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Vitamin E improved redox homeostasis in heart and aorta of hypothyroid rats

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Objectives. The objective of this study was to evaluate the effect of vitamin E on the oxidative stress parameters and antioxidant defense enzymes in the heart and aorta of 6-n-propylthiouracil (PTU)-induced hypothyroid rats.

Methods. The animals were divided into 4 experimental groups: Group 1 (Euthyroid) received tap water, Group 2 (Hypothyroid) received 0.05 % of PTU in dissolved in their drinking water, Group 3 (PTU+Vit E) hypothyroid rats treated with vitamin E, and Group 4 (Euthyroid+Vit E). Vitamin E was injected daily (20 mg/kg) to groups 3 and 4 via daily gavage for 6 weeks. Malondialdehyde (MDA) levels, total thiol levels, and the activities of Cu, Zn-superoxide dismutase (SOD) and catalase (CAT) were evaluated in the aortic and cardiac tissues.

Results. A significant decrease of thyroxine (T4) serum levels confirmed hypothyroidism in rats, which received PTU. The MDA level increased and total thiol level decreased in the hypothyroid group compared to control group ($p<0.001$). The activities of SOD and CAT significantly decreased in the hypothyroid rats in comparison to the control. Vitamin E treatment resulted in increased levels of total thiol, SOD, and CAT within aortic and cardiac tissues and decreased levels of MDA in comparison with the hypothyroid group ($p<0.01$–$p<0.001$).

Conclusions. PTU-induced hypothyroidism resulted in oxidative stress. Chronic administration of vitamin E to hypothyroid rats decreased the oxidative stress markers in the aortic and cardiac tissues.

Key words: hypothyroidism, oxidative stress, heart, aorta

Several studies have demonstrated that hypothyroid patients are prone to ischemic heart disease (Chu and Crapo 2001; Mcdermott and Ridgway 2001; Ranasinghe and Bonser 2010). Based on a Meta-analysis study, subclinical hypothyroidism has been shown to be associated with an increased prevalence of ischemic heart disease and cardiovascular mortality (Razvi et al. 2008). Hypothyroidism has been shown to increase the systemic vascular resistance (Klein and Danzi 2007, 2008). Thyroid hormones are one of the most prominent regulators of cellular respiration (Gustafsson et al. 1996) and maintain the metabolic homeostasis in mammalian (Sarkar 2002; Pacheco-Rosado and Alva-Sanchez 2007).

A deficiency in thyroid hormones decreases the vital cellular functions such as mitochondrial respiration, energy metabolism, and protein synthesis. These insufficiencies are mended by thyroid hormone

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supplementation (Satav and Katyare 1982). It has been reported that hypothyroidism is characterized by abnormal lipid metabolism, cardiac dysfunction, and atherosclerosis (Duggal et al. 2007). Hypothyroidism is a risk factor for coronary artery disease (Auer et al. 2003), vascular disease (Razvi et al. 2008), and alterations in cardiac function (Venditti and Di Meo 2006).

The effect of hypothyroidism on redox homeostasis is controversial. Recent researchers have reported that hypothyroidism induces oxidative stress which is characterized by an elevation in concentrations of reactive oxygen species (ROS) (Yilmaz et al. 2003; Mancini et al. 2012). However, other studies have demonstrated that hypothyroidism does not change lipid peroxidation levels (Das and Chainy 2001) and oxidative stress biomarkers (Sajadian et al. 2016).

Under homeostatic conditions, there is a balance between antioxidant defense and ROS production (Halliwell and Gutteridge 2015). A disruption to this balance occurs when ROS generation surpasses the cell's capacity to neutralize ROS (Sies 1997). Although ROS are generated under physiological conditions and are known mediators of intracellular signaling cascades, excessive ROS formation results in oxidative stress and can induce oxidative damage to biological macromolecules, especially the plasma membrane (Halliwell and Gutteridge 2015). Antioxidant defense systems contain Cu, Zn-superoxide dismutase (SOD), catalase (CAT), and non-enzymatic molecules like glutathione, thiols, vitamin C and vitamin E (Halliwell and Gutteridge 2015).

