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RESEARCH ARTICLE

PROTECTIVE ROLE OF VITAMIN-E IN MANGANESE INDUCED OXIDATIVE STRESS IN RAT CARDIAC TISSUE

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ABSTRACT

Oxidative stress is an essential biomarker in cell survival and death. Oxygen related radicals have a strong ability to cause long-term or even permanent damage to cellular components, particularly within the mitochondrial electron transport system (ETS). The present study addressed the effect of Manganese (Mn) on oxidative system in the cardiac tissue of rat. One month and three months old rats were exposed to Mn intraperitoneally at a concentration of low dose (2.5mg/kg body weight) and high dose (5mg/kg body weight) for a period of three weeks. A separate batch of low dose and high dose of Mn exposed rats received Vitamin-E intraperitoneally for a week. In this study, we assessed the biochemical end points indicative of oxidative stress and TCA cycle enzymes in mitochondrial fraction of cardiac tissue in rats at the age of two months and four months. We measured the activity levels of Mn-superoxide dismutase (Mn-SOD), Cu/Zn-superoxide dismutase (Cu/Zn-SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione dehydrogenase(GDH), succinate dehydrogenase (SDH) and isocitrate dehydrogenase (ICDH) which were decreased, whereas the lipid peroxidation (LP), glutathione-s-transferase (GST) showed significant increase in Mn exposed rats over control rats in a dose-dependent manner. The exposure to Vitamin-E, however, to low and high dose of Mn showed recovery which was observed in the increased activities of Mn-SOD, Cu/Zn-SOD, GPX, CAT, GDH, SDH, ICDH. Similarly, decreased levels of LP and GST were observed. The results may suggest that Mn-induced functional deficits in the oxidative enzymes of cardiac tissue and recovery through Vitamin E. As an antioxidant, Vitamin-E acts as a peroxyl radical scavenger, preventing the propagation of free radicals in tissues, by reacting with them and form a tocopheryl radical, which will then be reduced by a hydrogen donor and thus return to its reduced state. As it is incorporated into cell membranes, which protects them from oxidative damage. Thus Vitamin-E lesser the Mn burden in the cardiac tissue of rat as an effective chelating agent by decreasing the oxidative stress.

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INTRODUCTION

Oxidative stress is an essential biomarker in cell survival and death. Insignificant amounts of reactive oxygen species (ROS), as hydroxyl radicals, superoxide anions and hydrogen peroxide (H_2O_2), are constantly generated in aerobic organisms in response to both external and internal stimuli (Lee *et al*., 1998). Low levels of ROS may be indispensable in a plethora of processes, including intracellular messaging (Schulze-Osthoff *et al*., 1997) leading among others to proliferation or apoptosis (Vogt *et al*., 1998), immunity (Sun *et al*., 1998) and defense against micro-organisms (Lee *et al*., 1998). In contrast, high doses and/or inadequate removal of ROS result in oxidative stress, which may cause severe metabolic malfunctions and damage to biological macromolecules (Lledias *et al*., 1998).Manganese has been implicated in oxidative stress (DeSole *et al*., 1997), (Stokes *et al*., 2000). Studies have demonstrated that manganese has the capability of inducing oxidative stress and radical formation (Chen and Liao, 2002). ROS have been implicated in a variety of disease and physiological conditions. Mn toxicity is mediated by mitochondrial perturbations, initiating both apoptotic and necrotic cell death through the formation of reactive oxygen species (ROS) and oxidative stress (Milatovic *et al*., 2009; Roth and Garrick, 2003; Tamm *et al*., 2008; Zhang *et al*., 2004; Zhang *et al*., 2008). Manganese is a constituent of metalloenzymes such as arginase and functions as a cofactor for other enzymes, such as pyruvate carboxylase and manganese superoxide dismutase (Mn-SOD) (Hurley and Keen, 1987; NRC, 1989).Mammalian cells possess enzymatic antioxidant defense to cope with oxygen reactive species e.g.,

