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Intelligible and augmented influence of DL-α-tocopheryl acetate on lipid peroxidation and antioxidant status in various rat tissues

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ABSTRACT

Exposure of cells to everyday wear and tear inside the body caused by the reactions essential for aerobic life leads to the formation of reactive oxygen species (ROS) that are associated with cytotoxicity. Vit-E is the natural most effective lipid soluble antioxidant, which protects biological membranes and lipoproteins from oxidative stress. Therefore, the present study concentrates on the potency of Vit-E to reverse the changes caused by free radicals in Brain, Heart, Lung and Pancreas oxidative system. Swiss albino rats were orally administered with Vit-E (50mg / kg BW) alone via gavage, every other day for 12 days. The results revealed that treatment with Vit-E significantly reduced the oxidative changes in some parameters examined. Comparing the Vit-E supplemented group with the saline controls, no significant difference could be detected in brain, lung and heart SOD activities but pancreatic SOD level was found to be higher when compared to controls ($p<0.02$). TSH activity was found nonsignificant in all the tissues on comparing to controls. LPO was reversed in pancreas ($p<0.001$), but no change was detected in brain, lung and heart. CAT activities were seen to be higher in brain ($p<0.05$), but no change was analyzed in other tissues in comparison to controls. In conclusion oxidative stress, especially LPO may play an important role in tissue degeneration process and Vit-E may play an important role in eliminating oxidative damage as an effective antioxidant.

KEYWORDS

Brain;
Catalase;
Heart;
Lipid peroxidation;
Lung;
Pancreas;
Superoxide dismutase;
Total sulfhydryl.

INTRODUCTION

Oxidative stress is defined as a disruption of the prooxidant-antioxidant balance in favor of the former, leading to potential damage[1]. It is a result of one of the three factors: an increase in reactive oxygen species (ROS), an impairment of antioxidant defense systems or an insufficient capacity to repair oxidative damage. Damage induced by ROS includes alterations of cellular macromolecules such as membrane

lipids, DNA, and/or proteins. Oxidative stress in biological systems engenders as the repercussion of disproportion between the procreation of oxidizing species and cellular antioxidant defenses[2-4]. Under normal conditions, excessive formation of free radicals and concomitant damage at cellular and tissue concentrations is controlled by cellular defense systems. Copious enzymatic and non-enzymatic mechanisms including Vit-e and glutathione take place to escort the the cell in contrast to oxidative damage[4]. The antioxidant enzymes such as TSH, SOD and CAT may also have an important function in mitigating the toxic effects of ROS[5]. The radical chain reaction of lipid peroxidation looms to be an incessant physiological operation. This course if out of sway, can alter quintessential cell functions through changes in intracellular calcium or intracellular PH, and eventually can lead to cell demise[6-9]

Superoxide dismutase (SOD), Catalase and Total sulfhydryl (TSH) are the enzymes that provide cellular protection against the damage caused by free radicals and reactive oxygen species (ROS). Measurement of these enzyme activities is an indirect and noninvasive method that could be used to assess oxidant stress[10-12].

A grave benefactor to non-enzymatic bulwark against lipid peroxidation is Vit-e (α-tocopherol),a known free radical scavenger[4,13]. These are naturally occurring antioxidants that play important roles in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stressors. The antioxidant function of these micro-nutrients could, at least in part, enhance immunity by maintaining the functional and structural integrity of important immune cells[14,15]. It is well chronicled that Vit-e retaliate with lipid peroxyle radicals to form Vit-e radicals that are inapt of abstracting H˙ from the membrane lipids. The Vit-e radical then acts as a chain-terminator by barging chain reactions during lipid peroxidation[16] via its ability to form stable para-quinone[17]. Apart from scrounging property, Vit-e is also helpful in maintaining antioxidant status and in detoxification systems[18,19]. Vit-e is hauled in association with lipoproteins in the blood and taken up by the central nervous system through the blood-brain barrier[20]. Since the discovery of Vit-e in 1922 by H.M.Evans, when it was first described as an anti-sterility agent, many scientists and physicians have sought to elucidate its bio-chemistry, health benefits and clinical application[21].