Vitamin E is likely one of the most important lipid antioxidant in the human diet. Natural vitamin E consists of a family of 8 different compounds, 4 tocopherols, and 4 tocotrienols. All the tocopherols and tocotrienols are potent antioxidants. Evidence suggests antioxidant supplements such as vitamin E (tocopherols) reduce inflammation and oxidative stress. Inflammatory C reactive protein (CRP) is a biomarker which can be used as a predictor of morbidity and atherosclerosis among cardiovascular patients. Recent studies have shown that inflammatory CRP levels can be lowered via tocopherol supplementation (Soriano et al. 2007).

In vitro and *in vivo* animal experiments have led to the proposal that vitamin E, and α-tocopherol, in particular, possesses protective qualities that might help prevent cardiovascular disease. *In vitro* studies have shown that α-tocopherol can protect against oxidative stress, inflammation, and endothelial dysfunction, all of which are characteristic of atherosclerotic plaque development. For example, in the presence of α-tocopherol in cultured monocytes (Islam et al. 1998) and primary human monocytes, monocyte adhesion to the endothelium can be reduced (Devaraj et al. 1996). Some studies using *in vivo* animal models have demonstrated that vitamin E supplementation possesses protective properties, protecting LDL from peroxidation, thus possibly slowing atherosclerotic plaque development (Reaven et al. 1993). α-tocopherol scavenges through some ROS, thus preventing lipid peroxidation and the early stages of atherosclerosis. This also helps limit the damage resulting from ischemia reperfusion, where excessive ROS are produced (Droge 2002; Sagach et al. 2002).

The effects of vitamin E on oxidative stress in the cardiovascular system of hypothyroid rats have not been studied. Thus, this study attempted to elucidate the effect of vitamin E on redox homeostasis in cardiac and aortic tissues of PTU-induced hypothyroid rats.

Material and Methods

Animals. Twenty-eight male Wistar rats (55–65 g, three weeks old) were kept under standard conditions with a 12 h light/dark cycle at a temperature of 22±2°C. All procedures concerning animal treatment and experimentation were in accordance with the Guiding Principles in the Care and Use of Animals and approved by local Ethical Commission in Mashhad University of Medical Sciences.

Chemicals and drugs. The following drugs were used: propylthiouracil (PTU) and vitamin E were obtained from Sigma Chemical Company (U.S.A). All chemicals were of analytical grade (Merck, Germany).

Study design. The rats were randomly divided into the following groups (n=7 in each group): (1) control, (2) propylthiouracil (PTU)-treated, (3) propylthiouracil+vitamin E-treated (PTU+Vit E), and (4) vitamin E-treated (Vit E). All rats in groups 2 and 3 received 0.05% PTU in their drinking water over the course of 6 weeks. Vitamin E was injected daily (20 mg/kg) in groups 3 and 4 for 6 weeks. At the end of the study, the animals were anesthetized by ether and blood samples were subsequently collected from the retro orbital sinus and the heart and aorta were ablated for biochemical measurements.

Assessment of thyroxine. The blood samples were used to determine thyroid hormone status using the radioimmunoassay method (Daisource, T4-RIA-CT).

Determination of MDA. MDA, used as an index of lipid peroxidation, reacts with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS), and produces a reddish complex which has a peak absorbance at 535 nm. Briefly, 1 ml of supernatant was added to 2 ml of a solution consisting of TBA, trichlo-

roacetic acid (TCA), and hydrochloric acid (HCl). Using a water bath, it was then incubated in boiling water for 40 minutes. The solution was then centrifuged at 1000×g for 10 minutes. The absorbance was read at 535 nm (Ohkawa et al. 1979). The MDA levels were expressed as μM per gram of tissue.

