

Studies on Goitrogenic/ Antithyroidal Potentiality of Thiocyanate, Catechin and After Concomitant Exposure of Thiocyanate-Catechin

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ABSTRACT

Inadequate iodine intake including and a number of environmental agents can affect thyroid gland functions in man and animals. Thiocyanate and catechin that arise for the consumption of cyanogenic and flavonoids containing foods respectively, affect thyroid hormone synthesis but the data comparing the goitrogenic/ antithyroid potentiality of thiocyanate and catechin when act individually or synergistically on thyroid function found scanty. Experimental animals were divided into groups and orally administered either thiocyanate or catechin or both in combination for 30 days followed by analysis of thyroid gland weight, histology, thyroid peroxidase, sodium potassium ATPase, 5'-deiodinase- I activities, thyroid hormone profiles, urinary iodine and urinary thiocyanate of each group of animals. Enlarged thyroid showing hypertrophic and hyperplastic changes, decreased thyroid peroxidase, sodium potassium ATPase, 5'-deiodinase- I activities and thyroid hormone profiles were observed in the treated groups. Overall results reveal that goitrogenic/ antithyroid potentiality found highest after concomitant exposure of thiocyanate and catechin followed by thiocyanate and least after catechin.

Key words: Thiocyanate, catechin, goitrogen, thyroid hormone, TPO.

INTRODUCTION

Thyroid hormone plays a key role in many physiologic functions, and in the fetus and child, it is critical for brain and neurological development. Iodine is an essential trace element of great importance in human nutrition. The element is an integral part of the thyroid hormones¹. Plants are known to produce more than 200,000 different bio-active natural products (also denoted secondary metabolites) including cyanogens and polyphenols^{2,3}. Regular consumption of cyanogenic foods containing cyanogenic glucosides, glucosinolates, and thiocyanate (SCN) affect thyroid physiology and may lead to the development of endemic goitre, especially in iodine deficient environments⁴. Besides iodide, thiocyanate ion is also an attractive inorganic substrate because it is classified as a pseudo halide and bears many resemblances to iodide in its chemical behaviour. Thiocyanate has been shown to competitively inhibit iodide uptake by the sodium iodide symporter and blocking iodide uptake into the thyroid can decrease thyroid hormone production^{5,6}. It has previously been reported that a number of plant foods, including cauliflower, cabbage, mustard, turnip, and cassava, containing those substances may induce alterations in thyroid function as observed in *in vivo* and *in vitro* studies⁷⁻¹⁰. Polyphenols are widely distributed in plant-derived foods and possess a variety of biological activities including antithyroid effects in experimental animals and humans. Flavonoids can inhibit various enzymatic activities such as protein tyrosine kinase and DNA

topoisomerase I and II which are crucial for cellular proliferation^{11,12}. Apart from these effects flavonoids inhibit thyroid peroxidase (TPO) in *in vitro* and *in vivo* experimental animals and humans¹³⁻¹⁶ and also 5'-deiodinase activity *in vitro*^{11,12}. Certain plant foods contain both the naturally occurring goitrogenic/antithyroidal agents' viz. polyphenols and cyanogens having different types of action on thyroid. Though their individual antithyroid effect have been studied however the information after their individual exposure in equal concentrations or after combined exposure of these goitrogen in thyroid gland functions on comparative aspect found almost unavailable. In the present study a comparative evaluation of the effects of thiocyanate (potassium thiocyanate) as a source of cyanogenic plant foods and commercially available pure catechin as a source of polyphenols in equal concentrations or a combination of those two goitrogenic agents on thyroid gland morphology, histology, thyroid hormonal profiles (T3, T4 and TSH), thyroid peroxidase (TPO) activity, 5'-deiodinase- I activity, sodium potassium ATPase (Na⁺-K⁺-ATPase) activity and iodine nutritional status in experimental animals have been investigated.

