GPx in cadmium intoxicated rats (Casalino *et al.*, 2001; Iszard *et al.*, 1995). The present findings are also in correlation with that of the above observations.

α-Tocopherol is an important cellular antioxidant in biological systems. It inhibits the peroxidation of membrane lipids by scavenging lipid peroxyl radicals and

Table 2 : The hepatic biochemical parameters of control and experimental groups

Treatment	TBARS	GSH	SOD	CAT	GPx
Control	0.62 ± 0.02^{a}	5.31 ± 0.24^{a}	8.61 ± 0.83^{a}	87.52 ± 5.85^{a}	5.82 ± 0.17^{a}
Cd	1.75 ± 0.15^{b}	3.21 ± 0.12^{b}	5.75 ± 0.42^{b}	47.61 ± 3.11^{b}	3.24 ± 0.16^{b}
Vitamin C	1.25 ± 0.08^{c}	2.34 ± 0.16^{c}	4.36 ± 0.37^{c}	72.26 ± 5.14^{c}	4.52 ± 0.32^{c}
Vitamin E	1.01 ± 0.07^{d}	3.91 ± 0.22^{d}	5.67 ± 0.19^{d}	77.34 ± 5.18^{d}	4.93 ± 0.37^{d}
Cd + Vit C + Vit E	0.65 ± 0.05^{ae}	5.42 ± 0.26^{ae}	8.72 ± 1.73^{ae}	89.12 ± 5.62^{ae}	6.09 ± 0.48^{ae}

Values are mean ± SD for 6 rats in each group
Values bearing different superscripts in the same column are significantly different at $p < 0.05$ (DMRT)
TBARS – nmoles of MDA/mg protein
GSH – μg/mg protein
SOD – Units/mg protein (one unit is as 50% inhibition of NBT)
CAT – μmoles of H_2O_2 utilized /min/mg protein
GPx – μg of GSH consumed/min/mg protein

is converted into a tocopheroxyl radical (Valko *et al.*, 2007). The present results showed that combined treatment with vitamins C and E along with cadmium caused a highly significant decrease in serum parameters and levels of TBARS and an increase in SOD, CAT, GSH, GPx activities as compared with cadmium treated groups than the individual treatment regimen. The administration of vitamins C and E along with cadmium alleviated its most of the harmful effects on liver (Table 2). These results are in good accordance with those obtained by Valko *et al.* (2006) and El-Demerdash *et al.* (2004), who found that α-tocopherol might have quenched free radicals and inhibited LPO and ultimately decreased the burden of other cellular antioxidants. The protective role of these vitamins is greater when vitamins C and E were administered simultaneously. 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). Thus, vitamins E and C were reported to act synergistically, inhibit the oxidation of cellular antioxidants and prevent the cell destruction (Beyer, 1994; Chen and Tappel, 1995; Lass and Sohal, 2000).

Our study has clearly demonstrated that prior oral supplementation of vitamins C and E along with cadmium protects the liver from the deleterious effect of cadmium by decreasing the level of liver marker enzymes, hepatic lipid peroxidation and increases the level of antioxidant (GSH) and antioxidant enzymes (SOD, CAT and GPx). Combined treatment of vitamins C and E in cadmium intoxication appeared more effective than individual treatments with vitamins C and E. On the whole, the present data depict that vitamins C and E could act synergistically in preventing the oxidative damage induced by cadmium in the liver of rats.

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