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Goitrogenic/antithyroidal potential of green tea extract in relation to catechin in rats

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ABSTRACT

Catechins are flavonoids found in abundance in green tea, have elicited high interest due to their beneficial effects on health. Though flavonoids have been reported to have an antithyroid effect and also to be goitrogenic there have been no reports about the effect of green tea on rat thyroid. The present study was designed to examine whether high doses of green tea has any harmful effect on thyroid physiology. For this purpose green tea extract was administered orally to male albino rats for 30 days at doses of 1.25 g%, 2.5 g% and 5.0 g%, respectively. Similarly, pure catechin was administered at doses of 25, 50 and 100 mg/kg body weight which is equivalent to above doses of green tea extract. Lower body weight gain associated with marked hypertrophy and/or hyperplasia of the follicles was noted in the high dose of green tea and catechin treated groups. Decreased activity of thyroid peroxidase and 5′-deiodinase I and substantially elevated thyroidal Na,K + ATPase activity have been observed. Moreover, serum T3 and T4 levels were found to reduce followed by significant elevation of serum TSH. Taken together, these results suggest that catechin present in green tea extract might behave as antithyroid agent and possibly the consumption of green tea at high dose could alter thyroid function adversely.

1. Introduction

The last couple of decades have seen a tremendous increase in interest in the biological properties of natural products as a means to identify novel compounds that could have a potential in clinical medicine. To that end, flavonoids and flavonoid-like compounds percolate to the top due to their presence in diet constituents and reported beneficial effects on diverse biological processes and disease conditions. Tea is a natural polyphenolic compound consumed by over two thirds of the world's population. Among the various types of tea, green tea contains a relatively high level of polyphenols, which consist of flavanol monomers (flavan-3-ols), also referred to as catechins. The primary catechins in green tea (GTC) are epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG) (Higdon and Frei, 2003). Green tea also contains gallic acid (GA) and other phenolic acids such as chlorogenic acid, caffeine and flavonols such as kaempferol, myricetin, quercetin, quinic acids, carotenoids, trigalloylglucose, lignin, protein, chlorophyll, minerals and very small amounts of other methylxanthines such as theophylline, theobromine and theanine (Cabrera et al., 2006).

The potential health effects of catechins depend not only on the amount consumed but on their bioavailability which appears to be variable. In order to know the catechin bioavailability and metabolism, it is necessary to evaluate their biological activity within target tissues (Manach et al., 2004). Following oral administration of tea catechins to rats, the four principal catechins (EC, ECG, EGC, and EGCG) have been identified in the portal vein, indicating that tea catechins are absorbed intestinally. Absorbed tea catechins are biotransformed in the liver to conjugated metabolites, i.e. glucuronidated, methylated and sulfated derivatives; while (−)-epigallocatechin and (−)-epicatechin are mainly conjugated, (−)-epigallocatechin gallate is usually present in free form in human plasma (Chow et al., 2001).

Tea catechins have garnered considerable attention as a result of beneficial effects on health, such as their observed antioxidant activity and antimutagenic activity, as well as their ability to serve as an anti-carcinogenic agent, antiulcer, antiallergic, anti-inflammatory, anti-cardiovascular disease agent and hypolipidemic agent (Kasamatsu et al., 2008).

Although numerous beneficial effects of green tea catechins (GTC) for human health has been reported, but consumption of flavonoids and some phenolic acids by experimental animals caused enlargement and histological changes in the thyroid gland (Gaitan et al., 1995; Sartelet et al., 1996; Khelifi-Touhami et al., 2003; Ferreira et al., 2006). In the published literature, very few studies have explored the antithyroid/goitrogenic potential of green tea extract catechin (i.e. polyphenon 60) (Sakamoto et al., 2001; Satoh et al., 2002). On the contrary, daily oral administration

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of standardized and defined green tea polyphenols to humans for 28 days was found to be safe (Chow et al., 2003). In another dose–response study (Imai et al., 2008), dietary exposure of F344 rats to GTC at levels up to 5% in diet for 90 days resulted in no significant histopathological changes in the thyroid. Therefore, information about the effects of green tea catechin on thyroid physiology is not comprehensive. The purpose of this study was to evaluate whether high doses of green tea consumption has antithyroidal effect in rats. To substantiate the antithyroid activity of green tea, we conducted a comparative evaluation of the effects of green tea extract and commercially available pure catechin on serum levels of thyroid hormones, thyroid peroxidase, 5′-deiodinase I (5′-DI) and Na,K + ATPase activities and thyroid gland architecture.