Recently, Vit-e is being widely probed due to its action against oxidative stress[22-24], its protective role in biological membranes[25] and also related to its effect on impeding the manifestations of aging[26]. In the tissue of Vit-e deficient animals, it is divulged that lipid peroxidation is intensified suggesting that Vit-e plays a role as a physiological antioxidant based on its chemical properties[27]. Because of the health problems induced by many environmental pollutants, much efforts have been expended in evaluating the relative antioxidant potency of vitamin E[28,29].

Since it is known that Vit-e plays an important role in brindling the lipid peroxidation and is benevolent in perpetuating the antioxidant status, present study was contrived to appraise the protective role of low dose of Vit-e via measurement of free radical scavenging enzymes like superoxide dismutase (SOD), total sulfhydryl (TSH), catalase (CAT) and lipid peroxidation in various rat tissues.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used were of the highest purity available. Vitamin E (53% α- tocopherol acetate) manufactured by Codislait Sarl, 22120 Y ffiniac.

Animals

The animals care and handling was done according to the guidelines set by the institutional ethics committee, J.N.Medical College, AMU Aligarh. Twelve Male swiss albino rats weighing 180-200g obtained from central animal house, J.N.Medical College, AMU, Aligarh were used in the present experiment. Rats were housed in plastic cages, 3 per cage under standard conditions with free access to drinking water and basal diet. The animals were acclimatized to laboratory conditions for 7 days before use and were maintained in a room with controlled temperature (20-22ºC), relative humidity (50%) and 12 h light/dark cycle.

Treatment

Vitamin E dose was 50 mg / kg body weight every

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other day. This dose was used because of the previous studies of Alper[30], showed 50 mg vit E / kg BW was effective in enhancing renal and cardiac catalase activities in rats.

Experimental design

At the beginning of the experiment, rats were randomly divided into two groups of six rats each. The IST one was the untreated control group which received normal saline 0.2 ml per animal per day through gavage once a day. The 2nd group was treated with Vit- E (α-tocopherol) alone.

The total treatment time was 12 days. At the end of the experiment, animals were sacrificed by cervical dislocation. Brain, lung, heart and pancreatic tissues were removed and immediately rinsed in ice cold saline. Then homogenized with Teflon pestle glass homogenizer in 10% w/v ice cold 0.05M potassium phosphate buffer (PH 7.4) and the tissue homogenate was further processed accordingly to be used for the analysis.

Biochemical analysis

LPO activity was determined according to the method of Okhawa[31] based on the thiobarbituric acid (TBA) reaction with malondialdehyde (MDA) formed owing to the peroxidation of lipids. Superoxide dismutase (SOD) was assayed by the ability of the enzyme to inhibit autoxidation of pyragallol by the method of Marklund and Marklund[32]. Catalase (CAT) was assayed by the decomposition of H_2O_2, by the methods of Aebi[33]. Total sulfhydryl (TSH) content was estimated by the method of Sedlack and Lindsay[34], based on the development of a stable yellow color with 5',5' dithio,bis 2-nitro benzoic acid (DTNB, Ellman's reagent). Protein concentration was determined according to Lowry[35]. LPO and TSH were expressed as nmol/g tissue and μmol/g tissue respectively while as SOD and CAT were expressed as u/mg protein.

Statistical analysis

The results were expressed as mean ± SEM. All obtained results were compared with respect to the control animals (C), in order to elucidate a possible protective role of Vit E. Differences between means of control and treated rats were analyzed by using Student's t-test. The accepted level of significance in all the cases was $p<0.05$, $p<0.02$ highly significant and $p<0.001$ very highly significant. Student's t-test was done by using SPSS package program, version 10.01, SPSS, Chicago, IL.

RESULTS

TABLE 1 displays the effect of vitamin E (ά-tocopherol) on antioxidant enzymes activities and malondialdehyde concentration (as indicator for lipid peroxidation) in Brain, Heart, Lung and Pancreas of rats

TABLE 1 : Effect of vitamin E acetate on Brain, Lung, Heart and pancreas superoxide dismutase (SOD), Catalase (CAT), Total sulfhydryl (TSH) activities and malondialdehyde (MDA) concentration of rats as compared to control.

