Thyroxine induced stress and its possible prevention by catechin

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Free radicals are all known to damage cell components. The present study was designed to evaluate the free radical generation in the testis and liver and also to determine the testicular and hepatic antioxidant enzyme activities with and without catechin administration in thyroxine induced male Sprague-Dawley rats. The experimental animals were divided into four groups, six on each division. L-thyroxine (T4) (0.3 mg/kg body weight) was administered to experimental groups for 15 days. Another group (CAT-T4) was administered with L-thyroxine (T4) in the dose as mentioned and catechin (100mg/kg of body weight/day) simultaneously. Third group was administered only with catechin, and the remaining group was kept as control. Lipid peroxidation level (LPO) increased in L-thyroxine treated rats as compared to control, while LPO level was almost normal in L-thyroxine (T4) and catechin (CAT-T4) treated group. Superoxide dismutase (SOD) and catalase activities were increased in L-thyroxine (T4) treated rats as compared to control, where as there were almost at normal level in L-thyroxine (T4) and catechin (CAT-T4) treated groups. The results show that, thyroxine administration develops oxidative stress; the organism defends it against the effects of oxidative stress by increasing SOD and catalase activities as a protective mechanism and catechin, being an antioxidant, normalizes lipid peroxidation in testis and liver including SOD and catalase activities.

Keywords: Antioxidants, Catalase, Catechin, Free radical, Lipid peroxidation, Oxidative stress, Superoxide dismutase, Thyroxine

Thyroxine, or 3, 5, 3’, 5’-tetraiodothyronine (T4) a form of thyroid hormones is the major principle hormone secreted by the follicular cells of the thyroid gland. It is a prohormone and the reservoir for the active thyroid hormone i.e. triiodothyronine (T3). T4 is converted in the tissues to T3 by deiodinases. After administration of thyroxine, lipid peroxidation (LPO) level is increased and additional shifts in the location of intracellular lipid peroxidation being noted during hyperthyroidism1. Thyroid hormone can modulate the oxidative stress by acting on the mitochondrial electron transport system2. Oxidative stress increases the hepatic lipid peroxidation and protein oxidation3. Alteration in the thyroid state by T4 influences the antioxidant defense system in the liver, indicating that excess thyroxine (T4) has stress generating effects on liver and other organs4.

Catechins are polyphenolic plant metabolites that constitute about 25% of the dry weight of fresh tea leaf5. The health benefits of catechins have been reported extensively in humans and animal models. Reduction in atherosclerotic plaques was seen in animal models6. Reduction in carcinogenesis was observed in vitro7. Epigallocatechin-3-gallate, a constituent of catechin is an antioxidant that helps to protect the skin from UV radiation-induced damage and tumor formation8. Green tea catechins have also been shown to possess antibiotic properties due to their role in disrupting a specific stage of the bacterial DNA replication process9. An accumulated number of population studies also suggest that consumption of green and black tea beverages containing catechin may bring positive health effects10. Under normal physiological conditions, the generation and clearance of reactive oxygen species (ROS) in cells maintain a balanced state with the help of endogenic biological antioxidants, including glutathione, vitamin E, ascorbic acid, and some antioxidative enzymes. In addition, some dietary constituents are also important antioxidant sources including catechin11,12. In the present study, L-thyroxine has been selected for the generation of ROS in testicular and hepatocytic cells to investigate the antioxidant action of catechin especially in testicular and hepatic antioxidant enzyme profiles with or without catechin.

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administration in thyroxine induced experimental animals. For this purpose the dose of catechin used is relatively high based on earlier studies\textsuperscript{13-15} because the prime objective of the investigation is to find out whether thyroxine induced ROS formation may be prevented by administering such a dose of catechin.

Materials and Methods

Maintenance of experimental animals—For the present study twenty 24 mature albino rats of Sprague-Dawley strain weighing about 180±10 g were obtained from Indian Institute of Chemical Biology (IICB), Kolkata. The animals were caged in unheated well-ventilated (25±2°C with plenty of light and air) stainless steel cages and acclimatized to housing conditions for at least one week prior to experiment under 12:12 h L:D cycle. Animals were divided into following 4 groups of 6 each control, thyroxine (Sigma), catechin-thyroxine (CAT-T\textsubscript{4}) and catechin (E-Merck, Germany) treated groups. The rats were maintained on a standard diet (20% protein) mode 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\textsuperscript{16}. Approval for the animal study was provided by the Institutional Ethical Committee, Department of Physiology, University of Calcutta.

Selection of dose—Thyroxine treated rats were fed with normal diet and daily administration of 0.3 mg/kg of body weight L-thyroxine\textsuperscript{17} intraperitoneally for about 15 days. On the other hand, catechin treated rats were also fed with normal diet along with administration of catechin 100mg/kg of body weight/day using the references of earlier studies\textsuperscript{13-15}.

