

EFFECT OF VITAMIN E ACETATE SUPPLEMENTATION ON THYROID HORMONE-SENSITIVE ORGANS FOLLOWING EXOGENOUS L- THYROXINE TREATMENT

SABYASACHI SINHA, ARIJIT CHAKRABORTY, CHIRANJIT MONDAL, AMAR K CHANDRA*

Department of Physiology, Calcutta University, Kolkata - 700 009, West Bengal, India. Email: physiology.ac@gmail.com

Received: 02 November 2017, Revised and Accepted: 21 December 2017

ABSTRACT

Objective: L-thyroxine is used for control and prevention of many thyroidal diseases, though it may cause damages in thyroid hormone-sensitive organs, namely, liver and kidney. Reports on the protective effects of any antioxidants in L-thyroxine induced oxidative stress are scanty. Thus, L-thyroxine induced oxidative stress and its prevention by Vitamin E supplementation have been studied in the present investigation.

Methods: Adult, male Wister rats were divided into four groups of six animals each, and L-thyroxine (T_4) (0.3 mg/kg body weight) was administered intraperitoneally in the treated group. Similarly, L-thyroxine (T_4), at the above-mentioned dose, and Vitamin E acetate (100 mg/kg of body weight/day orally) coadministered simultaneously (T_4+V_E) in the next group. Third group was administered only with Vitamin E, and the remaining group kept as control. Treatment continued regularly for 15 and 30 days. Animals were sacrificed after completion of treatment. Lipid peroxidation (LPO) level, superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) activities were assayed in liver and kidney along with their histology. Obtained results were interpreted statistically against their respective control groups.

Results: Body weight was significantly decreased and relative kidney weight was increased after L-thyroxine administration as compared to control ($p < 0.05$). LPO level, SOD and catalase activities were significantly enhanced in L-thyroxine treated groups, whereas GPx activity was decreased. However, LPO level and the activities of those enzymes along with body weight and organ weights were almost restored their normal in L-thyroxine and Vitamin E coadministered group treated for 15 days and 30 days, respectively.

Conclusion: Exogenously administered L-thyroxine causes oxidative stress in liver and kidney that in turn generates reactive oxygen species resulting cell damages. Vitamin E acetate supplementation reduces these adverse effects on liver and kidney and thus acts as a beneficial health management agent.

Keywords: Catalase, Glutathione peroxidase, Lipid peroxidation, L-thyroxine, Reactive oxygen species, Superoxide dismutase, Thyroid hormone-sensitive organs, Vitamin E.

© 2018 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2018.v11i4.23415>.

INTRODUCTION

Now a days, use of antioxidants is a general health management criterion in different diseases as well as during stress conditions. Human-made or natural substances used as antioxidants may prevent or delay stress and cell damages. Antioxidants are generally found in many foods stuff, including fruits, vegetables, and natural products. They are also available as dietary supplements. However, identification of certain vitamins, like Vitamin E as antioxidants revolutionized the field and led to the realization of the importance of antioxidants in the biochemistry of healthy, living organisms [1]. Lifestyle habits sometimes produce substances called reactive oxygen species (ROS) that damage healthy living cells and organs [2]. Thus, antioxidants, in general, either prevent these reactive species from being formed or remove them partially before they can damage vital components of the cell [2,3]. Vitamin E refers to a group of eight fat-soluble compounds that include both tocopherols and tocotrienols [4]. Of the many different forms of Vitamin E, α -tocopherol is the most biologically active form of Vitamin E. This variant can be found most abundantly in wheat germ oil, sunflower, and safflower oils [4,5]. As a fat-soluble antioxidant, it stops the production of ROS formed when fat undergoes oxidation [6,7]. Vitamin E has many biological functions, the antioxidant function being the most important and best known [8]. As an antioxidant, Vitamin E acts as a peroxyl radical scavenger, preventing the propagation of free radicals in tissues, by reacting with them to form a tocopheryl radical that is reduced by a hydrogen donor and return to its reduced state [9]. Due to its solubility, it is incorporated into cell membranes, which protects them from oxidative damage.

Prolonged administrations of exogenous L-thyroxine on thyroid as well as different thyroid-sensitive organs produce free radicals. The present investigation has been thus undertaken to evaluate the ameliorating effect of Vitamin E on L-thyroxine-induced oxidative damages in different thyroid hormone-sensitive organs of experimental animals.

METHODS

Reagents

L-thyroxine (T_4) (T-2501) was purchased from Sigma-Aldrich, Germany. Vitamin E acetate (Di-alpha tocopheryl acetate; V_E) was purchased from E-Merck, Mumbai, India. Bovine serum albumin (BSA), thiobarbituric acid (TBA), nicotinamide adenine dinucleotide phosphate (NADPH), Triton X, triethanolamine, diethanolamine, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Company, Steinheim, Germany. Disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), sodium acetate, SODIUM azide, potassium iodide (KI), potassium chloride (KCl), trichloroacetic acid (TCA), glacial acetic acid, hydrogen peroxide (H_2O_2), sucrose, sodium chloride (NaCl), magnesium chloride ($MgCl_2$), hydrochloric acid (HCl), potassium dihydrogen phosphate (KH_2PO_4), ammonium molybdate, sodium bicarbonate (Na_2CO_3), copper (II) sulphate ($CuSO_4$), sodium potassium tartarate, formaldehyde, haematoxyline, eosin, paraffin wax (58°C–60°C), sodium metabisulphite, etc., were purchased from MERCK, Germany. Other necessary chemicals were purchased from SISCO Research Laboratory (SRL), Mumbai, India.