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superoxide dismutase (SOD) which catalyses the dismutation of superoxide onion to hydrogen peroxide (H_2O_2), Catalase (CAT) and glutathione peroxidase (GPX) that catalyse of H_2O_2 to H_2O. Manganese superoxide dismutase (Mn-SOD) is the principal antioxidant enzyme of mitochondria. Because mitochondria consume over 90% of the oxygen used by cells, they are especially vulnerable to oxidative stress. The superoxide radical is one of the reactive oxygen species produced in mitochondria during ATP synthesis. Mn-SOD catalyses the conversion of superoxide radicals to hydrogen peroxide, which can be reduced to water by other antioxidant enzymes (Leach *et al*., 1997).The generation of free radicals and peroxidation of lipids are extremely fast reactions, which are generally measured by the end products, mostly thiobarbituric acid reactive substances (TBARs), among which malondialdehyde (MDA) is the most common. TBARs enhancement is thought to be a marker of cell damage which indicates an increased production of free radicals and lipid peroxidation (Patockova *et al*., 2003). SDH and Isocitrate dehydrogenase (ICDH) are important enzymes in the tricarboxylic acid cycle, responsible for the formation of alpha ketoglutarate is a key participant in the detoxification of reactive oxygen species (ROS).Vitamin E is the primary liposoluble antioxidant which may have a significant role in scavenging free oxygen radicals and in stabilizing the cell membrane, thus maintaining its permeability (Bjorneboe *et al*., 1990; Navarro, 1999).The present study aimed to examine the alterations in oxidative enzymes of the mitochondrial fractions of cardiac tissue for low and high dose of Mn exposure in young and adult Albino rats and possible recovery of toxicity with α- tocopherol.

MATERIALS AND METHODS

Chemicals

Manganese and α-tocopherol were selected as test chemicals. Isopropanol, alpha keto glutaric acid, L-alanine, 2,4-dinitro phenyl hydrazine, L-aspartate, thiobarbituric acid (TBA), malonaldehyde (MDA), n-butanol, trimethoxy pentane (TMP), poly vinyl pyrolidone, EDTA, methionine, riboflavin, hydrogen peroxide (H_2O_2), 1-chloro-2,4-dinitro benzene (CDNB), glutathione (GSH), thioether, sodium azide, TCA, disodium hydrogen phosphate, sodium phosphate buffer, glutathione reduced, glutathione, folin-phenol reagent and other chemicals used in this study are procured from Sigma (USA), 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 $28^{o}\pm2^{o}$ C and relative humidity 60±10% with a 12hour 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. The young and adult albino rats (1 month and 3 months old) were exposed to a low dose of 2.5mg per kg body weight and a high dose of 5mg per kg body weight (Papas *et al*., 1997) through intraperitoneal injection for a period of 3 weeks left for a period of one week for supplementation with α-tocopherol at a dose of 5mg/kg body weight through intraperitoneal injection. A separate batch of low dose and high dose of Mn exposed rats received vitamin-E intraperitoneally for a week. The control animals received only deionized water without Mn. After the period of dosage, at the end of two months and four months, the animals were sacrificed through cervical dislocation and the tissues were stored at -80^{0}C for the further biochemical analysis.

Preparation of heart mitochondrial fractions

Heart mitochondria were isolated by the method followed previously (Gottipolu et al., 2008).The left ventricle piece of the heart was rapidly used for the isolation of mitochondria and cytosolic fractions. The tissues were minced and homogenized using a motorized Teflon pestle and glass homogenizer in 10 ml of buffer A (210 mM mannitol, 5mM MOPS, 70 mM sucrose, 1.0 mM EDTA, pH 7.4) on ice. After centrifugation at 300g for 20 min at 4^{0}C, the supernatant was carefully removed and recentrifuged at 10,000 x g for 20 min at 4C. The pellet was separated and suspended in 1ml buffer B separated (210mM mannitol, 5mM MOPS, 70mM sucrose) sonicated for 10 s and centrifuged at 800 x g for 10 min. The supernatant was separated (cytosolic fraction) and the pellets were suspended in 1 ml of buffer B (210mM mannitol, 5mM MOPS, 70mM sucrose) frozen at -80^{0}C and used for biochemical analysis.

Superoxide dismutase activity (EC 1.151.1)

Superoxide dismutase activity was measured as the inhibition of photoreduction of nitro blue tetrazolium (NBT) by the enzyme as per the method of Beachamp and Fridovich (1971). The tissues were homogenized (20%W/V) in potassium phosphate buffer pH (7.5) containing 1% poly vinyl pyrolidone and centrifuged at 16000g for 15 minutes. The supernatant was used as enzyme source. The total reaction mixture consisted of 1.5 ml of phosphate buffer pH (7.5), 0.3 ml of EDTA, 0.3ml of methionine, 0.3 ml of NBT, and 0.1 ml of riboflavin, 0.1 ml of enzyme source and 0.4 ml of distilled water. The reaction was initiated by the addition of riboflavin and the samples were placed under a tube light (fluorescence) for 30 minutes and the resulting colour was read at 560 nm against the reagent blank kept in a dark place. The activity of the enzyme was expressed as µg of formazan formed/mg of protein. One unit is equal to 50 % inhibition of the photoreduction.