At the end of the experimental periods of 15 days, after recording the body weight, animals were sacrificed by cervical dislocation and weight of the testis and lobe of the liver were noted. Just after sacrifice, testis and liver tissue were taken out and after removing the fats; tissues were weighed and preserved to assay different enzyme activity.

Biochemical tests—Level of Lipid peroxidation\textsuperscript{18}, activities of catalase\textsuperscript{19} and superoxide dismutase\textsuperscript{20} were assayed.

Statistical analysis—All data were statistically analyzed and presented as mean ± SD. Comparison among the groups were performed by ANOVA followed by multiple comparison t-test and the level of significance was expressed as \( P<0.05 \).

Results

Body weight—Weight of testis and the major stress producing organ i.e. adrenal along with body weight were measured. Testis weight and adrenal weight were significantly increased in thyroxine treated rats in contrast to control group when these are expressed in gm% and mg% of body weight respectively (Table 1). However the weights of these glands were almost normal in catechin-thyroxine (CAT-T\textsubscript{4}) treated rats.

Antioxidant enzyme profile and lipid peroxidation level—An increase in the status of antioxidant enzymes such as superoxide dismutase (SOD) and catalase with a concomitant rise in the rate of lipid peroxidation (LPO) was observed in testis and liver in thyroxine treated rats when compared to the control group in testis and liver (Table 2). The level of antioxidant enzyme activity and LPO level were normalized when thyroxine and catechin were administered simultaneously. There is also significant change in antioxidant enzyme activities as well as LPO level in catechin alone treated groups in liver and testis.

Discussion

The normal thyroid gland activity is concerned mainly with energy metabolism in nearly all tissues of the body. Development of the hyperthyroid state in vertebrates elevates basal metabolic rate due to increments in the rate of \( O_2 \) consumption in target

<table>
<thead>
<tr>
<th>Groups</th>
<th>Testis weight(TW) (g% of body weight)</th>
<th>Adrenal weight(AW) (mg% of body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.108 ± 0.015</td>
<td>0.014 ± 0.001</td>
</tr>
<tr>
<td>Thyroxine treated</td>
<td>1.457 ± 0.019\textsuperscript{a}</td>
<td>0.033 ± 0.002*</td>
</tr>
<tr>
<td>Catechin and Thyroxine treated (CAT-T\textsubscript{4})</td>
<td>1.219 ± 0.099\textsuperscript{ab}</td>
<td>0.018 ± 0.002*</td>
</tr>
<tr>
<td>Catechin treated</td>
<td>1.124 ± 0.017\textsuperscript{ab}</td>
<td>0.011 ± 0.001*</td>
</tr>
</tbody>
</table>

On One way ANOVA test followed by multiple comparison t tests was performed. Values bearing superscripts are significantly different by ANOVA at \( P<0.05 \).

\textsuperscript{a}control (TW) versus other groups; \textsuperscript{b}Thyroxine versus catechin and thyroxine treated (CAT-T\textsubscript{4}) group or only catechin treated group; \textsuperscript{c}Catechin versus catechin and thyroxine treated (CAT-T\textsubscript{4}) group

\textsuperscript{a}control(AW) versus other groups
tissues—an effect accomplished by both short term mechanism activating mitochondrial cytchrome oxidase through 3, 5-diiodothyronine signaling and long-term pathway involving changes in nuclear and mitochondrial gene expression through 3', 3, 5-triiodothyronine signaling. Alterations of thyroid hormone levels can be one of the main physiological modulators of in vivo cellular oxidative stress due to their known effects on mitochondrial respiration. These conditions determine a higher consumption of cellular antioxidants or inactivation of antioxidant enzymes, thus inducing oxidative stress with the concomitant increase in hepatic lipid peroxidation and protein oxidation along with the increase of SOD and catalase activity. The use of oxygen as part of the process for generating metabolic energy produces reactive oxygen species. Inadequate removal of ROS results in oxidative stress which can damage the biological tissue system.

On the other hand, antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation or oxidative stress. Catechin is an antioxidant present in green tea comprises epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC) and protects the liver and other essential organs from lipid peroxidation injury.

The available informations are not sufficient and thus the present study was undertaken to evaluate the generation of stress if any by measuring the oxidant form in the tissue system as well as the antioxidant enzyme profiles in liver and testis in thyroxine induced rats and its possible amelioration by administration of catechin, the important constituent of tea.

The weight of the testis and adrenal gland were increased significantly in thyroxine treated rats in contrast to the control group. During stress the weight of the adrenal gland and testis were increased significantly in thyroxine treated rats in comparison to control group and adrenal gland alone treated group, the weight of testis increased significantly in contrast to control group and adrenal gland alone treated group. The weight gain of such glands is not observed significantly in contrast to the control group. During stress the weight gain of the adrenal gland and testis were increased significantly in thyroxine induced rats and its possible amelioration by administration of catechin, the important constituent of tea.