Maintenance of animals

For the present study, 48 mature, adult, male albino rats (*Rattus norvegicus*) of Wister strain weighing about 170 ± 10 g were obtained from register breeder, Kolkata (Registration No. 1443/PO/b/11/CPCSEA). The animals were caged in well ventilated hygienic Terson cages ($25^\circ\text{C} \pm 2^\circ\text{C}$ with plenty of air and light) under 12 h light and 12 h dark cycle. The rats were maintained on a standard diet (20% protein) made of locally available wheat (70%), Bengal gram (20%), fish meal powder (5%), dry yeast powder (4%), refined till oil (0.75%) and shark liver oil (0.25%), and water was provided *ad libitum* [10]. The animals were acclimatized to housing conditions for at least 1 week before experiment and then were divided into following four groups of 6 on each - control, L-thyroxine (T_4) treated, L-thyroxine-Vitamin E- (T_4+V_E) treated and only Vitamin E (V_E) treated groups. L-thyroxine (T_4) treated rats were fed with normal diet with daily administration of 0.3 mg/kg of body weight L-thyroxine [11] intraperitoneally. On the other hand, Vitamin E treated rats were also fed with normal diet along with oral administration of Vitamin E acetate (100 mg/kg of body weight) [12-14] while L-thyroxine- Vitamin E treated group, L-thyroxine was administered intraperitoneally and Vitamin E orally at the above mentioned doses. The treatments as mentioned continued for 15 and 30 days, respectively.

At the end of the experimental periods of 15 days and 30 days, after recording the body weight, animals were sacrificed 24 h after the last treatment following standard protocol and ethical procedure. Just after sacrifice, blood samples for hormone assay were collected from the hepatic portal vein. Liver and kidneys (pair) were taken out after removing the fats; these were weighed and preserved to assay different enzyme activities as well as histological studies mentioned earlier. Approval for the animal study was provided by the Institutional Ethical Committee (IAEC/PROP/AC-3/2010, dated 16.11.2011), Department of Physiology, University of Calcutta.

Histological study

Immediately after the removal, liver lobes and kidney of the rat were fixed in 10% neutral buffered formalin, embedded in paraffin and block was prepared. The sections were cut and stained with Hematoxylin and Eosin and examined under light microscope.

Measurement of lipid peroxidation (LPO) level (in liver and kidney)

It was measured following the method of Ohkawa *et al.* [15] and modifying method of Buege and Aust [16] using TBA-TCA-HCl reagent. The level of LPO in the homogenate was measured based on the formation of TBA-reactive substances. Malondialdehyde (MDA) forms adducts with TBA, which was measured spectrophotometrically at 532 nm against the blank containing 50 mM phosphate buffer (pH 7.4). MDA, a product of LPO, was used as a standard. An extinction coefficient of $156,000 \text{ M}^{-1} \text{ cm}^{-1}$ was applied for calculation.

Assay of catalase activity (in liver and kidney)

Catalase activity was determined following the method of Aebi [17]. The tissue was homogenized with sucrose (0.25 M) solution at 4°C . It was centrifuged, and post-mitochondrial supernatant (PMS) was prepared. 100 μL of tissue sample containing PMS and phosphate buffer (50 mM, pH 7.8) were mixed in a cuvette and decrease in the absorbance was recorded at 240 nm for 5 min over a 60 s interval before the addition of 60 mM H_2O_2 . The change in absorbance was the measure of the catalase activity and was expressed as nmoles/mg of protein/sec. The extinction coefficient of H_2O_2 at 240 nm was $40 \text{ M}^{-1} \text{ cm}^{-1}$.

Assay of superoxide dismutase (SOD) activity (in liver and kidney)

SOD activity was assayed by the method of Marklund and Marklund [18], slightly modified by Paoletti and Mocali [19]. 100 mg of tissue was mixed with phosphate buffer (50 mM and pH 7.8) solution and tissue homogenate was prepared at 4°C . Tissue homogenate was centrifuged at 10,000 rpm for about 25 min. Supernatant was taken in a cuvette and the assay volume contain 100 mM triethanolamine - diethanolamine - HCl buffer (pH 7.4) along with 7.5 mM NADPH, 100 mM EDTA- 50 mM

MnCl_2 (pH 7.0). Then, the solution was kept at room temperature for about 5 min to stabilize. The decrease in absorbance was noted at 340 nm for 20 min over a 5 min interval at 25°C after the addition of 10 mM mercaptoethanol. In this assay system, 1 unit of SOD activity is defined as the amount of enzyme required to inhibit the rate of NADPH oxidation of the control by 50%.

Estimation of glutathione peroxidase (GPx) (in liver and kidney)

GPx was measured by the method described by Ahrens [20]. Briefly, tissue homogenized in 50 mM phosphate buffer (pH 7.8) and tissue homogenate was prepared. Tissue homogenate was centrifuged at 2000 rpm for about 10 min. Supernatant was taken for GPx activity with the supernatant. Then, 0.4 M sodium phosphate buffer (pH 7.0), 10 mM sodium azide, 4 mM reduced glutathione (GSH), and 2.5 mM hydrogen peroxide was mixed. The contents were incubated for 10 min at 37°C and 0.5 ml 10% TCA was added to stop the reaction. Mixture was again centrifuged at 1500 rpm for 10 min. The supernatant was assayed for GSH content using 0.3 M disodium hydrogen phosphate and Ellman's reagent (5, 5'-dithiobisnitrobenzoic acid dissolved in 0.1% sodium citrate). The color developed was read at 412 nm against reagent blank. The activities were expressed as μ moles of GSH consumed/min/mg protein.

Protein estimation

Proteins were estimated by the method of Lowry *et al.* [21] using BSA as the standard protein.

Statistical analysis

Results were expressed as the mean \pm standard deviation (SD). One-way analysis of variance (ANOVA) test was first carried out to test for any differences between the mean values of all groups. If differences between groups were established, the values of the treated groups were compared with those of the control group by a comparison *t*-test. A value of $p < 0.05$ was interpreted as statistically significant [22]. Statistical analyses were performed MS-Office Excel 2007 software packages.