MATERIALS AND METHODS

Animals and treatment

Three-month-old adult male albino rats of the Wistar strain weighing 150 ± 10g were used. The animals were maintained according to national guidelines and protocols,

and the study was approved by the Institutional Animal Ethics Committee. The animals were housed in clean polypropylene cages and maintained in a controlled environment at temperature $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and relative humidity (40% - 60%) in an animal house with a constant 12 hour light/12 hour dark schedule. The animals were fed a standardized diet which consisted of 70% wheat, 20% Bengal gram, 5% fish meal powder, 4% dry yeast powder, 0.75% refined sesame oil, 0.25% shark liver oil, and water ad libitum¹⁷. In the 30 days treatment, the experimental animals were divided into four groups of 8 animals each. First group was kept as control and administered sterile distilled water orally as vehicle. Second group animals were treated with pure catechin at a dose of 100 mg/kg body weight ($\equiv 15$ mg catechin per rat as used in this study) orally. Third group received 25 mg of potassium thiocyanate ($\equiv 15$ mg thiocyanate per rat as used in this study). Fourth group was subjected to pure catechin at a dose of 100 mg/kg body weight¹⁵ ($\equiv 15$ mg catechin per rat as used in this study) and 25 mg of thiocyanate¹⁸ ($\equiv 15$ mg thiocyanate per rat as used in this study) orally. Feed consumption, corrected for wasted feed, and body weight were measured every seven days. During the last week of the treatment animals in each group were kept in metabolic cages for 24 hr to collect urine over xylene for the analysis of iodine and thiocyanate. At the end of the experimental period the body weights of the rats were recorded and the animals were sacrificed at the end of the 30th days of the experiment, respectively. All the animals were sacrificed 24 hours after the last feeding (i.e. during 9 am to 10 am on the day of experiment to avoid any discrepancy that may arise for diurnal variation) following standard protocols and ethical procedures. Blood samples were collected and serum separated for hormone assay.

Measurement of urinary iodine and thiocyanate

The urine sample was digested followed by subsequent ashing, and iodide was measured by its catalytic action on the reduction of ceric ion Ce^{+2} to cerous ion Ce^{+3} (19), maintaining internal quality control. While thiocyanate concentrations in urine samples were measured using the method of Aldridge²⁰ as modified by Michajlovskij and Langer²¹.

Thyroid weight

Just after sacrifice, the rat thyroid glands were dissected out and weighed. The relative weight of thyroid gland (mg) was expressed per 100 g body weight.

ELISA of serum triiodothyronine (T3) and thyroxine (T4)

Just before sacrifice, blood samples were collected from each rat under ether anaesthesia and the serum was separated for the assay of T3 and T4. All the samples were stored at -50°C prior to measurement. Total serum T3 and T4 were assayed using ELISA kits obtained from RFCL Limited, India (Code no HETT 0210 and HETF 0914 respectively). The sensitivities of the T3 and T4 assays were 0.04 ng/ml and 0.4 mg/dl, respectively.

ELISA of serum thyroid stimulating hormone (TSH)

Thyroid stimulating hormone level was measured using Cusabio Biotech Limited, Rat TSH kit [Lot no.C0710270665]. This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody

specific for TSH has been pre-coated onto a microplate. Standards and samples (100 μl) are pipetted into the wells with 50 μl Horseradish (HRP) conjugated antibody specific for TSH. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of TSH bound in the initial step. The colour development is stopped and the intensity of the colour is measured and absorbance was read against 450 nm wavelength in ELISA reader (Merck).

Assay of thyroid peroxidase (TPO) activity

A 10% homogenate was prepared using thyroid tissue collected from the sacrificed animals in a phosphate buffer (pH 7.2, 100mM) and sucrose solution (500 mM) at 4°C . Homogenization was carried out in a glass homogenizer (Potter-Elvehjem, Germany). The homogenate was centrifuged at 1000g for 10 min and this low-speed supernatant was further centrifuged at 10,000 g for 10 min at 4°C to obtain the mitochondrial fraction. The microsomal fraction containing most of the peroxidase activity was obtained by centrifuging the post-mitochondrial supernatant at 1,05,000 g for one hour. Immediately after centrifugation the precipitate was dissolved in phosphate buffer. Thyroid peroxidase activity was measured in a 1 ml cuvette containing 0.9 μl 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) were added to start the reaction for assaying TPO activity ($\Delta\text{OD}/\text{min}/\text{mg}$ protein) in a spectrophotometer (UV-1240 Shimadzu) at 353 nm. The pooled sample was assayed in duplicate. Thyroid peroxidase activity was measured by the method of Alexander²². The tissue protein level was determined by the method of Lowry²³ using bovine serum albumin as a standard. The results are expressed as change in optical density ($\Delta\text{OD}/\text{min}/\text{mg}$ protein).