Glutathione peroxidase (GPx) (EC 1.11.1.9) activity

GPX activity in the mitochondrial fraction of rat Cardiac tissue was assayed as described by Rotruck *et al*. (1973). The reaction mixture consisted of 0.2 ml of EDTA, 0.2 ml of 4m M sodium azide, 0.2 ml of glutathione reduced, 0.2 ml of H_2O_2, 0.4 ml 0.32 M of buffer and 0.1 ml of enzyme source. The reaction mixture was incubated at 37º C for 10 min. The reaction was arrested by adding of 0.5 ml TCA. Then centrifuged at 2000 rpm for 10 min. 0.5 ml of supernatant was taken 3.0 ml of disodium hydrogen phosphate, 1.0 ml of DTNB were added. The reaction was read at 412 nm in spectrofluorometer.

Catalase activity (EC 1.11.1.6)

Catalase activity was measured following the method of Beers and Sizer (1952). The tissues were homogenized (10% W/V) in 50mM phosphate buffer (pH 7.0) and centrifuged at 16,000g

for 45minutes and again the supernatant was centrifuged at 1,05,000g for one hour and resulting supernatant was used as the enzyme source. The reaction mixture contained 1.9ml reagent grade water, 1.0ml of 0.059M hydrogen peroxide (H_2O_2) in buffer. The reaction mixture was incubated in spectrophotometer for 4-5 minutes to achieve temperature equilibration and to establish blank rate if any 0.1ml of diluted enzyme was added and decrease in absorbance was recorded at 240nm for 2-3 minutes. ΔA_{240} / min from the initial (45 second) linear portion of the curve was calculated. The enzyme activity was expressed as µmoles of H_2O_2 decomposed/mg protein/min.

Glutathione-S-Transferase (GST) (E.C. 2.5.11.8) activity

GST activity in the mitochondrial fraction of Cardiac tissue 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: 150mM phosphate buffer (pH 7.5), 1mM CDNB, 5mM 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 µ moles of thioether formed/mg protein/min, where one unit of enzyme activity is defined as one µ mole of thioether formed/mg protein/min.

Lipid peroxidation

The lipid peroxides were determined by the TBA method of Ohkawa *et al*. (1979). The tissues were homogenized in 1.5%KCl (20% W/V). To 1ml of tissue homogenate 2.5 ml of 20%TCA was added and the contents were centrifuged at 3,500g for 10 minutes and the precipitate was dissolved in 2.5ml of 0.05M sulphuric acid. To this, 3ml 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 4ml of n-butanol and the colour was read at 530nm in a spectrophotometer against the reagent blank. Trimethoxy pentane (TMP) was used as external standard. Values are expressed in µ moles of malanaldehyde formed/gm. wet weight of tissue/hour.

Glutamate Dehydrogenase activity

Glutamate Dehydrogenase activity was estimated by the method of Lee and Lardy (1965). The assay mixture final volume of 2ml contain 0.5 ml of phosphate buffer (pH 7.2), 0.4 ml of Sodium-L-glutamate, 0.4ml of INT, 0.1ml of NAD and 0.2ml of water. The reaction was initiated by addition of 0.4ml of cytosolic fraction. The contents were incubated for 30 min at 37^0 C. After incubation, the reaction was stopped by addition of 5ml of glacial acetic acid. Later 5ml of Toluene was added and kept for overnight. The formazan formed was extracted into 5.0ml of toluene and the colour was measured at 545 nm in spectrophotometer against toluene blank. The enzyme activity was expressed as µmoles of formazan formed/mg of protein/hr.

Succinate Dehydrogenase (SDH) activity: (EC 1.3.99.1)

SDH activity was estimated by the method of Nachlas *et al*., (1960) as modified by Prameelamma, (1976). The total reaction mixture contained 0.5ml of phosphate buffer (pH7.2), 0.4 ml of INT and 0.2ml of water in a final volume of 2ml.The reaction was initiated by addition of 0.5ml of mitochondrial fraction. The content was incubated for 30 min at 37^0 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 against toluene blank. The enzyme activity was expressed as µmoles of formazan formed /mg of protein/hr.