RESULTS

Body weight and organ weight

In L-thyroxine-treated group, body weight was decreased (4.37% for 15 days treatment and 8.39% for 30 days treatment) and relative kidney weight was increased as compared to control group ($p < 0.05$). However, in Vitamin E supplemented L-thyroxine cotreated group, the body weight was not regained to normal, but the trend was to become normal and was higher in only Vitamin E treated animals (11.64% for 15 days treatment and 13.08% for 30 days treatment). Inconsistent with this, in Vitamin E supplemented L-thyroxine cotreated group, the weight of kidney was significantly reduced, and the trend was also to become normal. However, in the Vitamin E supplemented group, kidney weight was almost at normal or at euthyroid level (Table 1).

LPO level in liver and kidney

LPO level was significantly increased ($p < 0.05$) in liver and kidney of L-thyroxine treated rats as compared to their respective control group (Fig. 1). LPO level in those organs was found to be ameliorated significantly in L-thyroxine and Vitamin E coadministered group (Fig. 1).

Antioxidant enzyme activities in liver and kidney

Figs. 2-4 demonstrates antioxidant enzyme activities, namely, catalase, SOD, and GPx in liver and kidney, respectively. L-thyroxine administration caused a statistically significant ($p < 0.05$) stimulation in the activity of SOD and catalase, whereas a decrease in GPx activities over their respective control values. However, the activities of those enzymes were almost restored to their normal levels in L-thyroxine and Vitamin E coadministered groups treated for 15 days and 30 days, respectively (Figs. 2-4).

Histopathological studies of liver and kidney

Histological assessments performed on studied organs from the different groups are presented in plates (Plates 1-4). Liver section of

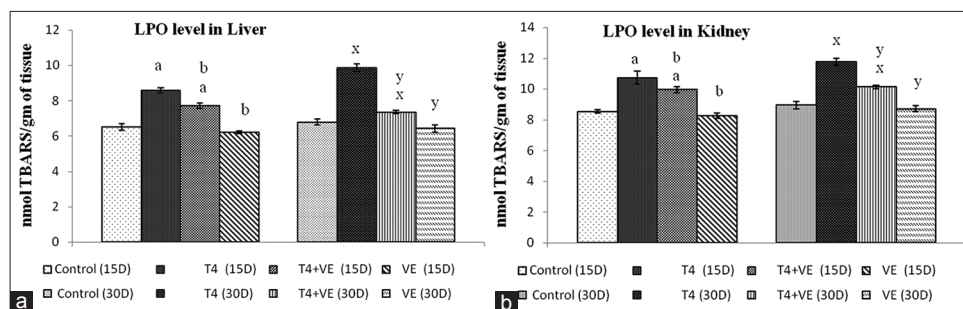


Fig. 1: (a and b) Changes in lipid peroxidation level in liver and kidney in control, L-thyroxine administered, L-thyroxine and Vitamin E coadministered and Vitamin E supplemented rats at different durations for 15 days and 30 days, respectively. Values are expressed as mean \pm standard deviation, n=6. Those bearing superscripts are significantly different by analysis of variance followed by multiple comparison *t*-tests. ^ap<0.05 when compared with 15 days control. ^bp<0.05 when compared with 15 days thyroxine-treated group. ^xp<0.05 when compared with 30 days control. ^yp<0.05 when compared with 30 days thyroxine-treated group

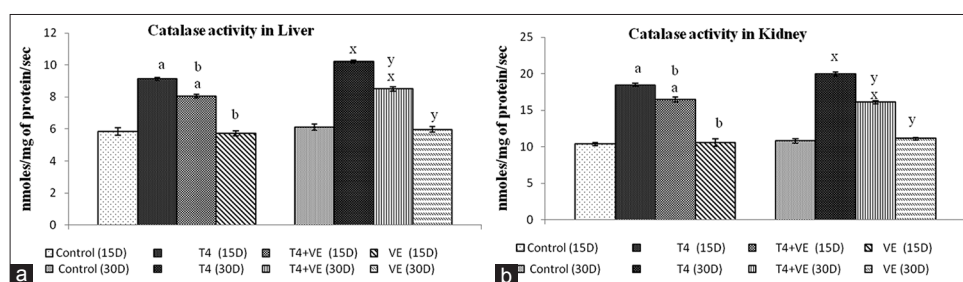


Fig. 2: (a and b) Changes in catalase activities in liver and kidney in control, L-thyroxine administered, L-thyroxine and Vitamin E coadministered and Vitamin E supplemented rats at different durations for 15 days and 30 days, respectively. Values are expressed as mean \pm standard deviation, n=6. Those bearing superscripts are significantly different by analysis of variance followed by multiple comparison *t*-tests. ^ap<0.05 when compared with 15 days control. ^bp<0.05 when compared with 15 days thyroxine-treated group. ^xp<0.05 when compared with 30 days control. ^yp<0.05 when compared with 30 days thyroxine-treated group

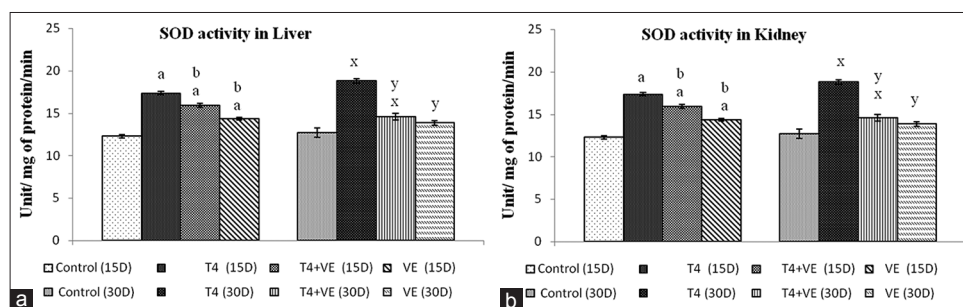


Fig. 3: (a and b) Changes in superoxide dismutase activities in liver and kidney in control, L-thyroxine administered, L-thyroxine and Vitamin E coadministered and Vitamin E supplemented rats at different durations for 15 days and 30 days, respectively. Values are expressed as mean \pm standard deviation, n=6. Those bearing superscripts are significantly different by analysis of variance followed by multiple comparison *t*-tests. ^ap<0.05 when compared with 15 days control. ^bp<0.05 when compared with 15 days thyroxine-treated group. ^xp<0.05 when compared with 30 days control. ^yp<0.05 when compared with 30 days thyroxine-treated group