5'-deiodinase I (5'-DI) assay

Iodothyronine 5'-deiodinase type I (5'-DI) activity was measured according to the method of Ködding²⁴ 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 mM T4 and 100-150 mg thyroid tissue protein in a final volume of 400 ml was incubated at 37°C for 30 min. The monodeiodination reaction of T4 to T3 was terminated by addition of 800 ml 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 enzyme-linked immune sorbent assay (ELISA). The activity of 5'-DI was calculated as the difference between the 0 and 30 min values and expressed in terms of pmoles T3 formed/mg protein. The pooled sample was assayed in duplicate. The validity of the assay method was determined by pre-incubation of the sample with the 5'-DI inhibitor, propylthiouracil (PTU), that resulted in >50% inhibition of the enzymatic activity. Conversion of T4 to rT3 by 5'-DI,

unlike T4 to T3 monodeiodination, cannot proceed under such simulated conditions, as rT3 formation can occur only under high pH and substrate concentrations.

Thyroidal (Na⁺-K⁺)-ATPase assay

Thyroidal Na⁺-K⁺-ATPase activity was measured by a modification of the method of Esmann²⁵. In brief, the microsomal fraction of the thyroid tissue homogenate was incubated in reaction mixtures of (i) 30 mM imidazole HCl, 130 mM NaCl, 20 mM KCl, 4 mM MgCl₂ and (ii) 1 mM ouabain (Sigma Chemical Co., St. Louis, MO 63178, USA) at pH 7.4 for 60 min at 0°C. The reaction was started by the addition of 4 mM Tris-ATP at 37°C and stopped with 0.1 mL of 20% SDS 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 Baginski²⁶. 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.

Protein estimation

Proteins were estimated by the method of Lowry²³ using bovine serum albumin (BSA) as the standard protein.

Statistical analysis

Results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was first carried out to test for differences across the mean values of all groups. If between-group differences were established, the values of the treated groups were compared with those of the control group using Tukey's procedure. A value of $p < 0.05$ was interpreted as statistically significant. Statistical analyses were performed using Origin 8 and MS-Office Excel 2007 software packages.

RESULTS

Thyroid weight

The weight of the thyroid glands was significantly increased ($P < 0.05$) after administration of catechin, thiocyanate (SCN) and catechin + thiocyanate (SCN) for 30 days as compared to their respective control groups. Intergroup variation was also noticed within the groups as shown in the Figure 1.

Thyroid peroxidase assay

Figure 2 compares the effect of catechin, thiocyanate (SCN) and catechin + thiocyanate (SCN) administration on thyroid peroxidase activity. Thyroid peroxidase activity was decreased significantly ($P < 0.05$) after administration of catechin, thiocyanate (SCN) and catechin + thiocyanate (SCN) for 30 days when compared with the control. More pronounced decrease in the activity of the enzyme observed after catechin + thiocyanate (SCN) treatment in comparison to other groups treated singly with SCN or catechin.

Thyroidal 5'-deiodinase I assay

Figure 3 demonstrates thyroidal 5'-deiodinase I (5'-DI) activity in control, catechin, thiocyanate (SCN) and catechin + thiocyanate (SCN) treated experimental animals. A statistically significant ($P < 0.05$) inhibition in the activity of 5'-DI found in treated groups.

Thyroidal (Na⁺-K⁺)-ATPase assay

Figure 4 compares the activity status of thyroidal (Na⁺-K⁺)-ATPase in control and experimental animals. (Na⁺-K⁺)-ATPase activity was significantly ($P < 0.05$) decreased after administration of thiocyanate (SCN) and catechin + thiocyanate (SCN) for 30 days when compared to controls however increased (Na⁺-K⁺)-ATPase activity was found in catechin treated group.