Determination of total thiol levels. Total thiol levels were determined via a spectrophotometric method using Ellman's reagent. DTNB (2,2'-dinitro-5,5'-dithiodibenzoic acid) reacts with the SH group found in thiol molecules. The product formed is yellow in hue with a peak absorbance at 412 nm. Briefly, 50 μl of supernatant was added to 1 ml Tris-EDTA (ethylenediaminetetraacetic acid) buffer (pH=8.6) and the absorbance was read at 412 nm against Tris EDTA buffer alone (A1). Then, 20 μl of 10 mM solution of DTNB (10 mM in methanol) was mixed with heart or aorta homogenate and the absorbance was read again (A2). The absorbance of DTNB reagent was also read as blank (B). Total thiol was expressed as mM per gram of tissue (Abbasnezhad et al. 2016; Riddles et al. 1983).

Assay of SOD activity. Cu, Zn-SOD activity was measured using the procedure of Madesh and Balasubramanian (1998). A colorimetric assay involves the use of pyrogallol, which undergoes autooxidation to produce a superoxide anion. This anion reduces tetrazolium dye, MTT [3-(4,5-dimethylthiazol-2-yl)2, 5-diphenyltetrazolium bromide] to its formosan. Cu, Zn-SOD inhibits this reaction and its activity can be measured using spectrophotometry measured at 570 nm wavelength. The amount of enzyme causing 50% inhibition in the MTT reduction rate was defined as one unit of SOD activity.

Assay of CAT activity. The Aebi's method was used to measure CAT activity. This assay requires the determination of the rate constant, k, (dimension: s-1, k), of hydrogen peroxide decomposition. The rate constant of the enzyme was determined by measuring the reduction in absorbance at 240 nm per minute (Aebi 1984).

Statistical analyses. All data were expressed as means ± SEM and were compared using one-way ANOVA followed by Tukey's post hoc comparison test. Differences were considered statistically significant when $p<0.05$.

Results

Hypothyroidism was confirmed by measuring thyroxin levels. The PTU group demonstrated a significantly lower thyroxin concentration in their serum compared to that of control (2.44±0.04 vs. 5.34±0.092 μg/dl; $p<0.001$), which confirms the hypothyroid status of PTU-administered rats. The results also showed that the serum thyroxin level in the PTU+Vit E group (3.14±0.22 μg/dl) was significantly lower than in control group ($p<0.05$). Treatment with vitamin E improved thyroxin level (5.60±0.12 μg/dl) compared to PTU group ($p<0.001$) (Figure 1).

Effect of vitamin E on MDA. In the aortic and cardiac tissues of PTU group, MDA levels were elevated compared to the control group ($p<0.001$). Additionally, the groups treated with PTU and vitamin E (PTU+Vit E) and vitamin E (Vit E) showed only a significant reduction of MDA levels in the aortic and cardiac tissues ($p<0.001$). However, there were no statically significant differences seen in MDA levels between the control, PTU+Vit E, and Vit E groups (Figure 2).

Effect of vitamin E on total thiol. Total thiol levels in the aortic and cardiac tissues of PTU group were lower than control group ($p<0.001$), and an elevation of this antioxidant factor was observed in aortic and cardiac tissues of PTU+Vit E and Vit E groups in comparison with PTU group ($p<0.001$). Moreover, there were no statically significant differences found in total thiol levels between control, PTU+Vit E, and Vit E groups in aortic tissue. However, in cardiac tissue the total thiol in PTU+Vit E group was significantly lower than control group ($p<0.01$). Total thiol levels were also elevated in Vit E group compared to the control group ($p<0.001$) (Figure 3).

Effect of vitamin E on SOD activity. The results demonstrated that the activity of SOD in PTU group