control rats showed normal liver lobules and central vein, but cellular damage and oval cells accumulation were seen in liver sections in L-thyroxine treated group. Renal capsular damage, shrinkage of glomerulus and hemolysis were seen in L-thyroxine-treated group, but control kidney showed its normal appearance. Liver lobules and renal tubular damages were significantly reduced when L-thyroxine was coadministered with Vitamin E as noted in histological sections of liver and kidney. Ameliorative changes were much more prominent in 30 days treatment group as compared to 15 days treatment group.

DISCUSSION

The health benefits of antioxidants have been studied extensively in humans and animal models since past few decades. The range of antioxidant defense systems available within the cell and extracellular

components should be adequate to protect against oxidative damage. However, the balance may be interrupted when free radical production is large. Vitamin E is such a kind of antioxidant which scavenges the free radicals to protect the cells from oxidative damage. In the present study, it has been found that after L-thyroxine administration, body weight was decreased after 15 days treatment, but the diminution was more in the group treated for longer duration (30 days) post-treatment groups (Table 1); while at the same duration, body weight of control group was increased progressively. The result was almost similar with the earlier observations found by earlier researchers [23,24], where body weights were significantly decreased after L-thyroxine administration. Administration of excess L-thyroxine may lead to the development of hyperthyroid state in rats. Development of the hyperthyroid state in vertebrates elevates basal metabolic rate due to increments in the rate of O₂ consumption in target tissues [25]. Alterations of thyroid hormone

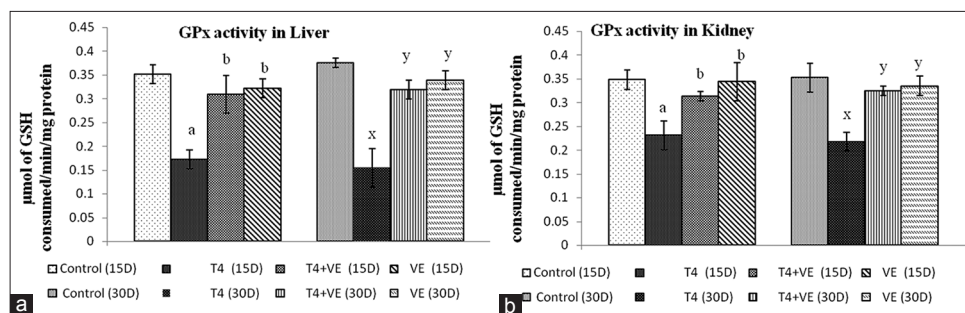


Fig. 4: (a and b) Changes in glutathione peroxidase activities in liver and kidney in control, L-thyroxine administered, L- thyroxine and Vitamin E coadministered and Vitamin E supplemented rats at different durations for 15 days and 30 days, respectively. Values are expressed as a mean \pm standard deviation, n=6. Those bearing superscripts are significantly different by analysis of variance followed by multiple comparison *t*-tests. ^a*p*<0.05 when compared with 15 days control. ^b*p*<0.05 when compared with 15 days thyroxine-treated group. ^x*p*<0.05 when compared with 30 days control. ^y*p*<0.05 when compared with 30 days thyroxine-treated group

Table 1: Changes in body weight and relative kidney weights in control, L-thyroxine administered, L- thyroxine and Vitamin E coadministered and Vitamin E supplemented rats at different durations for 15 days (A) and 30 days (B), respectively

Groups	Duration	Body weight (g) (pre-treatment)	Body weight (g) (post-treatment)	Body weight gain (%)	Relative kidney weight (g/100 g of body weight)
Control	A	174.55 \pm 9.41	190.55 \pm 11.27	9.17	0.772 \pm 0.02
	B	173.33 \pm 9.10	198.45 \pm 16.21	14.49	0.851 \pm 0.12
T ₄ treated	A	172.77 \pm 9.74	165.22 \pm 10.74 ^a	-4.37	1.027 \pm 0.03 ^a
	B	(pre-treatment)	161.44 \pm 12.19 ^x	-8.39	1.145 \pm 0.07 ^x
T ₄ +VE treated	A	176.22 \pm 8.11 (pre-treatment)	168.33 \pm 8.55 ^{a,b}	-5.08	1.001 \pm 0.03 ^a
	B	177.33 \pm 7.85 (pre-treatment)	169.78 \pm 11.51 ^{x,y}	-3.09	0.975 \pm 0.06 ^{x,y}
Only V _E treated	A	172.41 \pm 8.22 (pre-treatment)	192.47 \pm 8.22 ^{a,b}	11.64	0.817 \pm 0.02 ^{a,b}
	B	174.49 \pm 9.47 (pre-treatment)	197.31 \pm 9.29 ^{x,y}	13.08	0.926 \pm 0.06 ^{x,y}

Values are expressed as mean \pm SD, n=6. Those bearing superscripts are significantly different by ANOVA followed by multiple comparison *t*-test. ^a*p*<0.05 when compared with (A) 15 days control. ^b*p*<0.05 when compared with (A) 15 days thyroxine-treated group. ^x*p*<0.05 when compared with (B) 30 days control. ^y*p*<0.05 when compared with (B) 30 days thyroxine-treated group. SD: Standard deviation, ANOVA: Analysis of variance