Thyroid hormone level

Figures 5 and 6 show serum T3 and T4 levels in control, catechin, thiocyanate (SCN) and catechin + thiocyanate treated animals. The serum T3 and T4 levels were significantly ($P < 0.05$) decreased in animals after administration of catechin, thiocyanate (SCN) and catechin + thiocyanate as compared to controls. Intergroup variation was also noticed as shown in the figure. Like thyroid hormone profiles, significant alteration ($P < 0.05$) was also noted in serum TSH level in the treated groups as compared to control (Figure 7).

Urinary iodine and urinary thiocyanate concentration

Urinary excretion of iodine and thiocyanate were significantly ($P < 0.05$) increased in the catechin, thiocyanate (SCN) and catechin + thiocyanate treated groups when compared against control. Intergroup variation between the treated groups was also recorded as depicted in Figure 8.

Histological studies of thyroid

Histological assessments performed on thyroid sections from the different groups of animals are presented in Plate 1. In control rats, thyroid follicles were lined by low cuboidal epithelial cells filled with colloid and all the follicles were almost equal and regular in size. In the thyroids of the catechin, thiocyanate (SCN) and catechin + thiocyanate treated rats; there was an increase in the number of irregularly-shaped small follicles filled with relatively less colloid, including hypertrophied and hyperplastic follicular epithelial cells.

Morphometric / histometric analysis

The data of morphometric / histometric analysis of thyroid gland of the control and treated groups are depicted in Table-1. that showed increase in the areas of both the follicular cells as well as colloid in the treated groups than that of the control group. Semiquantitative assessment of thyroid follicles of control group was shown mostly to be made up of small and medium sized follicle with a relatively few large follicle while the large and medium sized follicles increased in catechin, thiocyanate (SCN) and catechin + thiocyanate exposed group of animals showed in Table-1. Maximum changes were found in catechin + thiocyanate treated group followed by thiocyanate (SCN) and catechin that indicates a development of catechin, thiocyanate and thiocyanate + catechin induced cytomorphological modifications.

DISCUSSION

Factors other than iodine deficiency are known to interfere with thyroid metabolism. Antithyroid effects of feeding goitrogenic foods containing thiocyanate precursor are well established^{8,10,27,28}. It has been also reported that tea extracts, both green and black, have potent antithyroidal activity for the presence of flavonoids^{15,16}. In different

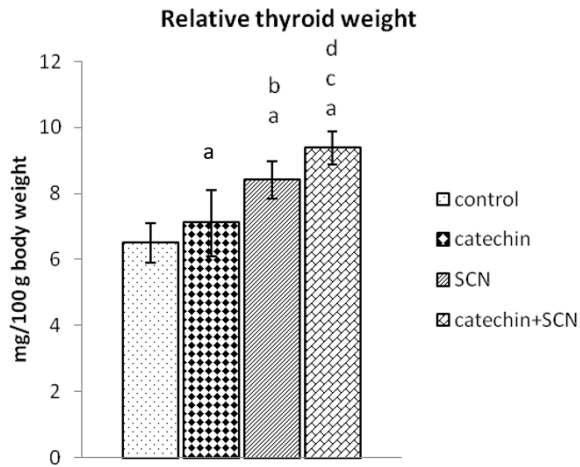


Figure 1. Changes in the relative thyroid gland weight of rats administered with catechin, thiocyanate (SCN) and catechin + thiocyanate (SCN) for 30 days. Each bar denotes mean ± SD, n = 8. One-way analysis of variance (ANOVA) test followed by Tukey's post hoc test was performed to determine differences across means of different groups. ^a Control versus others groups (p<0.05); ^b catechin versus thiocyanate (SCN) (p<0.05); ^c thiocyanate (SCN) versus thiocyanate (SCN) + catechin (p<0.05); ^d thiocyanate (SCN) + catechin versus catechin (p<0.05).