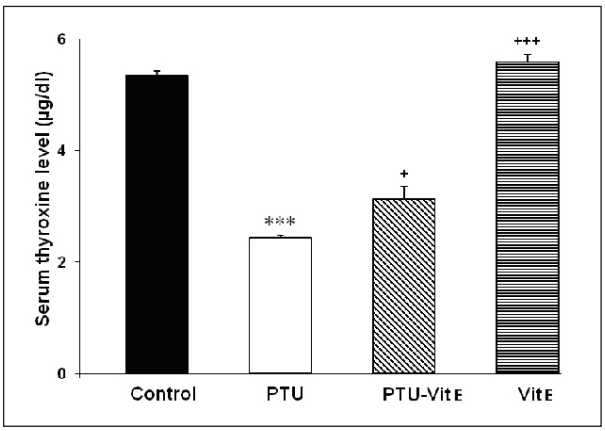

Figure 1. The serum thyroxine level in control (C), propylthiouracil (PTU), propylthiouracil+vitamin E (PTU+Vit E) and vitamin E (Vit E) groups. Data are presented as mean ± SEM, n=5. ***$p<0.001$ vs. control group; +$p<0.05$, +++$p<0.001$ vs. PTU group.

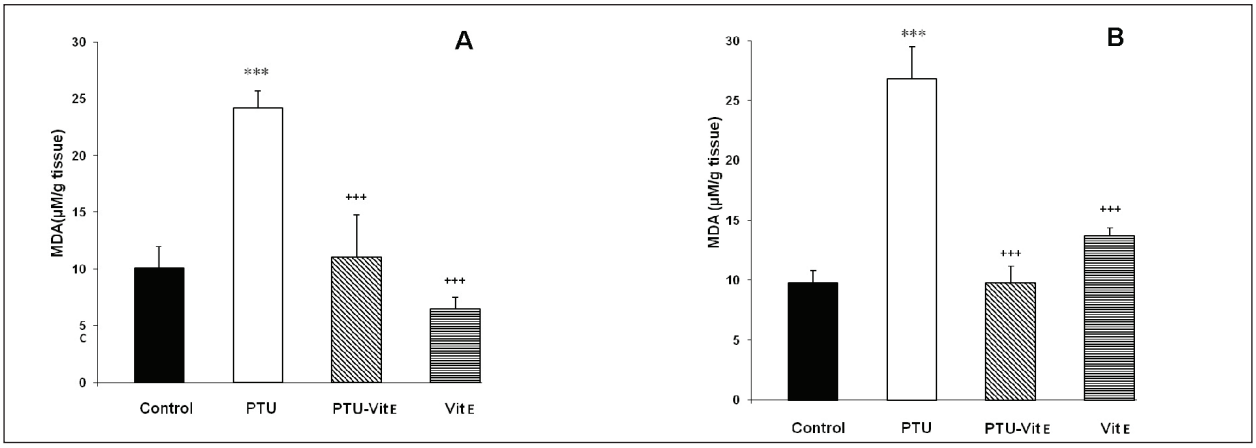

Figure 2. The malondialdehyde (MDA) levels of aortic (a) and cardiac (b) tissues in control (C), propylthiouracil (PTU), propylthiouracil+vitamin E (PTU+Vit E) and vitamin E (Vit E) groups. Data are presented as mean ± SEM, n=7. ***p<0.001 vs. control group; +++p<0.001 vs. PTU group.