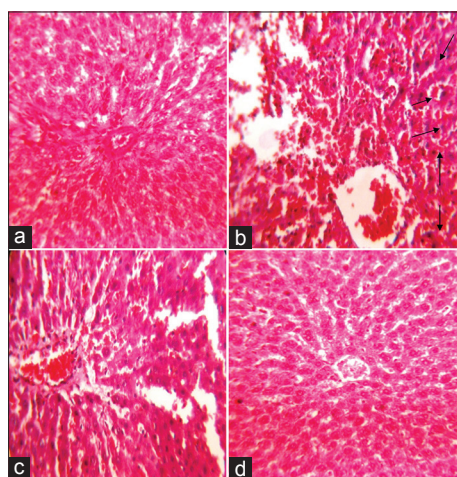


Plate 1: Photomicrographs of paraffin-embedded Hematoxylin and Eosin - stained rat liver sections (400 \times) showing (a) liver section from control animals of 15 days vehicle-treated group. (b) Liver section from L-thyroxine treated animals for 15 days. (c) Liver section from T₄ + V_E treated animals for 15 days. (d) Liver section from only V_E treated animals for 15 days

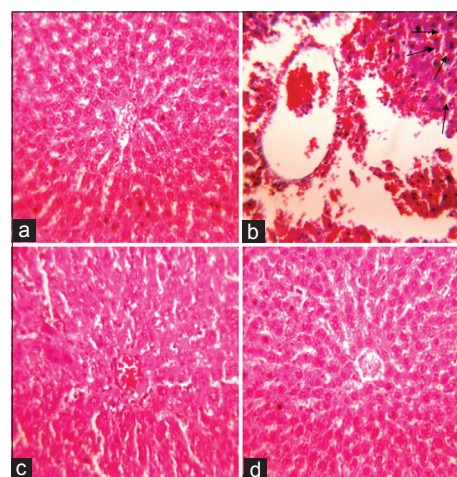


Plate 2: Photomicrographs of paraffin-embedded Hematoxylin and Eosin - stained rat liver sections (400 \times) showing (a) liver section from control animals of 30 days vehicle-treated group. (b) Liver section from L-thyroxine treated animals for 30 days. (c) Liver section from T₄+V_E treated animals for 30 days. (d) Liver section from only V_E treated animals for 30 days

levels can be one of the main physiological modulators of *in vivo* cellular oxidative stress due to their known effects on mitochondrial respiration [26]. It has also found that L-thyroxine treatment causes some short of hazardous effects on different organs which lead to oxidative stress [11]. During oxidative stress, body weight was

significantly decreased [27]. The observed decrease in body weight could be due to the direct effect of stress on the food intake behavior of the rats [28]. Stress might have increased the protein catabolism and impaired the food consumption during the stress period, thereby causing a decrease in body weight.

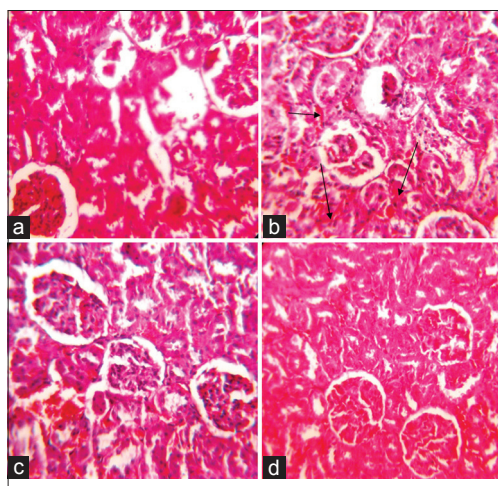


Plate 3: Photomicrographs of paraffin-embedded Hematoxylin and Eosin - stained rat kidney sections (400×) showing (a) kidney section from control animals of 15 days vehicle-treated group. (b) Kidney section from L-thyroxine treated animals for 15 days. (c) Kidney section from $T_4 + V_E$ treated animals for 15 days. (d) Kidney section from only V_E treated animals for 15 days

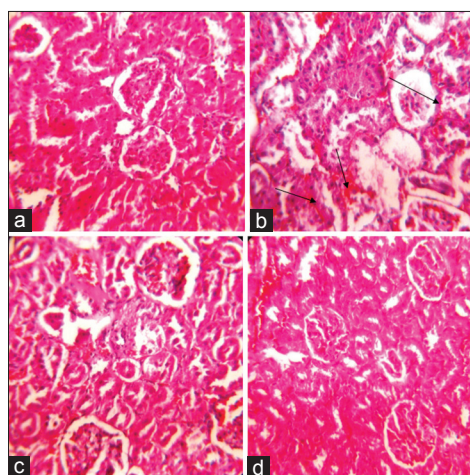


Plate 4: Photomicrographs of paraffin-embedded Hematoxylin and Eosin - stained rat kidney sections (400×) showing (a) kidney section from control animals of 30 days vehicle-treated group. (b) Kidney section from L-thyroxine treated animals for 30 days. (c) Kidney section from $T_4 + V_E$ treated animals for 30 days. (d) Kidney section from only V_E treated animals for 30 days

It has also been found in our experiment that relative kidney weight was increased significantly in L-thyroxine treated group when compared with control. It might be due to development of renal hypertrophy in rats due to L-thyroxine administration. L-thyroxine administration possibly augmented the generation of stress in kidney, which is the main cause for the renal hypertrophy. This result is in agreement with the earlier studies which have reported that renal hypertrophy takes place after the generation of stress in kidney [29,30]. The mouse kidneys are enlarged after the administration of thyroxine, and this influence is not mediated through androgens [31]. The administration of excess thyroxine increased the weight of kidney not only by direct mechanism but also stimulates the activity of adrenals indirectly and evokes hyperadrenocorticism which could be related to the action of adrenocortical steroids on kidney function and kidney growth [31]. The kidney weight was increased in our experiment due to the generation of stress by L-thyroxine administration. Vitamin E acetate coadministration along with L-thyroxine, body weight and

kidney weight were recovered toward normal range for the extra protective mechanism of this antioxidant. It has also been reported that daily supplement of Vitamin E with diet reduces age-related muscular degeneration [32] which in turn helps in the growth of those organs. Thus, body weight and weight of the kidney almost regained their normal size and weight with the increment of the muscle mass after Vitamin E supplementation along with L- thyroxine.

