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Research Article

PROTECTIVE EFFECT OF VITAMIN E TREATMENT ON COLD RESTRAINT INDUCED OXIDATIVE STRESS IN RATS

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ABSTRACT
Free radicals and reactive oxygen species are electrically charged molecules produced spontaneously during normal metabolic processes. They attack and damage the lipids, proteins, nucleic acids and other biologically important molecules. They are neutralized by antioxidant defence systems. Disturbance of oxidant-antioxidant equilibrium and exposure to cold leads to the accumulation of free radicals and oxidative stress which is involved in tissue injury associated with a number of diseases. The present study was done to evaluate the effect of vitamin E supplementation on acute and chronic cold exposure induced oxidative stress in rats. Rats were divided into control, acute and chronic stress groups with and without vitamin E treatment. Tissue total antioxidant concentration and tissue lipid peroxide were measured in all groups. Statistical analysis was done using the computer software program prism version 3.3. Results showed that the total antioxidant levels were decreased in acute cold restraint group as compared to control while there was a significant increase in antioxidant levels in vitamin E treated group. Chronic cold restraint produced non-significant decrease of total antioxidant levels as compared to control but there was a significant increase of total antioxidant levels in chronic cold restraint rats administered with vitamin E. It was also found that the chronic cold group with vitamin E treatment produced maximum increase in plasma total antioxidant levels above control, acute, chronic groups. The lipid peroxide levels were increased significantly in acute cold restraint group without vitamin E while the values were similar to control in vitamin E treated group. However Vitamin E produced significant decrease in lipid peroxide levels in chronic cold restraint group.

Key Words: *Stress, Enzymes, Metabolism, Temperature, Animal Experimentation*

INTRODUCTION
Free radicals and reactive oxygen species are continuously produced by the cells as a part of metabolic processes. These are electrically charged molecules which spontaneously attack lipids, proteins, nucleic acids and other biologically important molecules (Davydov and Shvets, 2001).
These free radicals are neutralized by a well established antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and numerous non-enzymatic antioxidants, including vitamins A, E and C, glutathione, ubiquinone, and flavonoids (Urso and Clarkson, 2003). An imbalance between free radical generation and various antioxidant defense systems leading to the accumulation of free radicals leads to oxidative stress (Elewa *et al.,* 2012).
Free radicals and oxidative stress are involved in aging, tissue injury and a number of diseases including rheumatoid arthritis, atherosclerosis, immunological disorders, cataract, diabetes mellitus, cancer, hepatic diseases, psoriasis, and ischemia-reperfusion injury of the heart, brain, pancreas, and other organs (Abd Ellah, 2011).
Exposure of the whole body to cold environment is reported to reduce cognitive performance in terms of memory, vigilance and concentration (Palinkas, 2001; Pilcher *et al.,* 2002). Previous studies have indicated that there is an elevation of BMR (Selman *et al.,* 2002), an imbalance in the oxidant-antioxidant

defence system (Kaushik and Kaur, 2003), an increased generation of free radicals and production of oxidative stress (Sohal *et al.*, 2000) as a result of whole body cold exposure.

Vitamin E is a lipid-soluble antioxidant that can react directly with the free radicals, including hydrogen peroxide and superoxide produced by oxidative stress (Asha and Ravi, 2004). Moreover, another study by Al-Attar (2011) suggested that vitamin E might be a useful in preventing the body against the toxic effect of heavy metals at least partly due to its antioxidant properties (Al-Attar, 2011).

Malondialdehyde which is the end product of lipid peroxidation is a good marker of free radical-mediated damage and oxidative stress (Burke, 2007) and it has been reported by previous studies that vitamin E supplementation in the cold-exposed rats leads to reduced levels of MDA (Asha *et al.*, 2012).

This study is designed to evaluate the effect of vitamin E supplementation on cold restraint induced acute and chronic oxidative stress in rats.