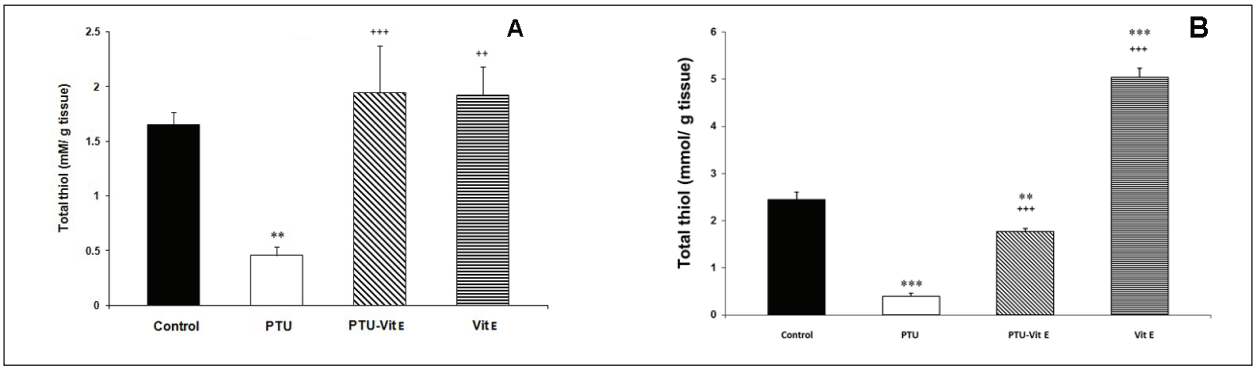

Figure 3. The total thiol levels of aortic (a) and cardiac (b) tissues in control (C), propylthiouracil (PTU), propylthiouracil+Vitamin E (PTU+Vit E) and vitamin E (Vit E) groups. Data are presented as mean ± SEM, n=7. **p<0.01 and ***p<0.001 vs. control group; ++p<0.01 and +++p<0.001 vs. PTU group.

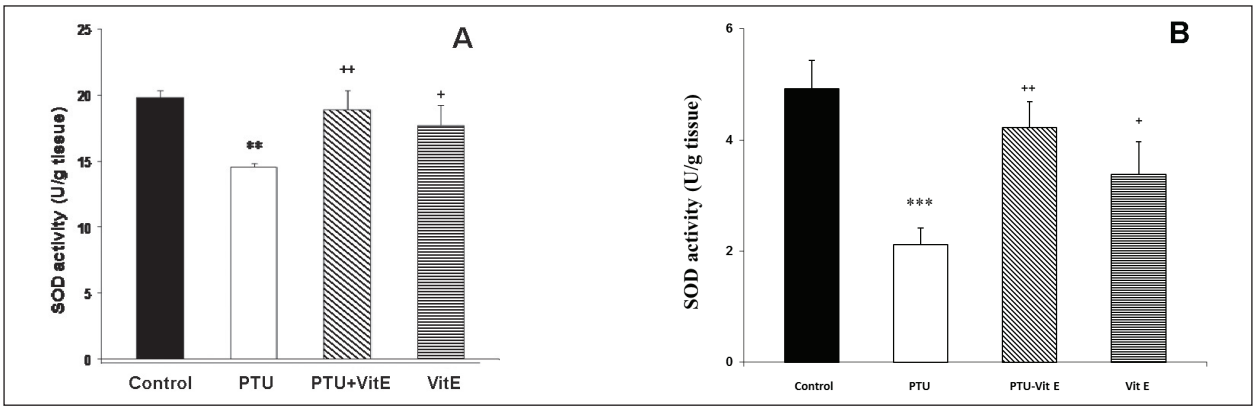

Figure 4. The superoxide dismutase (SOD) activity (U/g tissue) of aortic (a) and cardiac (b) tissues in control (C), propylthiouracil (PTU), propylthiouracil+vitamin E (PTU+Vit E) and vitamin E (Vit E) groups. Data are presented as mean ± SEM, n=7. **p<0.01 and ***p<0.001 vs. control group; +p<0.05 and ++p<0. 01 vs. PTU group.

was lower than in the control group in aortic and cardiac tissues (p<0.01 and p<0.001, respectively). SOD activity in aortic and cardiac tissues of PTU+Vit E and Vit E groups were significantly higher than in PTU group (p<0.01 and p<0.05, respectively). However, a significant difference in SOD activity was not observed between Vit E, PTU+Vit E, and control groups (Figure 4).

Effect of vitamin E on CAT activity. The catalase activity in aortic and cardiac tissues of PTU group was lower in comparison to the control group ($p<0.001$). A negligible elevation in catalase activity within aortic tissue in PTU+Vit E in comparison with PTU was observed. However, a significant difference was observed in cardiac tissue ($p<0.01$). CAT activity in cardiac tissue of Vit E group was lower than the control group ($p<0.01$; Figure 5).

Discussion

The results showed that MDA levels, used as an indicator of oxidative damage, were increased and the total thiol levels, an endogenous antioxidant, were decreased in the heart and aorta of PTU-induced hypothyroid rats. Figure 2 clearly demonstrates that vitamin E supplementation effectively reduced MDA levels and increased the total thiol levels in the heart and aorta of hypothyroid rats, which indicates that vitamin E possesses antioxidant properties.