Isocitrate Dehydrogenase (ICDH) activity: (EC 1.1.1.41)

Isocitrate Dehydrogenase activity was estimated by the method of Lee and Lardy (1965). The assay mixture final volume of 2ml contain 0.5ml of phosphate buffer (pH7.2), 0.4ml of INT, 0.1 ml of MgCl2, 0.1ml of ADP and 0.2ml of NAD. The reaction was initiated by addition of 0.3ml of mitochondrial fraction. The contents were incubated for 30 min at 37^0C. After incubation, the reaction was stopped by addition of 5ml of glacial acetic acid. Later 5 ml of toluene was added and kept for overnight. The formazan formed was extracted into 5.0ml of toluene and colour was measured at 545 nm in spectrophotometer against toluene blank. The enzyme activity was expressed as µmoles of formazan formed/mg of protein/hr.

Statistical Analysis

The mean, Standard deviation, analysis of variance (Two-way ANOVA) and test of significance were calculated using standard statistical software package.

RESULTS

Mn-SOD activity

In the existing study, the levels of Mn-SOD activity in 2 months and 4 months rats showed decrease when compared to control rats. From the results (Fig.1) it is evident that the decrease of Mn-SOD activity in Mn treated rats in both the doses i.e., low dose and high dose of Mn exposure. The decrease was more in high dose of Mn exposure than low dose when compared to control rats. A maximal decrease in Mn-SOD activity levels were observed in 2 months low dose and high dose of Mn exposed rats. However, the administration of α-tocopherol along with Mn showed recovery from Mn toxicity in the levels of Mn-SOD activity in low dose and high dose of Mn exposure.The four months old rats also showed similar decrease in Mn-SOD activity levels. The four months Mn treated rats showed decrease in low dose and high dose when compared to controls. The administration of α-tocopherol showed recovery from Mn toxicity in low dose high dose and showed increase in the level of Mn-SOD activity respectively.

Cu/Zn-SOD activity

In the present study, from the results (Fig.2) it is evident that the levels of Cu/Zn-SOD activity in mitochondrial fraction of Mn treated rats in both the age groups (2 months and 4 months) showed decrease when compared to control rats.Low dose and high dose of Mn exposure showed decreased Cu/Zn-SOD activity compared to control. Among the two different

exposure of Mn, high dose has more effect on Cu/Zn-SOD activity in both ages. However, partial recovery was observed in the administration of α-tocopherol along with Mn from Mn toxicity in the activity levels of Cu/Zn-SOD activity in low dose and high dose of Mn exposure.From the results (Fig.2) it is evident that the Mn- exposure decreased the levels of Cu/Zn-SOD activity maximal decrease observed in 2 months rats over 4 months rats. At the same time 2 months Mn exposed rats showed more recovery with α- tocopherol over 4 months Mn exposed rats.

GPx activity

In the contemporary study, the activity of GPx in 2 months and 4 months control and experimental rats. From the results (Fig.3) it is evident that decrease of GPx activity in Mn treated rats in both the age groups (2 months and 4 months) and both concentrations i.e., low dose and high dose when compared to control rats.Mn exposure decreased the activity of GPx in 2 months and 4 months more than control. A maximal decrease in GPx activity levels observed in 2 months low dose and high dose of Mn exposed rats. However, the administration of α-tocopherol along with Mn showed recovery from Mn toxicity in the activity of GPx in low dose and high dose of Mn exposure. Similarly, in four months old rats also showed decrease in GPx activity levels. The four months Mn treated rats showed decrease in low dose and high dose when compared to controls. The administration of α-tocopherol showed recovery from Mn toxicity in low dose and high dose showed increase in the level of GPx activity. From the results (Fig.3) it is evident that the Mn- exposure decreased the levels of GPx activity maximal in 2 months rats over 4 months rats. At the same time 2 months Mn exposed rats showed more recovery with α- tocopherol over 4 months Mn exposed rats.

Catalase activity

In the present study, from the results (Fig.4) it is evident that Catalase activity was decreased in Mn treated rats in both the age groups (2 months and 4 months) when compared to control rats. Low and high dose of Mn exposure decreased Catalase activity than control. A maximal decrease Catalase activity was observed in 2 months low dose and high dose of Mn exposed rats. However, the administration of α-tocopherol showed recovery from Mn toxicity in the Catalase activity in low dose and high dose of Mn exposure. The four months old rats also showed similar decrease in Catalase activity. The four months Mn treated rats showed decrease in low dose and high dose when compared to controls. The administration of α-tocopherol showed recovery from Mn toxicity in low dose and high dose showed increase Catalase activity.