MATERIALS AND METHODS

Animals Model

Adult male albino rats of Sprague-Dawley strain (n=90), weighing 200-250 grams at the beginning of the experiment were purchased from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdul Aziz University, Jeddah, Saudi Arabia. The experimental animals were acclimatized to the laboratory conditions for two weeks. The rats were housed individually under standard conditions of temperature and humidity and a 12-h light/dark cycle (lights on at 08:00 a.m.) with free access to food and water. All animals were maintained under constant conditions for 14 days before stress. All experiments were performed during the same time of day, between 9 AM and 3 PM to avoid variations due to diurnal rhythms (Noriyoshi *et al.*, 2000). All of the procedures regarding the care and use of animals and animal experimentation in this study were conducted in accordance with ethical guidelines of the Animal Care and Use Committee of King Abdulaziz University.

Animal Groupings / Treatment

The rats were divided into five sub-groups: a) Control, b) Acute cold restraint, c) Acute cold restraint with vitamin E treatment, d) Chronic cold restraint, and e) Chronic cold restraint with vitamin E treatment. Rats in control group were left in animal house without exposure to any form of stress. Acute cold restraint was induced by fixing the four limbs to a wooden board and placing it in a refrigerator at 4°C for six hours one time while chronic cold restraint was induced by placing the board once daily for 9 days (Zaidi *et al.*, 2005). Vitamin E (50 IU/kg of body weight) was administered orally prior to and after 6 hours of restraint stress exposure one time in acute groups and once daily for 9 days in chronic groups. Control, Acute and Chronic stress groups without treatment were given distilled water (DW) orally (3 ml/kg body weight) for the same duration accordingly (Zaidi and Banu, 2004).

Collection of Samples

Animals were killed immediately using sodium pentobarbital after the last session of immobilization (still in the restrainer). A weighed portion of the liver and brain were put in a test tube containing 5 ml of acidified butanol. The mixture was homogenized in a conical tube immersed in ice. The homogenate was centrifuged at 1000 rpm for 5 min. Then 2.5 ml of the supernatant was transferred to a test tube and used for measurements (Zaidi *et al.*, 2005).

Biochemical Measurements

Determination of Tissue Total Antioxidant Concentration: Total antioxidant concentration was assayed by spectrophotometer. One ml of the chromogen is added to 20µl of double deionized water to form the reagent blank, and one ml of chromogen to the 20µl of standard to prepare the standard. Then samples are measured after addition of one ml of chromogen to the 20µl of sample. Initial absorbance was measured. Then 200µl of diluted substrate was added to each sample, standard and blank. Mixture was measured after exactly 3 minutes.

Determination of Tissue Lipid Peroxides: The total amount of lipid peroxides was assayed by the thiobarbituric acid method described by Okhawa *et al.*, (1979). This measures the malondialdehyde

equivalent substances. MDA (malondialdehyde) is a major secondary breakdown product of LPO (lipid peroxides) which reacts with TBA (thiobarbituric acid) to generate a coloured product with maximum absorption at 532 nm.

Statistical Analysis of Experimental Data

Statistical analysis was done using the computer software program prism version 3.3. Data are expressed as mean ± SEM. Statistical significance for data was determined using a one-way analysis of variance (ANOVA) with Bonferroni's Multiple Comparison Test to find inter-group significance. Statistical significance was fixed at $P<0.05$ while a value of $p<0.001$ was considered highly significant.

RESULTS AND DISCUSSION

Results

The mean± SE values of total antioxidant and lipid peroxide levels of all groups are represented in table 1. The findings showed that the acute cold restraint decreased the total antioxidant levels below the control but there is a significant increase in antioxidant levels of the group in which vitamin E is administered as compared to control. On the other hand, chronic cold restraint produces non significant decrease of total antioxidant levels below control levels. There is a significant increase of total antioxidant level in chronic cold restraint rats treated with vitamin E compared to chronic cold restraint rats not given vitamin E. Overall, the increase in plasma total antioxidant levels was highest in chronic cold restraint group treated with vitamin E as compared to control, acute, chronic groups.

Regarding the lipid peroxide levels, result show that the lipid peroxide levels are increased significantly in acute cold restraint group without vitamin E as compared to control. Administration of vitamin E produces a significant decrease in lipid peroxide levels in acute cold restraint group. On the other hand, lipid peroxide levels show a non significant increase in chronic cold restraint group as compared to control which is reduced significantly when vitamin E is administered.