It has been observed that the use of oxygen as part of the process for generating metabolic energy produces ROS [33]. Superoxide dismutase (SOD) and catalase (CAT) together constitute an important defense against ROS. Catalase catalyzes hydrogen peroxide to water and oxygen, thus removing the toxic H_2O_2 . SOD catalyzes the conversion of superoxide anion free radical to H_2O_2 through dismutation reaction [34]. Inadequate removal of ROS results in oxidative stress which can damage the biological tissue system [35]. These conditions determine a higher consumption of cellular antioxidants or inactivation of antioxidant enzymes, thus inducing oxidative stress [36,37] with the concomitant increase in hepatic LPO and protein oxidation [25] along with the increase of SOD and catalase activities [38]. For this reason, in this study, LPO level along with SOD and catalase in liver and kidney were significantly raised in L-thyroxine-treated both the groups under treatment for 15 days and 30 days (Figs. 1-3), respectively, in contrast, to control group ($p < 0.05$). This data have also been supported in our earlier study that during L-thyroxine-induced oxidative stress, SOD and catalase activities were also increased due to higher consumption of antioxidant enzymes along with increased LPO [39]. On the other hand, antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the LPO or oxidative stress [40]. Vitamin E is the most important lipid-soluble antioxidant in the membrane [41] and blood plasma [42] that acts as a potent antioxidant in different tissues [43]. It has been reported that Vitamin E protects the cells against oxidative stress and inhibit the DNA damage [44]. In this study, it has been observed that LPO, SOD, and catalase activities in liver and kidney almost regain at this normal level in L-thyroxine and Vitamin E cotreated group as compared with control due to its potent antioxidant effects. In one of our earlier study, it has been reported that Vitamin E acts as an ameliorating agent against the oxidative stress [45]. Hence, it might be depicted that Vitamin E acetate supplementation in L-thyroxine-treated rats prevents the adverse toxic effects of free radicals by inhibiting oxidative stress.

GPx is an important selenium-containing antioxidant enzyme [46] involved in protection against oxidative stress and uses GSH as a substrate. GSH participates in amino acid transport through the plasma membrane, scavenges hydroxyl radical and singlet oxygen directly, detoxifying hydrogen peroxide, and lipid peroxides by the catalytic action of GPx [47]. GPx is also a free radical scavenging enzyme and known to inactivate LPO reactions and oxidative damage [48]. It inhibits free radical-mediated LPO [49]. Decreased activity of GPx in L-thyroxine treated group (Fig. 4) as observed in this study might be due to free radical-mediated inactivation of the enzyme which was clearly reported by Asayama *et al.* [50]. At the same, the inactivation was withdrawn to a certain extent when Vitamin E coadministered with L-thyroxine. Therefore, after Vitamin E supplementation, GPx activity was almost normalized during stress [51]. Smoking-induced GPx activity was restored to normal after Vitamin E supplementation [52]. Thus, Vitamin E supplementation has beneficial effects on hepatic and renal GPx activities under the influence of exogenous L-thyroxine exposure.

The microphotographs showed the liver lobules were disintegrated in L-thyroxine induced groups when compared with control (Plates 1 and 2). Oval cell accumulation occurs in L-thyroxine-treated groups in liver as observed, shown by arrows, which has also been evidenced by Roskams *et al.* [53]. Distorted renal morphology, loss of renal tubular structure and abnormal lumen were also observed in L-thyroxine-treated group as a contrast to control group (Plates 3 and 4). In addition, it has been noted that renal hemolysis occurred in L-thyroxine-treated

group, as shown by arrows, and this renal morphological alteration was supported by other authors [54]. Renal morphology was altered during oxidative stress [55]; in our earlier study, it was found that after amidarone exposure (excess iodide containing drug) there developed oxidative stress that in turn had shrunk glomerulus of renal tubule and disrupted the architecture of kidney [56]. All these changes were neutralized when Vitamin E acetate cosupplemented with L-thyroxine. There are reports that Vitamin E acts as an antioxidant and improve the histology of male rat liver and kidney following cadmium-induced toxicity [57]. Sajitha *et al.* [58] also reported that administration of Vitamin E has protected the histopathological and biochemical alterations induced by lead (Pb) intoxication in female Sprague-Dawley albino rats. Thus, Vitamin E improved the histological changes in L-thyroxine-induced liver and kidney of the experimental rats acting as a potent antioxidant.

All the observations as observed in this study may lead to the conclusion that exogenous L-thyroxine induces oxidative stress in different organs including liver and kidney, while Vitamin E acetate supplementation prevents such stress-induced condition to an extent. As a result, improvement found in the histological structure of those organs, which further substantiate the beneficial antioxidant properties of Vitamin E. Pathologically people sometimes expose or treated with excess L-thyroxine which causes organ toxicity and enzymatic imbalance. In such condition, supplementation of Vitamin E acetate regularly reduces these harmful effects on thyroid hormone-sensitive organs, namely, liver and kidney and may act as a beneficial health management agent.

ACKNOWLEDGEMENT

The author appreciatively acknowledges the financial assistance received from BI (92) of University of Calcutta. Deotima Sarkar, Senior DST INSPIRE Research Fellow is acknowledged for assistance in preparation of the certain parts of the manuscript.