Glutathione- S-transferase (GST) activity

From the results (Fig.5) it is evident that increase of GST was showed in Mn treated rats in both the age groups (2 months and 4 months) when compared to control rats.Mn exposure increased activity levels of GST in 2 months and 4 months more than control. A maximal increase was observed in 2 months low dose and high dose of Mn exposed rats. However, the administration of α-tocopherol showed partial recovery from Mn toxicity in the activity levels of Glutathione S transferase in low dose and high dose of Mn exposure. In four months old rats also showed similar increase in GST activity levels. The four months Mn treated rats showed increase in low dose and high dose when compared to controls. The administration of α-tocopherol showed recovery from Mn toxicity in low dose and high dose showed increase in the level of Glutathione- S- transferase.

Lipid peroxidation

From the results (Fig.6) it is evident that increase of Lipid peroxidation in Mn treated rats in both the age groups (2 months and 4 months) when compared to control rats. Low dose and high dose of Mn exposure showed increase in the levels of Lipid peroxidation more than control rats. Among two different exposure of Mn, high dose has more effect on Lipid peroxidation. However, partial recovery was observed in α-tocopherol supplemented rats along with Mn.From the results (Fig.6) it is evident that the Mn- exposure decreased the levels of Lipid peroxidation was maximal in 2 months rats over 4 months rats. At the same time 2 months Mn exposed rats showed more recovery with α- tocopherol over 4 months Mn exposed rats.

Glutamate dehyhyrogenase activity (GDH)

In the present study, the activity of GDH in two months and four months control rats showed gradual increase compared to Mn exposed rats. From the results (Fig.7) it was evident that the decrease in GDH activity was observed at both concentrations i.e., low dose and high dose of Mn treated 2 months and 4 months rats when compared to control rats. However, the administration of α-tocopherol showed recovery from Mn toxicity in the activity levels of GDH in low dose and high dose of Mn exposure.In this study, it was evident that the Mn- exposure decreased the activity of GDH maximum in two months rats over four months rats. At the same time two months Mn exposed rats showed more recovery with α-tocopherol over four months Mn exposed rats.

Succinate dehydrogenase (SDH) activity

Similar to GDH, SDH activity also increased in four months old rats when compare to two months old rats. The SDH activity levels were also decreased with Mn exposure (Fig.8). The decrease was more conspicuous in high dose Mn exposed rats than low dose (Fig.8).The increase in SDH activity was more significant at both age groups at both concentrations i.e., low dose and high dose of Mn. However, the activity of SDH was increased with α-tocopherol administration along with Mn. The α-tocopherol administration increased SDH activity in two months low dose and high dose of Mn as well as four months low dose and high dose of Mn exposure.

Isocitrate dehydrogenase (ICDH) activity

In the present study, the activity of ICDH in two months and four months control rats showed gradual increase compared to Mn treated rats. From the results (Fig.9) it was observed that the decrease of ICDH activity in Mn treated rats in both the age groups (two months and four months) when compared to control rats.The decrease was observed in the activity of ICDH in two months low dose and high dose of Mn exposed rats. However, the administration of α-tocopherol showed recovery from Mn toxicity in the activity of ICDH in low dose and high dose of Mn exposure in two months old rats. The four months

old rats also showed similar decrease in ICDH activity. The four months Mn treated rats showed decrease in ICDH activity in low dose and high dose when compared to control rats. The administration of α-tocopherol showed recovery from Mn toxicity in low dose and high dose showed increase in the activity of ICDH in four months old rats. From the results (Fig.9) it was evident that the Mn exposure decreased the activity levels of ICDH maximum in two months rats over four months rats. At the same time two months Mn exposed rats showed more recovery with α- tocopherol over four months Mn exposed rats.

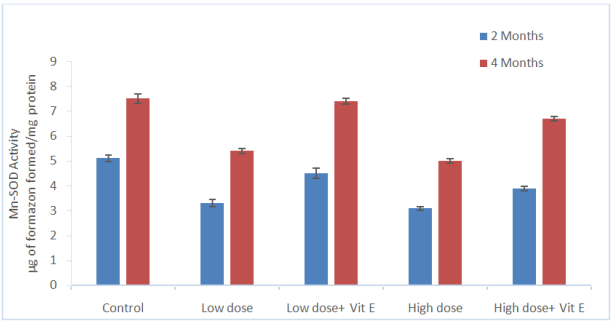

Fig. 1. Effect of manganese on Mn-SOD activity in heart mitochondria of control and Mn-exposed rats to low dose (2.5mg/bw) and high dose (5mg/bw) through intraperitoneal injections for 3 weeks. The recovery of manganese toxicity in two separate groups of albino rats exposed to manganese intraperitoneally low and high dose treated with α-tocopherol (5mg/bw) for a week. All values are mean of values of six albino rats and significant at p<0.05, except the values marked with asterisk (*)