2. Materials and methods

2.1. Materials

Pure catechin {2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3,5,7-triol; catechol; 3,3′4′5,7-flavan pentol; catechoic acid; cyanidol; $C_{15}H_{14}O_6$}was purchased from E-Merck, Darmstadt, Germany. DTE, Tris–HCl and Na_2ATP was purchased from Sisco Research Laboratories (SRL), Mumbai, India Ouabain, PTU, T4, bovine serum albumin, EDTA, $MnCl_2$ was purchased from Sigma Chemical Company, Steinheim, Germany. SDS was purchased from LOBA Chemie Pvt. Ltd., Mumbai, India.

Green tea was collected from the Institute of Himalayan Bioresource Technology (IHBT), Palampur, Himachal Pradesh. The composition of green tea is EC (Epicatechin) – 1.55%, EGCG (Epigallo catechin gallate) – 9.00%, ECG (Epi catechin gallate) – 4.8%, EGC (Epigallo catechin) – 5.0%, Caffeine – 2.38% as mentioned by the producer. Preparation of aqueous extract of green tea was done following the method of Wei et al. (1999). Briefly, 2.5 g green tea was added to 100 ml of boiling water and was steeped for 15 min. The infusion was cooled to room temperature and then filtered. The tea leaves were extracted a second time with 100 ml of boiling water and filtered and two filtrates were combined to obtain a 1.25 g% green tea aqueous extract (1.25 g tea leaf/100 ml water). By the same procedure 2.5 g% and 5.0 g% green tea aqueous extract was prepared. The total catechin content of the green tea is 20.35% (wt/wt) (1.55% + 9.0% + 4.8% + 5.0%). The green tea extract was fed to animals orally once daily at a dose of 1 ml/100 g body weight. Simultaneous pure catechin was administered to experimental animals at the dose of ~25 mg/kg body weight (Dose I), ~50 mg/kg body weight (Dose II) and ~100 mg/kg body weight (Dose III) which was equivalent to natural catechin present in green tea extract.

2.2. Animals and treatment

In the present study, three months old adult male albino rats of Sprague Dawley strain weighing 200 ± 10 g were obtained from Indian Institute of Chemical Biology (IICB), Kolkata. The animals were maintained as per national guidelines and protocols, approved by the Institutional Animal Ethics Committee (PHY/CU/IAEC/07 dated 25.07.2007). The animals were housed in clean polypropylene cages and maintained in a controlled environment at a temperature 22 ± 2 °C and a relative humidity of 40–60% in an animal house with constant 12 h light and 12 h dark schedule. The animals were fed on standardized diet which consisted of 70% wheat, 20% Bengal gram, 5% fish meal powder, 4% dry yeast powder, 0.75% refined til oil and 0.25% shark liver oil and water *ad libitum* (Chandra et al., 2007).

In the 30 days treatment, the study animals were divided into five groups of 8 animals each. The groups were as follows:

Group 1: Eight animals from the first group were administered with sterile distilled water orally as vehicle and considered as control; **Group 2**, **3** and **4**: In addition to the standard diet, the animals in these group received green tea extract (1.25%, 2.5 g% and 5.0 g%) at a dose containing ~25 mg, ~50 mg and ~100 mg natural catechins/kg body weight, respectively; **Group 5**, **6** and **7**: The experimental animals were treated with pure catechin at a dose of 25 mg, 50 mg and 100 mg/kg body weight orally. All the animals were sacrificed 24 h after the last treatment following protocols and ethical procedures. Blood samples were collected and serum separated for hormone assay.

2.3. Body weight and thyroid weight

The body weight (g) of the experimental animals was recorded on the first day before treatments start (pre-treatment) and the day of sacrifice (post-treatment). Just after sacrifice, the thyroid glands were taken out, trimmed off the attached tissues and weighed. The relative weight of thyroid gland (mg) was expressed per 100 g body weight.

2.4. Histological study

Immediately after removal, the thyroid gland of each rat was fixed in 10% neutral buffered formalin, embedded in paraffin and sections were stained with Hematoxylin and Eosin (HE) and Periodic acid-Schiff (PAS) staining and examined under a light microscope.