Table 1: Effects of vitamin E administration on total antioxidant (mmol/l) and lipid peroxide (mean ±SE) levels in cold restraint induced acute and chronic stress compared with control non-stressed group

Parameter	Total Antioxidant (mean ±SE)	Lipid Peroxide (mean ±SE)
Control	$5.04 \pm 0.71^{\#o}$	$0.04 \pm 0.003^{\#o}$
Acute cold restraint	$2.01 \pm 0.91^{\#o}$	$0.18 \pm 0.006^{\#o}$
Acute cold restraint with vit. E	$3.82 \pm 0.10^{\#*o}$	$0.05 \pm 0.006^{\#*o}$
Chronic cold restraint	5.01 ± 0.53^{o}	$0.05 \pm 0.02^{\#*}$
Chronic cold restraint with vit. E	$5.98 \pm 0.20^{\#*o}$	$0.01 \pm 0.005^{\#*o}$

*$^{\#}$Significant with control, *significant with acute cold restraint, ^{o}significant with chronic cold restraint*

Discussion

The data from the results show that the total antioxidant levels are significantly decreased in acute cold restraint stress group as compared to control group while these changes are not significant in the chronic stress groups. These findings are in accordance with previous studies by Zaidi *et al.,* (2005), Hussain *et al.,* (2009) and our past study (Elewa *et al.,* 2012).

Studies by Kaushik and Kaur (2003) and Sohal *et al.,* (2000) also reported an imbalance in the oxidant-antioxidant defence system and an increased generation of free radicals and production of oxidative stress by cold restraint. Moreover, a study by Vaz *et al.,* (2011) explained that hormones released by acute stress

(glucocorticoids and catecholamines) produce metabolic changes in the enzyme activities of glucose and phosphate pathways. Thus a decreased activity of glucose 6- phosphate dehydrogenase leads to diminished production of NADPH (glucose-6-phosphate dehydrogenase regenerates NADPH) and eventually the decreased blood antioxidant levels. Another possible mechanism is that, under normal circumstances, there is an equilibrium of $O^{\cdot}_2$ free radical generation and antioxidant activity of enzyme systems of the body. In acute stress, this equilibrium is disturbed so that the production of reactive species overwhelms the capacity of the endogenous antioxidant defense system to remove the excess free radicals. Such imbalance results in $O^{\cdot}_2$ mediated damage (Dede *et al.,* 2002).

In the present study, there is a significant increase in lipid peroxides levels in acute cold restraint stress groups as compared to control group. This finding also is in accordance with our previous study (Elewa *et al.,* 2012), and some other similar studies (Zaidi *et al.,* 2005; Hussain *et al.,* 2009). Cold stress causes the release of reactive oxygen species in cell membrane leading to the production of lipid peroxide (Dede *et al.,* 2002). The increase of free radicals during acute cold stress is associated with a decrease in mitochondrial respiratory control, loss of sarcoplasmic reticulum integrity, and increased levels of peroxidation products and lipid peroxidation (Concepción *et al.,* 2004). Our findings also show a non-significant increase in lipid peroxides levels in chronic cold restraint stress group which was previously confirmed by McEwen (2007) who reported that cessation of chronic stress returns the body to its original equilibrium. Lipid peroxide determination is an important parameter for the evaluation of oxidative damage, since membrane phospholipids are the most important target (Katz *et al.,* 1996). Burke (2007) reported that Malondialdehyde (MDA), the end product of lipid peroxidation, is a good marker of free radical-mediated damage and oxidative stress which is the basis for our methodology.

As evident by table 1, vitamin E supplementation significantly decreased the lipid peroxide levels and increased the antioxidant levels as compared to positive controls. These findings are well endorsed by the previous study by Venditti *et al.,* (2011). They found that cold exposure increased and vitamin E reduced the levels of hydroperoxides in liver homogenates and mitochondria.

The possible mechanism involved in antioxidant activity of vitamin E is not only limited to a direct action on cellular lipid and protein components, but also through a reduction in reactive oxygen species generation by muscle mitochondria (Venditti, 2009) scavenging them, and by promoting their decomposition.

Conclusion

This study reinforces the antioxidant activity of vitamin E in cold restraint induced oxidative stress. Further studies can be done to decipher the precise and comprehensive pharmacological basis of therapeutics of vitamin E and other vitamins in myriad disorders related to oxidative stress.

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