The activity of SOD and CAT of PTU-induced hypothyroid rats were significantly reduced in aortic and cardiac tissues. Vitamin E supplementation improved SOD activity in the heart and aorta in hypothyroid induced rats. The activity of CAT following vitamin E supplementation increased significantly in the cardiac tissue of hypothyroid rats. However, an insignificant elevation was observed in aorta.

Lipid peroxidation (LPO) is triggered by hydroxyl radicals, leading to a series of free radical reactions and ultimately membrane breakage. LPO facilitates the alteration in the protein structure and function and promotes generation of free radicals (Bouderbala et al. 2008). MDA is used as a marker of LPO. Total thiols are composed of both intracellular and extracellular thiols. The total thiol pool constitutes a major portion of the total antioxidants levels within the body and plays major role in the defense against ROS (Jones 2008). Intracellular thiols such as glutathione (GSH) and thioredoxin play an important role in maintaining the highly reduced environment inside the cell (Jones et al. 2000). All varieties of thiol groups are very susceptible to oxidation and are considered one of the most important antioxidants defense systems. When cells are exposed to oxidative stress, thiol groups are the first antioxidants that are consumed (Halliwell and Gutteridge 1990).

MDA is reported to be high in hyperlipidemia, which is a consistent biochemical marker in hypothyroidism (Nanda et al. 2008). A study has shown that MDA levels in subclinical hypothyroid patients was similar to that in normal controls (Kebapcilar et al. 2007), while another study found increased MDA in hypothyroid patients (Konukoglu et al. 2002). Ates et al. (2016) have shown that serum total thiol levels were lower in patients with Hashimoto's thyroiditis than in healthy controls. In accordance with our results, previous studies have shown increased MDA levels in the heart (Messarah et al. 2011), testis and kidney, and decreased GSH level in the testis and kidney in animal models of hypothyroidism (Baltaci et al. 2013).

A spectrum of antioxidants controls the harmful biological effects that free radicals have on lipids, proteins, and DNA. Several enzyme systems, such as superoxide dismutase (SOD) and catalase (CAT), provide enzymatic protection against reactive oxygen species (ROS) (Halliwell and Gutteridge 2015). SOD catalyzes the dismutation of the superoxide

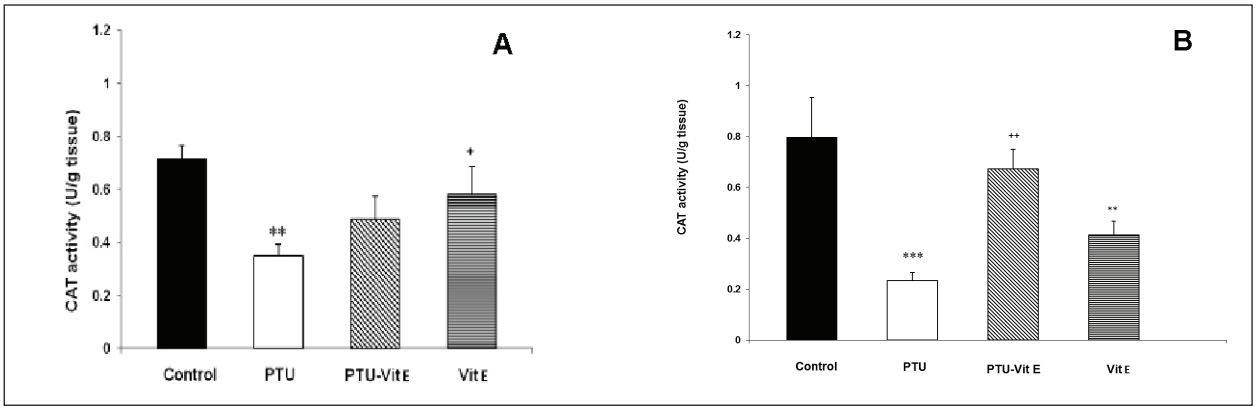

Figure 5. The catalase activity (U/g tissue) of aortic (a) and cardiac (b) tissues in control (C), propylthiouracil (PTU), propylthiouracil+vitamin E (PTU+Vit E) and vitamin E (Vit E) groups. Data are presented as mean ± SEM, n=7. **p<0.01 and ***p<0.001 vs. control group; +p<0.05 and ++p<0.01 vs. PTU group.