2.5. Thyroid peroxidase (TPO) assay

Thyroid peroxidase (TPO) activity was measured by a method of Alexander (1962). For TPO activity, 10% homogenate was prepared using pooled thyroid tissues collected from the sacrificed animals; in phosphate buffer (pH 7.2, 100 mM) and sucrose solution (500 mM) at 4 °C. Homogenisation was carried out in a glass homogeniser (Potter–Elvehjem) for 45–60 s at 4400 g and about 15 strokes/min. The homogenate was centrifuged at 1000*g* for 10 min. This low speed supernatant was further centrifuged at 10,000*g* for 10 min at 4 °C to get the mitochondrial fraction. The microsomal fraction containing most of the peroxidase activity was obtained by centrifuging the post mitochondrial supernatant at 105,000*g* for one hour. Immediately after centrifugation the precipitate was solubilized in phosphate buffer (pH 7.2).Thyroid peroxidase activity was measured in a 1 ml cuvette containing 0.9 ml acetate buffer (pH 5.2, 50 mM), 10 μl potassium iodide (1.7 mM), 20 μl microsomal fraction of thyroid tissue and freshly prepared 20 μl hydrogen peroxide (0.3 mM) was added lastly to start the reaction for assaying the TPO activity (ΔOD/min/mg protein) in a spectrophotometer (UV-1240 Shimadzu) at 353 nm. The pooled sample was assayed in duplicate.

2.6. Thyroidal sodium, potassium adenosine triphosphatase (Na,K + ATPase) assay

Thyroidal sodium, potassium adenosine triphosphatase (Na,K + ATPase) activity was measured by a modification of the method of Esmann et al. (1988). In brief, microsomal fraction of thyroid tissue homogenate was incubated in reaction mixtures of (i) 30 mM imidazole HCl, 130 mM NaCl, 20 mM KCl, 4 mM $MgCl_2$ and (ii) 30 mM imidazole HCl, 4 mM $MgCl_2$ and 1 mM ouabain (Sigma Chemical Co., USA) at pH 7.4 for 60 min at 0 °C. The reaction was started by addition of 4 mM Tris–ATP (hydroxymethyl aminomethane hydrochloride adenosine triphosphate) at 37 °C and stopped with 0.1 ml of 20% SDS (Sodium dodecyl sulphate) after 10 min. The inorganic phosphate (Pi) liberated was determined by reading the absorbance at 850 nm in a UV-mini1240 Spectrophotometer, Shimadzu, Japan by the method of Baginski et al. (1967). The enzyme activity was expressed as nmols of Pi liberated per hour per mg protein calculated from a standard curve of potassium dihydrogen phosphate. The pooled sample was assayed in duplicate.

2.7. 5′-Deiodinase I (5′-DI) assay

Iodothyronine 5′-deiodinase type I (5′-DI) activity was measured according to the method of Ködding et al. (1986) with slight modifications. Briefly, a substrate solution of 0.1 M Tris–HCl buffer (pH 7.4), 3 mM EDTA and 150 mM DTE containing 0.4 μM T4 and 100–150 μg pooled thyroid tissue protein in a final volume of 400 μl was incubated at 37 °C for 30 min. The monodeiodination reaction of T4 to T3 was terminated by addition of 800 μl ice-cold absolute ethanol, followed by shaking for 8 min at 4 °C. The reactants were then centrifuged at 10,500*g* at 4 °C for 8 min and the ethanol supernatants were collected for measurement of T3 content. For all samples, values for zero time were prepared by adding the thyroid tissue to the substrate containing T4 after the addition of alcohol. The concentration of T3 in the ethanolic extract after 0 and 30 min of incubation were estimated by ELISA. The activity of 5′-DI was calculated as the difference of the 0 and 30 min values and expressed in terms of pmoles T3/mg protein. The pooled sample was assayed in duplicate.

The validity of the assay method has been justified by preincubation of the sample with the 5′-DI inhibitor, propylthiouracil (PTU), that resulted in >50% inhibition of the enzymatic activity. It needs to be mentioned here that conversion of T4–rT3 by 5′-DI cannot proceed under such simulated conditions, as rT3 formation can occur only under high pH and substrate concentration, unlike T4–T3 monodeiodination, as found in our experimental condition.

2.8. Protein estimation

Proteins were estimated by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as the standard protein.

2.9. ELISA of serum tri-iodothyronine (T3) and thyroxin (T4)

Just before sacrifice, blood samples were collected for each rat under ether anesthesia and the serum was separated for the assay of tri-iodothyronine (T3) and thyroxin (T4). All the samples for measurement were preserved at −20 °C. Total serum tri-iodothyronine and thyroxin were assayed using an ELISA kits obtained from RFCL Limited, India (Code no HETT 1108 and HETF 0908, respectively). The sensitivity of the T3 and T4 assay were 0.04 ng/ml and 0.4 μg/dl, respectively.