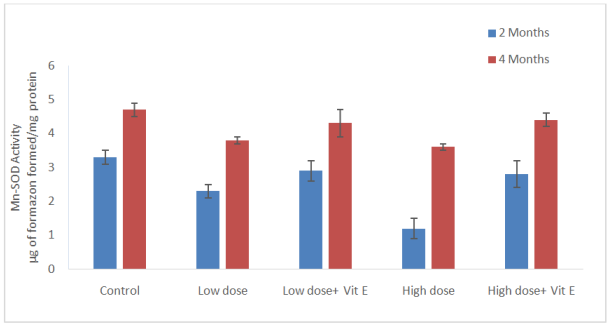

Fig. 2. Effect of manganese on Cu/Zn-SOD activity in heart mitochondria of control and Mn-exposed rats to low dose (2.5mg/bw) and high dose (5mg/bw) through intraperitoneal injections for 3 weeks. The recovery of manganese toxicity in two separate groups of albino rats exposed to manganese intraperitoneally low and high dose treated with α-tocopherol (5mg/bw) for a week. All values are mean of values of six albino rats and significant at p<0.05, except the values marked with asterisk (*)

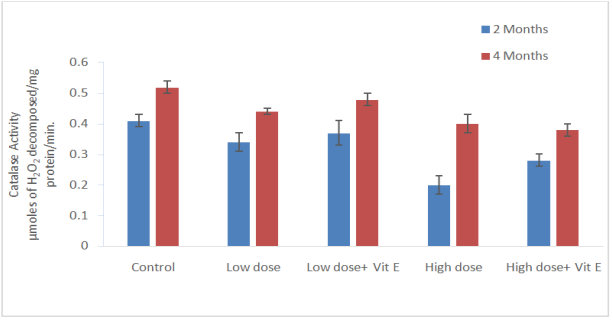

Fig. 3. Effect of manganese on Catalase activity in heart mitochondria of control and Mn-exposed rats to low dose (2.5mg/bw) and high dose (5mg/bw) through intraperitoneal injections for 3 weeks. The recovery of manganese toxicity in two separate groups of albino rats exposed to manganese intraperitoneally low and high dose treated with α-tocopherol (5mg/bw) for a week. All values are mean of values of six albino rats and significant at p<0.05, except the values marked with asterisk (*)

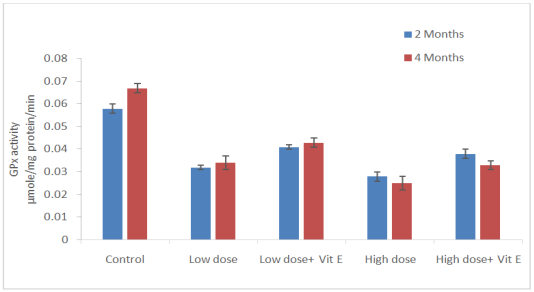

Fig. 4. Effect of manganese on GPx activity in heart mitochondria of control and Mn-exposed rats to low dose (2.5mg/bw) and high dose (5mg/bw) through intraperitoneal injections for 3 weeks. The recovery of manganese toxicity in two separate groups of albino rats exposed to manganese intraperitoneally low and high dose treated with α-tocopherol (5mg/bw) for a week. All values are mean of values of six albino rats and significant at p<0.05, except the values marked with asterisk (*)

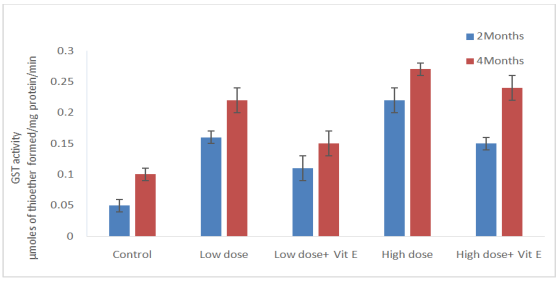

Fig. 5. Effect of manganese on GST activity in heart mitochondria of control and Mn-exposed rats to low dose (2.5mg/bw) and high dose (5mg/bw) through intraperitoneal injections for 3 weeks. The recovery of manganese toxicity in two separate groups of albino rats exposed to manganese intraperitoneally low and high dose treated with α-tocopherol (5mg/bw) for a week. All values are mean of values of six albino rats and significant at p<0.05, except the values marked with asterisk (*)