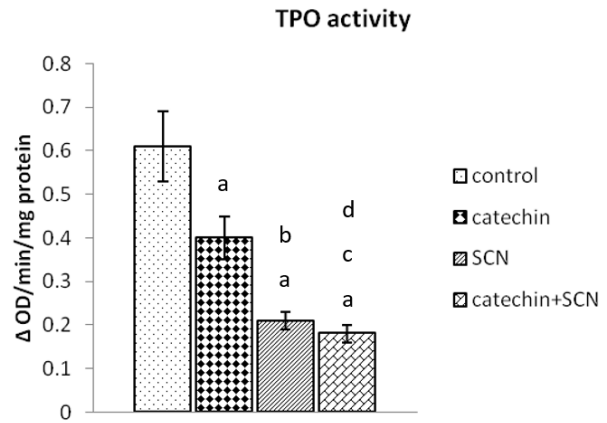


Figure 2. Thyroid peroxidase (TPO) activity of catechin, thiocyanate (SCN) and catechin + thiocyanate (SCN) treated animals for 30 days. Each bar denotes mean ± SD of three pooled samples. Each pool contained a mixture of three thyroid glands isolated from three individual rats. One-way analysis of variance (ANOVA) test followed by Tukey's post hoc test was performed to determine differences across means of different groups. ^a Control versus others groups (p<0.05); ^b catechin versus thiocyanate (SCN) (p<0.05); ^c thiocyanate (SCN) versus thiocyanate (SCN) + catechin (p<0.05); ^d thiocyanate (SCN) + catechin versus catechin (p<0.05).

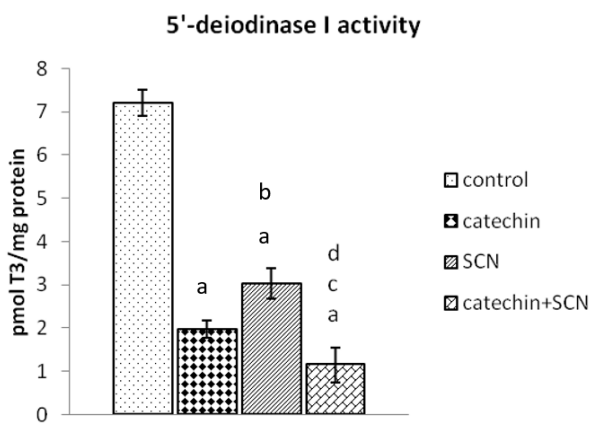


Figure 3. Thyroid 5'-deiodinase I (5'-DI) activity of catechin, thiocyanate (SCN) and catechin + thiocyanate (SCN) treated animals for 30 days. Each bar denotes mean ± SD of three pooled samples. Each pool contained a mixture of three thyroid glands isolated from three individual rats. One-way analysis of variance (ANOVA) test followed by Tukey's post hoc test was performed to determine differences across means of different groups. ^a Control versus others groups (p<0.05); ^b catechin versus thiocyanate (SCN) (p<0.05); ^c thiocyanate (SCN) versus thiocyanate (SCN) + catechin (p<0.05); ^d thiocyanate (SCN) + catechin versus catechin (p<0.05).

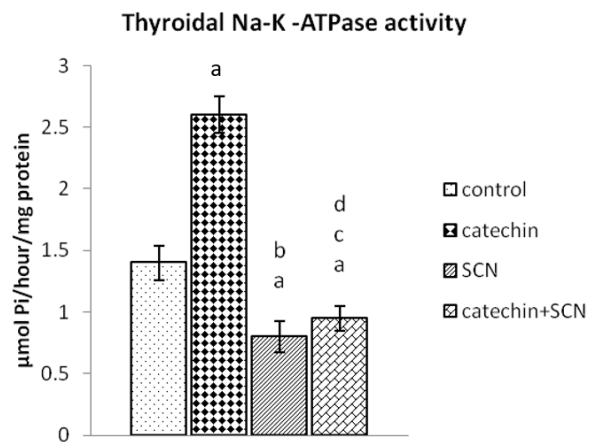


Figure 4. Thyroidal (Na⁺-K⁺)-ATPase activity of catechin, thiocyanate (SCN) and catechin + thiocyanate (SCN) treated animals for 30 days. Each bar denotes mean ± SD of three pooled samples. Each pool contained a mixture of three thyroid glands isolated from three individual rats. The assay was repeated twice. One-way analysis of variance (ANOVA) test followed by Tukey's post hoc test was performed to determine differences across means of different groups. ^a Control versus others groups (p<0.05); ^b catechin versus thiocyanate (SCN) (p<0.05); ^c thiocyanate (SCN) versus thiocyanate (SCN) + catechin (p>0.05); ^d thiocyanate (SCN) + catechin versus catechin (p<0.05).