AUTHORS CONTRIBUTION

Sabyasachi Sinha: Data collection and analysis, drafting article, writing manuscript. Arijit Chakraborty: Data collection and analysis, critical revision of the article. Chiranjit Mondol: Data collection and analysis. Prof. Amar K Chandra: Design of the work, data analysis, critical revision of the article, final approval of the version to be published.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

- Jacob R. The eras of vitamin C discovery. *Subcell Biochem* 1996;25:1-16.
- Davis PJ, Davis FB. Hyperthyroidism in patients over the age of 60 years. Clinical features in 85 patients. *Medicine (Baltimore)* 1974;53:161-81.
- Sies H. Oxidative stress: Oxidants and antioxidants. *Exp Physiol* 1997;82:291-5.
- Brigelius FR, Traber MG. Vitamin E: Function and metabolism. *FASEB J* 1999;13:1145-55.
- Reboul E, Richelle M, Perrot E, Desmoulins CM, Pirisi V, Borel P. Bioaccessibility of carotenoids and vitamin E from their main dietary sources. *J Agric Food Chem* 2006;54:8749-55.
- Herrera E, Barbas C. Vitamin E: Action, metabolism and perspectives. *J Physiol Biochem* 2001;57:43-56.
- Packer L, Weber SU, Rimbach G. Molecular aspects of alpha-tocotrienol antioxidant action and cell signalling. *J Nutr* 2001;131:369S-73S.
- Bell EF. History of vitamin E in infant nutrition. *Am J Clin Nutr* 1987;46:183-6.
- Traber MG, Stevens JF. Vitamins C and E: Beneficial effects from a mechanistic perspective. *Free Radic Biol Med* 2011;51:1000-13.
- Sarkar D, Chakraborty A, Bhattacharya C, Singh LH, Chandra AK. Exploration of goitrogenic/antithyroidal potentiality of bamboo-shoots in relation to thiourea. *Int J Pharm Pharm Sci* 2017;9:7-12.
- Mohamadin AM, Hammad LN, El-Bab MF, Abdel Gawad HS. Attenuation of oxidative stress in plasma and tissues of rats with experimentally induced hyperthyroidism by caffeic acid phenylethyl ester. *Basic Clin Pharmacol Toxicol* 2007;100:84-90.
- Upaganlawar A, Gandhi H, Balaraman R. Effect of vitamin E alone and in combination with lycopene on biochemical and histopathological alterations in isoproterenol-induced myocardial infarction in rats. *J Pharmacol Pharmacother* 2010;1:24-31.
- Shaker ME, Houssem ME, Hashem AE, Ibrahim TM. Comparison of vitamin E, L-carnitine and melatonin in ameliorating carbon tetrachloride and diabetes induced hepatic oxidative stress. *J Physiol Biochem* 2009;65:225-33.
- Konopacka M, Widel M, Rzeszowska-Wolny J. Modifying effect of vitamins C, E and beta-carotene against gamma-ray-induced DNA damage in mouse cells. *Mutat Res* 1998;417:85-94.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxidases in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1989;95:351-8.
- Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol* 1978;52:302-10.
- Aebi H. Catalase. In: Bergmeyer HU, editor. *Methods in Enzymatic Analysis*. New York: Academic Press; 1974. p. 673-8.
- Marklund S, Marklund G. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 1974;47:469-74.
- Paoletti F, Mocali A. Determination of superoxide dismutase activity by purely chemical system based on NAD(P)H oxidation. *Methods Enzymol* 1990;186:209-20.
- Ahrens RA. Glutathione peroxidase: A role for selenium (Rotruck 1972). *J Nutr* 1997;127:1052S-1053S.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193:265-75.
- Fisher RA, Yates R. *Statistical Tables for Biological Agricultural and Medical Research*. London: Longman Group; 1974.
- Shinohara R, Mano T, Nagasaka A, Hayashi R, Uchimura K, Nakano I, *et al.* Lipid peroxidation levels in rat cardiac muscle are affected by age and thyroid status. *J Endocrinol* 2000;164:97-102.
- Venditti P, Balestrieri M, Meo SD, Leo TD. Effect of thyroid state on lipid peroxidation, antioxidant defenses and susceptibility to oxidative stress in rat tissues. *J Endocrinol* 1997;155:151-7.
- Videla LA. Energy metabolism, thyroid calorigenesis, and oxidative stress: Functional and cytotoxic consequences. *Redox Rep* 2000;5:265-75.
- Guerrero A, Pamplona R, Portero-Otín M, Barja G, López-Torres M. Effect of thyroid status on lipid composition and peroxidation in the mouse liver. *Free Radic Biol Med* 1999;26:73-80.
- Larco DO, Cruthirds DF, Weiser MJ, Handa RJ, Wu TJ. The effect of chronic immobilization stress on leptin signaling in the ovariectomized (OVX) rat. *Endocrine* 2012;42:717-25.
- Marti O, Gavalda A, Jolin T, Armario A. Effect of regulatory exposure to chronic immobilization stress on the circadian pattern of pituitary adrenal hormones, growth hormone and thyroid stimulating hormone in the adult male rat. *Psychoneuroendocrinol* 1993;18:67-77.
- Mansouri E, Panahi M, Ghaffari MA, Ghorbani A. Effects of grape seed proanthocyanidin extract on oxidative stress induced by diabetes in rat kidney. *J Iran Biomed* 2011;15:100-6.
- Chang LT, Kras K, Suzuki K, Strasburg G, Rodger CD, Schemmel RA. Voluntary running in S5B/P1 rat fed high fat or carbohydrate diets. *Physiol Behav* 1995;57:501-8.
- Broulik PD, Marek J, Schreiber V. The effect of experimental hyperthyroidism on renal and adrenal weight increase in mice. *Physiol Res* 1991;40:527-32.
- Age-Related Eye Disease Study Research Group, SanGiovanni JP, Chew EY, Clemons TE, Ferris FL 3rd, Gensler G, *et al.* The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS report no 22. *Arch Ophthalmol* 2007;125:1225-32.
- Raha S, Robinson BH. Mitochondria, oxygen free radicals, disease and ageing. *Trends Biochem Sci* 2000;25:502-8.
- Ghosh D, Firdaus SB, Mitra E, Dey M, Bandyopadhyay D. Protective effect of aqueous leaf extract of *Murraya koenigi* against lead induced oxidative stress in rat liver, heart and kidney: A dose response study. *Asian J Pharm Clin Res* 2012;5:54-8.
- Georgeson GD, Szony BJ, Streitman K, Varga IS, Kovács A, Kovács L, *et al.* Antioxidant enzyme activities are decreased in preterm infants and in neonates born via caesarean section. *Eur J Obstet Gynecol Reprod Biol* 2002;103:136-9.
- Sies H. Biochemistry of oxidative stress. *Angew Chem Int*