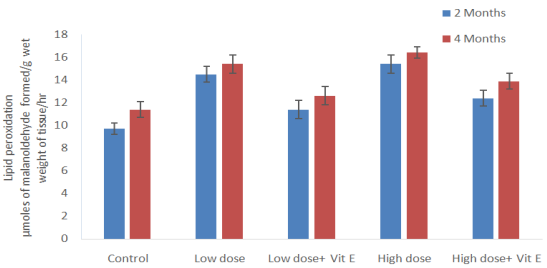

Fig. 6. Effect of manganese on Lipid peroxidation in heart mitochondria of control and Mn-exposed rats to low dose (2.5mg/bw) and high dose (5mg/bw) through intraperitoneal injections for 3 weeks. The recovery of manganese toxicity in two separate groups of albino rats exposed to manganese intraperitoneally low and high dose treated with α-tocopherol (5mg/bw) for a week. All values are mean of values of six albino rats and significant at p<0.05, except the values marked with asterisk (*)

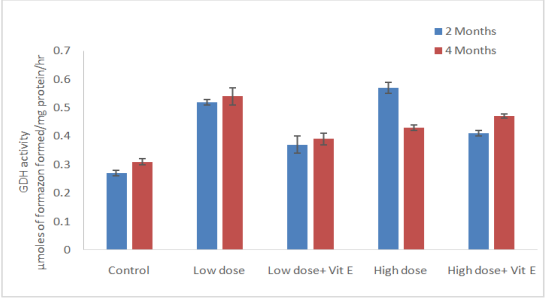

Fig. 7. Effect of manganese on GDH activity in heart mitochondria of control and Mn-exposed rats to low dose (2.5mg/bw) and high dose (5mg/bw) through intraperitoneal injections for 3 weeks. The recovery of manganese toxicity in two separate groups of albino rats exposed to manganese intraperitoneally low and high dose treated with α-tocopherol (5mg/bw) for a week. All values are mean of values of six albino rats and significant at p<0.05, except the values marked with asterisk (*)

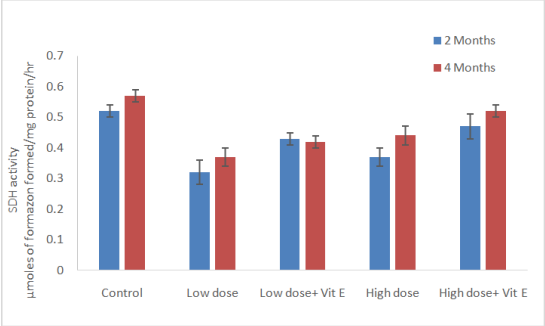

Fig. 8. Effect of manganese on SDH activity in heart mitochondria of control and Mn-exposed rats to low dose (2.5mg/bw) and high dose (5mg/bw) through intraperitoneal injections for 3 weeks. The recovery of manganese toxicity in two separate groups of albino rats exposed to manganese intraperitoneally low and high dose treated with α-tocopherol (5mg/bw) for a week. All values are mean of values of six albino rats and significant at p<0.05, except the values marked with asterisk (*)

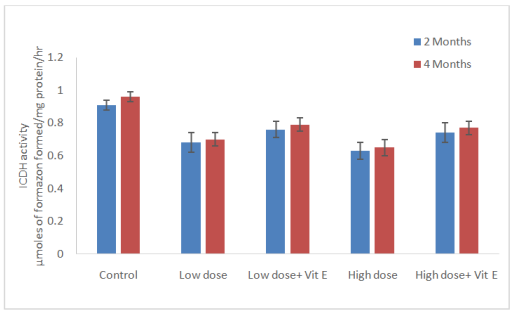

Fig. 9. Effect of manganese on ICDH activity in heart mitochondria of control and Mn-exposed rats to low dose (2.5mg/bw) and high dose (5mg/bw) through intraperitoneal injections for 3 weeks. The recovery of manganese toxicity in two separate groups of albino rats exposed to manganese intraperitoneally low and high dose treated with α-tocopherol (5mg/bw) for a week. All values are mean of values of six albino rats and significant at p<0.05, except the values marked with asterisk (*)