The weight of the testis and adrenal gland were increased significantly in thyroxine treated rats in contrast to the control group. During stress the weight of the adrenal gland and testis were increased, whereas other investigators found that the weight gain of such glands is not observed significantly. The probable mechanism for increasing the weight in those glands in hyperthyroid condition might be for the development of stress as observed in the present study as also reported by the earlier workers. It is also found that after administration of catechin (polyphenols and related compounds) the weight of such glands almost returned to normal. In catechin alone treated group, the weight of testis increased significantly in contrast to control group and adrenal activity decreased with respect to its control. It has been found that testis performs its spermatogenic and steroidogenic activity in all time, and these process are much more vulnerable to oxidative stress.

### Table 2—Thyroxine induced alteration of lipid peroxidase and antioxidant enzymes profiles in testis (A) and liver (B) in thyroxine administered rats with and without catechin supplementation for 15 days

<table>
<thead>
<tr>
<th></th>
<th>Lipid peroxidation level (mol TBARS/g of tissue)</th>
<th>Catalase activity (moles/mg/sec)</th>
<th>SOD activity (Unit/mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6.75 ± 0.07</td>
<td>5.35 ± 0.03</td>
<td>12.24 ± 0.04</td>
</tr>
<tr>
<td>B</td>
<td>5.84 ± 0.03</td>
<td>4.75 ± 0.02</td>
<td>12.43 ± 0.02</td>
</tr>
<tr>
<td>Thyroxine treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>7.77 ± 0.20</td>
<td>8.68 ± 0.02</td>
<td>19.44 ± 0.03</td>
</tr>
<tr>
<td>B</td>
<td>7.54 ± 0.03</td>
<td>6.47 ± 0.03</td>
<td>18.07 ± 0.04</td>
</tr>
<tr>
<td>Catechin and Thyroxine treated (CAT-T₄)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>5.29 ± 0.03abc</td>
<td>5.85 ± 0.04ab</td>
<td>17.21 ± 0.03abc</td>
</tr>
<tr>
<td>B</td>
<td>6.97 ± 0.06xyz</td>
<td>7.32 ± 0.02xyz</td>
<td>15.99 ± 0.04xyz</td>
</tr>
<tr>
<td>Catechin treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>5.89 ± 0.17abc</td>
<td>4.75 ± 0.03abc</td>
<td>14.97 ± 0.04abc</td>
</tr>
<tr>
<td>B</td>
<td>5.08 ± 0.08xyz</td>
<td>4.42 ± 0.04xyz</td>
<td>11.26 ± 0.04xyz</td>
</tr>
</tbody>
</table>

One way ANOVA test followed by multiple comparison t tests was performed. Values bearing superscripts are significantly different by ANOVA at P<0.05.

*control(A) versus other groups; †Thyroxine versus catechin and thyroxine treated (CAT-T₄) group or only catechin treated group; 2Catechin versus catechin and thyroxine treated (CAT-T₄) group.

Control (B) versus other groups; †Thyroxine versus catechin and thyroxine treated (CAT-T₄) group or only catechin treated group; 2Catechin versus catechin and thyroxine treated (CAT-T₄) group.
these reasons, excess catechin and the antioxidant system in testis together may increase the weight of testis. It is also seen that in catechin treated group the weight of adrenal gland decreased in contrast to control group. All the results indicate that catechins in green tea not only help to burn calories and lower LDL cholesterol but also able to mildly reduce body fat and adrenal steroid level as well as the adrenal weight.

The lipid peroxidation (LPO) activity was increased both in liver and testis because thyroxine administration causes oxidative stress in these tissues. Severe oxidative stress produces ROS and induces uncontrolled lipid peroxidation. Following lipid peroxidation aldehydic products, such as free fatty acids, malondialdehyde (MDA) also referred to as thiobarbituric acid-reacting substances (TBARS), are generated. Cell membranes consist primarily of lipids, thus uncontrolled lipid peroxidation may cause cell injury and death via DNA damage and directly inhibiting proteins, such as Na\textsuperscript{+}/K\textsuperscript{+}-ATPase and glutamate transporters.

Oxidative stress may lead to lipid peroxidation; and the oxidative stress may be prevented by daily intake of antioxidants. Mice fed with catechins showed decreased levels of aging; oxidative stress was lowered in cell mitochondria, as well as increased in mRNA transcription of mitochondria related proteins. Probably for this reason LPO level is normalized in thyroxine-catechin induced rats. LPO level was also decreased in only catechin treated animals. Thus catechin, being an antioxidant, decreases thyroxine induced stress. It has been reported that malondialdehyde, a marker of oxidative stress, also decreases after green tea intake.