goitre endemic regions people are exposed to thiocyanate (SCN), polyphenols or both for the consumption of goitrogenic foods that are used as common vegetables and

beverage. Many plant foods contain both the goitrogenic constituents viz. cyanogens and polyphenols and thus act synergistically on thyroid glands disrupting thyroidal

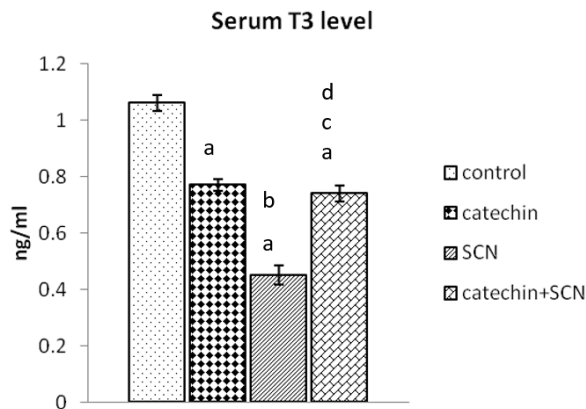


Figure 5. Serum T3 level of catechin, thiocyanate (SCN) and catechin + thiocyanate (SCN) treated animals for 30 days. Each bar denotes mean± SD. One-way analysis of variance (ANOVA) test followed by Tukey's post hoc test was performed to determine differences across means of different groups. ^a Control versus others groups ($p < 0.05$); ^b catechin versus thiocyanate (SCN) ($p < 0.05$); ^c thiocyanate (SCN) versus thiocyanate (SCN) + catechin ($p < 0.05$); ^d thiocyanate (SCN) + catechin versus catechin ($p > 0.05$).

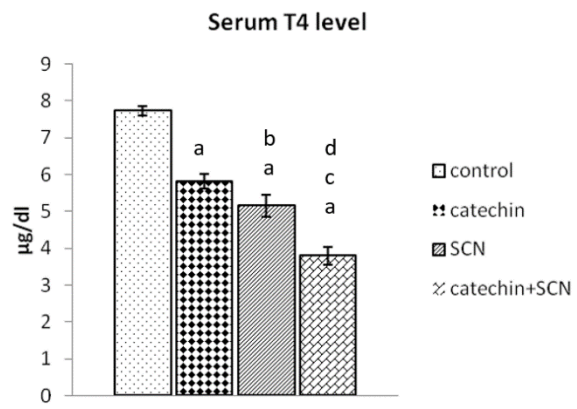


Figure 6. Serum T4 level of catechin, thiocyanate (SCN) and catechin + thiocyanate (SCN) treated animals for 30 days. Each bar denotes mean± SD. One-way analysis of variance (ANOVA) test followed by Tukey's post hoc test was performed to determine differences across means of different groups. ^a Control versus others groups ($p < 0.05$); ^b catechin versus thiocyanate (SCN) ($p < 0.05$); ^c thiocyanate (SCN) versus thiocyanate (SCN) + catechin ($p < 0.05$); ^d thiocyanate (SCN) + catechin versus catechin ($p < 0.05$).

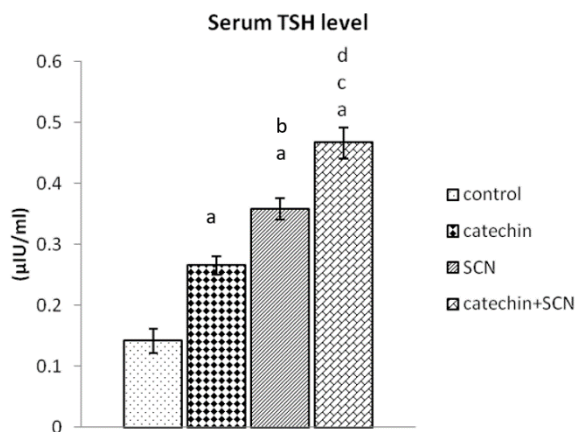


Figure 7. Serum TSH level of catechin, thiocyanate (SCN) and catechin + thiocyanate (SCN) treated animals for 30 days. Each bar denotes mean± SD. One-way analysis of variance (ANOVA) test followed by Tukey's post hoc test was performed to determine differences across means of different groups. ^a Control versus others groups ($p < 0.05$); ^b catechin versus thiocyanate (SCN) ($p < 0.05$); ^c thiocyanate (SCN) versus thiocyanate (SCN) + catechin ($p < 0.05$); ^d thiocyanate (SCN) + catechin versus catechin ($p < 0.05$).