Tissues & groups	(SOD) (U/mg protein)		(CAT) (U/mg protein)		(TSH) (μmol/g tissue)		(LPO) (nmol/g tissue)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Brain (C)	5.39	0.47	6.99	0.41	47.86	5.46	16.87	1.93
(Vit E)	7.67	2.15 (42.30)	11.46	2.37 (63.94)***	49.93	9.98 (4.32)	16.44	2.18 (-2.54)
Lung (C)	6.99	1.52	1.88	0.23	55.30	10.84	4.99	0.73
(Vit E)	7.01	0.47 (0.28)	2.41	0.65 (28.19)	57.88	4.98 (4.66)	4.27	0.74 (-14.42)
Heart (C)	9.60	1.91	4.12	0.80	55.71	6.65	7.25	2.50
(Vit E)	10.35	1.14 (7.81)	4.57	1.26 (10.92)	63.03	6.82 (13.13)	5.97	1.21 (-17.65)
Pancreas (C)	7.16	1.00	2.83	0.73	39.46	5.94	5.50	0.86
(Vit E)	11.85	1.11 (65.50)**	4.48	0.68 (58.30)	43.45	8.56 (10.11)	3.64	0.60 (-33.81)*

Values represent Mean and SEM of six rats per group in brain, lung, heart and pancreas. Paired samples t-test was performed to compare the parameters of control (C) and Vit-E acetate (Vit E) groups. Values: *** p<0.05; ** p<0.02; * p<0.001 are significant. Figures in parentheses indicate percent decrement compared to controls.

In comparison to the controls no significant difference could be detected in brain, lung and heart SOD activities of Vit-e group (TABLE 1). But SOD activity of pancreas was found higher in Vit-e group when compared to control rats (65.50%, $p<0.02$). Interestingly, TSH remained unaltered in all the tissues of α-tocopheryl acetate group compared to controls. The rate of lipid peroxidation show a prominent decrease in pancreas (33.81%, $p<0.001$) but remained unchanged in all other tissues of Vit-e administered group. Also the higher CAT activity was noticeable in the brain (63.94%, $p<0.05$) but manifest no significant differences in other tissues of Vit-e supplemented group with respect to controls. The increase in pancreatic SOD activity is demonstrated in figure 3, while increase in brain tissue CAT activity is demonstrated in figure 4.

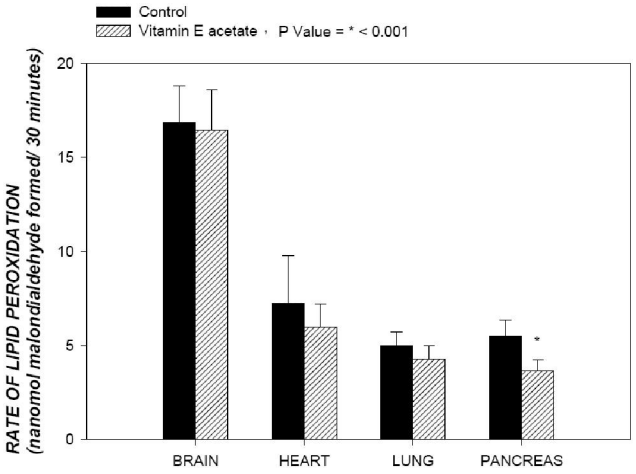

Figure 1 : Effect of vitamin E acetate on the rate of lipid peroxidation in different tissues of rat. Values represent ± SEM

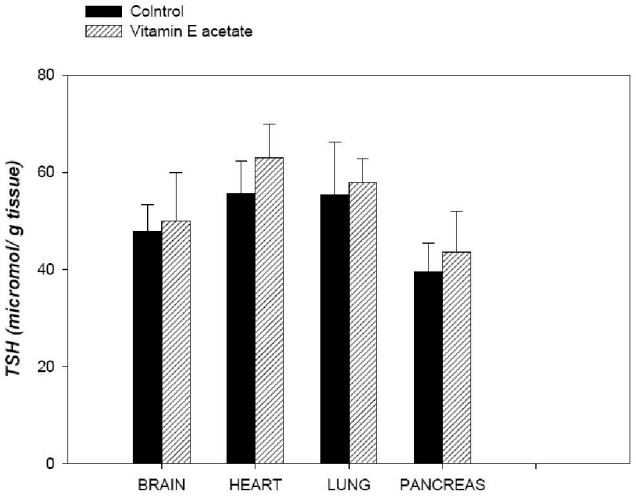

Figure 2 : Effect of vitamin E acetate on total sulfhydryl (TSH) activity in different tissues of rat. Values represent ± SEM.