DISCUSSION

The current study provides evidence of oxidative damage to the cardiac tissue in two months and four months old rats after low and high dose of manganese intra peritoneal administration. In this study the alterations in the activity levels of cardiac marker enzymes of related to oxidative stress and TCA cycle like Mn-SOD, Cu/ Zn-SOD, CAT, GPX, GST, GDH, ICDH, SDH and Lipid peroxidation levels were observed in cardiac tissue of control and experimental rats. Oxidative stress has been implicated as a contributing mechanism by which manganese can be toxic to cells (Aschner, 1997; Galvani *et al*., 1995). There are several possible mechanisms for this iron related decrease in Mn-SOD activity. One probable reason is increased peroxidative stress. Many studies indicate that heavy metals act as catalysts in the oxidative reactions of biological macromolecules therefore the toxicity with oxygen reactive species, with these metals might be due to oxidative tissue damage (Cuypers *et al*., 1999; Flora *et al*.,2008; Leonard *et al*., 2004; Hultberg *et al*.,1999; Stohs and Bagchi, 1993). Lipid peroxidation is one of the main manifestations of oxidative damage and has been found to play asignificant role in the toxicity of many xenobiotics, and it alters physiological and biochemical characteristics of biological systems (Anane and Creppy, 2001). Our study supports the similar increase in Lipid peroxidation with intra peritoneal administration of Mn. Cytosolic glutathione peroxidase (GPx, GPx1) is a selenoprotein, first described as an enzyme that protects hemoglobin from oxidative degradation in red blood cells (Mills, 1957). GPx requires several secondary enzymes (glutathione reductase and glucose-6-phosphate dehydrogenase) and cofactors (reduced glutathione, NADPH, and glucose 6-phosphate) to function at high efficiency. CAT is responsible for breakdown of hydrogen peroxide, an important ROS produced during metabolism, reduced activity of CAT observed in the present study could be due to exhausted or over utilization of the enzyme during the oxidative stress caused by Mn-exposure. The decrease in CAT activity could also be due to an increase in MDA production, which can cross link with the amino group of the protein, there by inactivating several membrane bound enzymes (Kikugawa *et al*., 1984). More over Rister and Bachner (1976) have speculated that during oxidative stress, CAT activity decreases as H_2O_2 accumulates. The increased sensitivity of transfected enriched catalase cells to adriamycin, bleomycin and paraquat is attributed to the ability of catalase in cells to prevent the drug-induced consumption of O_2. Thus, capturing H_2O_2 before it can escape the cell and converting it to O_2. In this way, catalase can maintain the concentration of O_2either for repeated rounds of chemical reduction or for direct interaction with the toxin (Speranza *et al*., 1993). In the contemporary study CAT showed similar decrease with Mn exposure this may due to increase in MDA production. The α-tocopherol administration reversed Mn toxicity. Mn exposure significantly decreased the activity levels of SDH and ICDH enzymes. The decrease in the SDH activity in the heart of rats exposed to Mn indicates mitochondrial dysfunction. The decrease in SDH activity indicates reduction in the conversion of succinate to fumarate and fluctuations of oxidative metabolism reflects the turnover of carbohydrates and energy output. Thus, the decreased SDH activity observed in the heart of rats treated with rats Mn. The decrease was more in high dose than low dose with age groups two months and four months rats, α-tocopherol increased enzyme activity and reduced the Mn toxicity. Similar decrease in SDH and ICDH activities was observed in alcohol treated rats (Maruthappan *et al*., 2010), in carboplatin and cisplatin exposed rats (Kishor Reddy *et al*., 2010). Glutathione-S-transferase, and Lipid peroxidation levels were increased more than control when exposed to Mn. The α-tocopherol decreased enzyme activity and reduced manganese toxicity. Hassan and Awad (2007) showed that the exposure to Cd caused marked elevation in the level of lipid peroxidation and a decline in SOD, glutathione peroxidase (GSH-Px) and CAT activities accompanied by an increase in the rate of hemoglobin autoxidation in Swiss albino rats. Additionally, they demonstrated that the treatment with vitamin E significantly reduced the changes caused by Cd exposure in all examined parameters. Moreover, they suggested that these results indicate that alterations caused by Cd are connected with free radicalsgeneration and used antioxidants effectively to protect against Cd intoxication.

Conclusion

In conclusion, the present study showed that α-tocopherol has protective effect on Mn induced oxidative stress and injuries. This study therefore suggests that α-tocopherol may be a useful preventive agent against the effect of the manganese at least partly due to its antioxidant properties.

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