anion into hydrogen peroxide (H_2O_2), which is then converted into water (H_2O) by catalase or glutathione peroxidase (GPx) (Lardinois et al. 1996; Torun et al. 2009). In accordance with our findings, previous studies using an animal model of hypothyroidism have demonstrated reduced SOD activity in the hippocampus (Pan et al. 2013; Guo et al. 2014), serum (Pan et al. 2013) and kidney (Jena et al. 2012). Additionally, some studies have demonstrated reduced CAT activity in the kidney (Jena et al. 2012) and liver (Subudhi and Chainy 2012). Jena et al. (2012) have demonstrated that MDA levels increased but SOD activity remained unaltered in the cerebral cortex of PTU-induced hypothyroid rats. Jena et al. (2012) have also reported significantly increased activity of SOD and expression of SOD1 in the cerebral cortex of PTU-induced hypothyroid rats. This study demonstrated that SOD and CAT activity was reduced in cardiac and aortic tissues in the PTU group. These controversies in SOD activity may be related to the specific tissue under investigation in each particular study.

Other studies on vitamin E supplementation in animal models of hypothyroidism have revealed decreased MDA levels in serum (Sarandol et al. 2005) and hippocampus (Pan et al. 2013). Serum GSH levels were also elevated in rats with hypothyroidism (Sarandol et al. 2005). Recently, Jena et al. (2012) have demonstrated that administration of vitamin E to hypothyroid rats resulted in elevated renal CAT gene expression; however, CAT activity was unaltered in response to vitamin E. Guo et al. (2014) have shown that vitamin E in PTU-induced hypothyroid rats increased the SOD activity in hippocampus. Pan et al. (2013) have also demonstrated that vitamin E administration to hypothyroid rats significantly increased SOD levels and decreased MDA levels in serum and hippocampal tissues. All these studies have concluded that vitamin E improves oxidative stress in hypothyroid rats which is in agreement with our current study.

Vitamin E supplementation reduced oxidative stress in hippocampus of PTU-induced hypothyroid rats (Pan et al. 2013; Guo et al. 2014). Vitamin E supplementation increased expression of antioxidant genes: SOD, CAT, glutathione peroxidase (GPx) and glutathione reductase (GR) in PTU-induced hypothyroid rat liver (Subudhi and Chainy 2012). Furthermore, vitamin E supplementation caused an increase in the gene expression of CAT in kidney of PTU-induced hypothyroid rats; however, the gene expression of SOD, GPx and GR were not affected (Jena et al. 2012). Another study has suggested that vitamin E supplementation in PTU-induced hypothyroid rat enhances oxidative stress parameters and hyperlipidemia (Subudhi et al. 2009). Oxidative stress in hypothyroidism can lead to cardiovascular disease and vitamin E supplementation, which can act as a potent antioxidant, may play a protective role.

Our results showed the CAT and SOD activities in heart of Vit E group were lower than in control group. Recent studies have revealed that vitamin E at higher doses may exert a pro-oxidant activity (Rietjens et al. 2002; Pearson et al. 2006). This may explain the decrease in CAT and SOD activity observed in the cardiac tissues between Vit E and control groups. However, this decrease was statistically not significant. The higher MDA levels in heart of Vit E group in comparison with the control group also indicated higher lipid peroxidation in Vit E group. However, this decrease was statistically not significant. It is possible that the dose, rout of vitamin E administration (injection rather than diet) or duration period of administration (6 week) in our study may be involved in the results. Further studies are needed to clear these results.

Conclusions

The results of the present study demonstrate that PTU-induced hypothyroidism resulted in oxidative stress in the heart and aorta tissues and that the chronic administration of vitamin E to hypothyroid rats decreased the oxidative stress markers in the aortic and cardiac tissues.

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