- 1986;25:1058-71.
37. Fernandez V, Llesuy S, Solar L, Kipreos K, Videla LA, Boveris A. Chemiluminescence and respiratory responses related to thyroid hormone-induced liver oxidative stress. *Free Radic Res Commun* 1986;5:77-84.
 38. Asayama K, Dobashi K, Hayashibe H, Kato K. Effects of beta-adrenergic blockers with different ancillary properties on lipid peroxidation in hyperthyroid rat cardiac muscle. *Endocrinol Jpn* 1989;36:687-94.
 39. Chandra AK, Sinha S, Choudhury SR. Thyroxine induced stress and its possible prevention by catechin. *Indian J Exp Biol* 2010;48:559-65.
 40. Youdim KA, Jooseph JA. A possible emerging role of phytochemicals in improving age-related neurological dysfunction: A multiplicity of effects. *Free Rad Biol Med* 2001;30:583-94.
 41. Machlin LJ, Bendich A. Free radical tissue damage: Protective role of antioxidant nutrients. *FASEB J* 1987;1:441-5.
 42. Halliwell B. Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. *Free Radic Res* 1996;25:57-74.
 43. Senthil JK, Banudevi S, Sharmila M, Murugesan P, Srinivasan N, Balasubramanian K, et al. Effects of vitamin C and E on PCB (Aroclor 1254) induced oxidative stress, androgen binding protein and lactate in rat sertoli cells. *Reprod Toxicol* 2004;19:201-8.
 44. Bagchi D, Hassoun EA, Bagchi M, Stohs SJ. Protective effects of antioxidants against endrin-induced hepatic lipid peroxidation, DNA damage, and excretion of urinary lipid metabolites. *Free Radic Biol Med* 1993;15:217-22.
 45. Chandra AK, Ghosh R, Chatterjee A, Sarkar M. Amelioration of vanadium-induced testicular toxicity and adrenocortical hyperactivity by vitamin E acetate in rats. *Mol Cell Biochem* 2007;306:189-200.
 46. Arthur JR. The glutathione peroxidases. *Cell Mol Life Sci* 2000;57:1825-35.
 47. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 2006;160:1-40.
 48. Levy U, Zaltzber H, Ben-Amotz A, Kanter Y, Aviram M. β -carotene affects antioxidant status in non-insulin dependent diabetes mellitus. *Pathophysiology* 1999;6:157-61.
 49. Chance B, Grenstein DS, Roughton RJ. The mechanism of catalase action 1-steady state analysis. *Arch Biochem Biophys* 1952;37:301-21.
 50. Asayama K, Dobashi K, Hayashibe H, Megata Y, Kato K. Lipid peroxidation and free radical scavengers in thyroid dysfunction in the rat: A possible mechanism of injury to heart and skeletal muscle in hyperthyroidism. *Endocrinology* 1987;121:2112-8.
 51. Zal F, Mostafavi-Pour Z, Amini F, Heidari A. Effect of vitamin E and C supplements on lipid peroxidation and GSH-dependent antioxidant enzyme status in the blood of women consuming oral contraceptives. *Contraception* 2012;86:62-6.
 52. Hoshino E, Shariff R, Van Gossam A, Allard JP, Pichard C, Kurian R, et al. Vitamin E suppresses increased lipid peroxidation in cigarette smokers. *J Parenter Enteral Nutr* 1990;14:300-5.
 53. Roskams T, Yang SQ, Koteish A, Durnez A, DeVos R, Huang X, et al. Oxidative stress and oval cell accumulation in mice and humans with alcoholic and nonalcoholic fatty liver disease. *Am J Pathol* 2003;163:1301-11.
 54. Kamboj P, Aggarwal M, Puri S, Singla SK. Effect of aqueous extract of *Tribulus terrestris* on oxalate-induced oxidative stress in rats. *Indian J Nephrol* 2011;21:154-9.
 55. Reddy TU, Lakshmi SM, Kumar AC, Prathyusha S, Kumar AD. Protective effect of *Abutilon indicum* L. (*Malvaceae*) against acetaminophen induced nephrotoxicity in rats. *Innovare J Lif Sci* 2013;1:40-3.
 56. Chakraborty A, Mondal C, Sinha S, Mandal J, Chandra AK. Amiodarone induced oxidative stress in stress - Vulnerable organs of adult male rats. *Asian J Pharm Clin Res* 2014;7:177-83.
 57. Ahmadizadeh M, Baghpa AR. The preventive effect of vitamin E on cadmium chloride induced toxicity in rat liver and kidney. *Sci Med J* 2008;6:404-13.
 58. Sajitha GR, Jose R, Andrews A, Ajantha KG, Augustine P, Augusti KT, et al. Garlic oil and vitamin E prevent the adverse effects of lead acetate and ethanol separately as well as in combination in the drinking water of rats. *Indian J Clin Biochem* 2010;25:280-8.