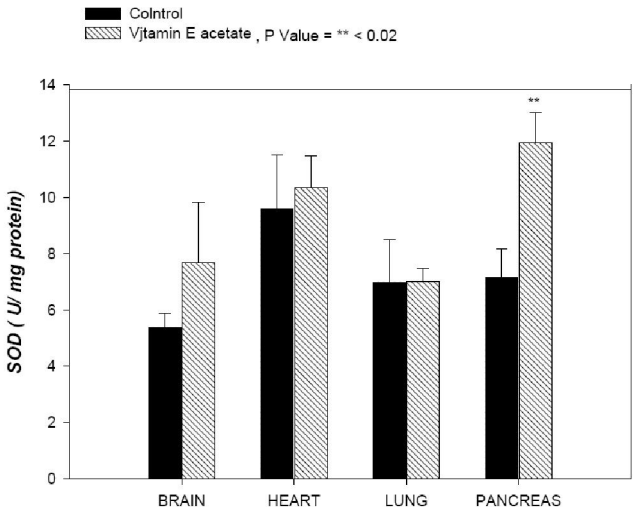

Figure 3 : Effect of vitamin E acetate on superoxide dismutase (SOD) activity in different tissues of rat. Values represent ± SEM.

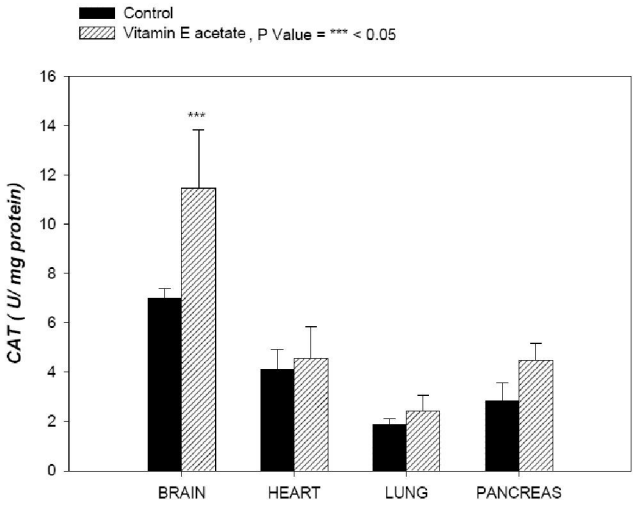

Figure 4 : Effect of vitamin E acetate on catalase (CAT) activity in different tissues of rat. Values represent ± SEM.

DISCUSSION

Epidemiological studies provide increasing evidence related to the importance of the human antioxidant defense system in assessing the risk of chronic and degenerative diseases. In recent years, several such investigations have proved strong circumstantial evidence for the beneficial effects of Vit-e and have shown a highly significant correlation between lower risk to ischemic heart disease mortality and higher plasma Vit-E levels. Beneficial effects of Vit-e supplementation on human health are also noted in various chronic diseases and some acute clinical conditions[21].

Recently, it has become clear that, Vit-e is also

necessary for the maintenance of normal neurological structure and function. In vivo, Vit-e is the only-well recognized, lipid soluble, chain- breaking antioxidant and may therefore be expected to play an important role in protecting lipid rich structures such as the brain from free radical damage[36]. It has been reported that enrichment of brain membranes with Vit-e by dietary supplementation provides a higher protection of brain membranes against free radical oxidation[37].

Chaudière J[38] reported that despite the fact that Vit-e concentration was 12 times lower in the brain of Vit-e deficient rats; no significant change in CAT activity in cerebral tissue was found between the controls and Vit-e deficient groups. These results suggest that the central nervous system (CNS) is still substantially protected when its Vit-e content has been decreased to 3 μg/g fresh weight[38].