The activities of antioxidant enzymes in liver and testis were assayed. It was found that after thyroxine administration in rats SOD and catalase level were increased in contrast to control ($P<0.05$). SOD activity was increased in the cardiac muscle and liver in hyperthyroid rats. Also, an increase in SOD activity has been shown in the blood of patients with hyperthyroidism. Products of free radical-mediated damage to lipids, protein and DNA have been identified in biological materials such as plasma, urine and blood cells and proposed as biomarkers for oxidative damage. The protecting feature of SOD, one of the antioxidant enzymes, against oxidative stress bears an essential role for life. Tamotoxin (an antiestrogenic drug) treatment caused a significant increase in the mitochondrial lipid peroxidation (LPO) and the protein carbonyls (PCs). It also caused a significant increase in superoxide radical production. Pretreatment of mice with catechin showed significant protection as demonstrated by marked attenuation of increased oxidative stress parameters such LPO, PCs, and superoxide production. It also restored the decreased nonenzymatic and enzymatic antioxidants of mitochondria.

SOD activity and catalase level are increased during stress to prevent the ROS generation. Catechin, being an antioxidant, tries to maintain the normal antioxidant enzyme level. Reverse transcriptase–polymerase chain reaction analysis indicated that the antioxidant enzymatic activities of catalase and SOD were accompanied by up-regulation of genes for catalase, copper–zinc containing SOD and manganese-containing SOD. Green tea catechin reduced oxidative DNA damage in mice and rats. Green tea is a chemopreventive agent for hepatocarcinogenesis in the absence of chronic hepatocyte damage. Similar results were reported in animals treated with aflatoxin; green tea inhibited initiation and promotion steps. Moreover, daily ingestion of green tea prevented hepatotoxicity (increase in serum glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase; decrease in hepatic glycogen, serum triglyceride, and lactate dehydrogenase) and cell proliferation in the liver in rats after administration of 2-nitropropane.

In only catechin alone treated group, LPO level was decreased significantly. After catechin treatment LPO level are decreased because catechin prevents unwanted oxidative damage in liver and certain other organs. Catechin is a natural antioxidant and shown to have anti tumor effect in basic and epidemiological studies. In the present study it was also observed that, in catechin alone treated group SOD level was increased significantly with respect to control group in testis. Catechin can increase SOD level and MnSOD gene expression in pheochromocytoma cells (PC-12) which may have beneficial effect in tumor prevention in short period of time.

Surprisingly the catalase level in liver and testis and SOD activity in liver were decreased in only catechin treated group as compared with control. This is probably because of administration of relatively high dose of catechin or for the pharmacologic effects of catechin on these parameters to an extent. The
intake of foods and beverages (e.g. tea) containing flavonoids is recommended, although it is too early to make recommendations on daily flavonoid intake as suggested by Nijveldt et al.\textsuperscript{50}.

The catechin exerts its more prompt antioxidant effects or reversing effects of TBARS on testis than liver. This is because spermatogenesis is an active replicative process that takes place in testis, capable of generating approximately 1000 sperms a second. The high rates of cell division imply correspondingly high rates of mitochondrial oxygen consumption by the germinal epithelium. However, the poor vascularization of the testis means that oxygen tensions in this tissue are low\textsuperscript{51} and that competition for this vital element within the testis is extremely intense. Since both spermatogenesis\textsuperscript{51} and Leydig cell steroidogenesis\textsuperscript{2,33} are vulnerable to oxidative stress, the low oxygen tension that characterizes this tissue may be an important component of the mechanisms by which the testis protects itself from free radical-mediated damage. In addition, the testis contains an elaborate array of antioxidant enzymes and free radical scavengers to ensure that the twin spermatogenic and steroidogenic functions of this organ are not impacted by oxidative stress. These antioxidant defense systems and catechin together create these reverse effects upon testis against the T\textsubscript{4} induced oxidative stress.

On the other hand, liver is the essential organ for metabolism and it consists of numerous numbers of mitochondria. The mitochondria are the most important source of ROS, mainly because of auto-oxidation of ubisemiquinone at the level of complex III. This occurs to some extent under physiological, aerobic conditions. But it may take several hours to recover this oxidative stress. Therefore for the short duration of treatment (approximately 2 weeks) the mitigating action is found more in testis than liver.

The results of the present study reveal that thyroxine administration causes oxidative stress both in testis and liver as evidenced by enhanced LPO level associated with increased SOD and catalase activities to defend against the effects of oxidative stress. Simultaneous administration of catechin with thyroxine, maintains the LPO level, SOD and catalase activities almost at normal. Therefore the developed stress caused by thyroxine administration may be overcome by simultaneous catechin supplementation.

Conflict of interest
Authors declare there is no conflict of interest.

Acknowledgement
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