In a study Matsuo M[39] reported that, since the Vit-e concentration in brain was found fairly stable, there might be a mechanism by which brain antioxidant capacity is maintained and optimized despite the possible influence of oxidative stress. In concordance with the results mentioned above, in our study CAT activities were found higher in brain of Vit-e group in respect to control animals (TABLE 1) but no significant change could be seen in lung, heart and pancreatic tissues of treated group. So here in our case brain antioxidant capacity is optimized by Vit-e.

According to Tappel[40], biomembranes and subcellular organelles are the major sites of lipid peroxidation damage. The peroxidative changes triggered by free radical in brain fatty acids and phospholipids may be of importance in the development of brain cell damage. Free radicals, i.e. highly reactive molecules with an unpaired electron in an outer orbital, are constantly being formed in various reactions essential for aerobic life. The best studied effect of free-radical attack is that causing lipid peroxidation i.e. oxidation of α-methylene bridges of unsaturated fatty acids, resulting in the formation of lipid peroxides and hydroperoxides, finally leading to fragmentation of lipids. As biomembranes are rich in unsaturated fatty acids, such reaction may lead to the disintegration of membrane structure and finally to irreversible cell damage. Vit-E (α-tocopheryl acetate) is a well known antioxidant and reported to stabilize plasma membranes[41] as well as lysosomal membranes[42] and mitochondrial membranes[43]. Discussing our results with respect to brain tissue LPO activity, no significant variation could be noted in the Vit-E supplemented rats. This might be due to low dose of Vit-E, insufficient to cause reversible change in brain LPO of treated rats. As to the TSH and SOD of brain of Vit-E group, here also no change could be seen in respect to controls may be due to the same reason mentioned above. Interpretting our findings with respect to lung, heart and pancreas, it was noted that LPO and antioxidant enzymes showed no change in lung tissue of Vit-E inoculated rats. Matsuo M[39] also could not detect any change in Vit-E deficient rats. Discussing about the heart LPO and antioxidant status, here also no change was found in treated group. L. Packer[21] indicates that, there is a highly significant correlation between ischemic heart disease, angina pectoris and low plasma Vit-E levels. He stresses that Vit-E is inversely related to the risk of angina, independent of the other antioxidants. These observations which are in concordance with our results postulate the fact that long term and high dosage Vit-E administration might be beneficial in patients carrying the risk of coronary heart diseases. Pancreatic tissue manifests a prominent variation in LPO and SOD activities in Vit-E supplemented rats. Thus it is not unreasonable to postulate that the protective effect of α-tocopheryl acetate is affected by its free radical scavenging, antioxidative and membrane stabilizing effects.

Based on the fact that it usually takes several days or weeks to substantially increase the Vit-E content of membranes[21] and also taking into consideration the marked influences of the health status, life style and environmental on the requirements of the organism for Vit-E[21], therefore Vit-E supplementation sufficient to protect the organism from toxic agents and free radical damage is a time consuming process. It is concluded that Vit-E is an essential component of the pancreas for the protection of this tissue against peroxidative damage even at the low dose. Since Vit-E is the least toxic among fat soluble vitamins and no toxicity has been observed at high doses[44], our results indicate that long term and high dose Vit-E supplementation may be beneficial in the prevention of brain, heart and lung toxicity.

CONCLUSION

The present study demonstrated that treatment with vitamin E acetate caused a significant decline in the levels of MDA and an increase in SOD activity in pancreas and Catalase activity of the brain was also elevated by the antioxidant as compared to control animals. The observed levels of these parameters were found higher than the normal values of the control (TABLE 1). This postulates that Vitamin E acetate proved to be beneficial in decreasing the levels of free radicals in the above mentioned organs concluding that using diets rich in vitamin E acetate on regular basis could be beneficial in alleviating oxidative stress in Pancreas and Brain even at low doses, but for heart and lung this dose level was found low to reverse the changes caused by reactive oxygen species thus confirming that for these organs Vit E intake should be higher than 50 mg / kg BW per day in order to enhance the activity of endogenous antioxidant enzymes as well as for decrement in MDA.

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