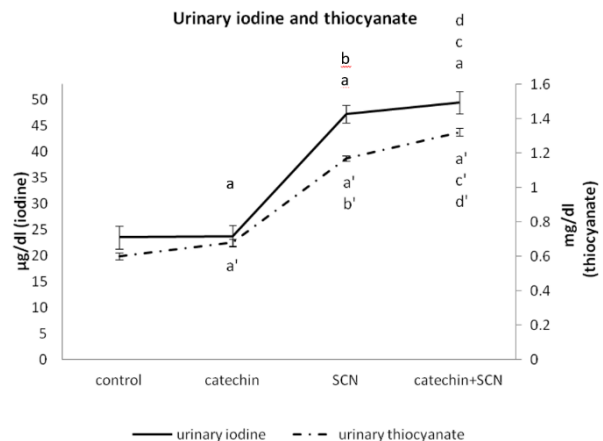


Figure 8. Urinary excretion of iodine and urinary thiocyanate of catechin, thiocyanate (SCN) and catechin + thiocyanate (SCN) treated animals for 30 days. Each bar denotes mean± SD, n = 8. One-way analysis of variance (ANOVA) test followed by Tukey's post hoc test was performed to determine differences across means of different groups. ^a Control versus others groups ($p < 0.05$); ^b catechin versus thiocyanate (SCN) ($p < 0.05$); ^c thiocyanate (SCN) versus thiocyanate (SCN) + catechin ($p < 0.05$); ^d thiocyanate (SCN) + catechin versus catechin ($p < 0.05$). ^{a'} Control versus others groups ($p < 0.05$); ^{b'} catechin versus thiocyanate (SCN) ($p < 0.05$); ^{c'} thiocyanate (SCN) versus thiocyanate (SCN) + catechin ($p < 0.05$); ^{d'} thiocyanate (SCN) + catechin versus catechin ($p < 0.05$).

synthesizing and secretory functions. However the information on the goitrogenic potency in comparative aspect after ingestion of equal concentration of thiocyanate, polyphenols (catechin) and both the

thiocyanate and polyphenols in combination is scanty. In the present investigation therefore a comparative evaluation of the goitrogenic / antithyroid potency of thiocyanate (potassium thiocyanate), catechin

Table 1: Morphometric/histometric and semiquantitative assessment of thyroid follicles of experimental animals administered with catechin, thiocyanate (SCN) and catechin + thiocyanate (SCN) for 30 days.

Groups	Parameters					
	Mean individual follicular area (mm ²)	Mean colloidal area in individual follicle (mm ²)	Small follicles (%)	Medium follicles (%)	Large follicles (%)	Immature follicles (%)
Control	1.38 ± 0.03	1.26 ± 0.02	43	37	16	4
Catechin	1.47 ± 0.04*	1.29 ± 0.03*	35	39	21	5
Thiocyanate (SCN)	1.62 ± 0.05*	1.44 ± 0.04*	28	39	30	3
Thiocyanate (SCN) + Catechin	1.90 ± 0.06*	1.77 ± 0.04*	22	40	35	3

One-way analysis of variance (ANOVA) test followed by Tukey's post hoc test was to determine differences across means of different groups. Values with asterisk (*) are significantly different by ANOVA at $p < 0.05$ when compared to control for 30 days. Data are presented as the mean \pm SD, $n = 8$. Small follicle, follicular diameter of 5–10 μm ; medium follicle, follicular diameter of 11–15 μm ; large follicle, follicular diameter of 16–20 μm ; and immature follicle having follicular diameter 0–5 μm .

(polyphenols) and their synergistic effect on thyroid in experimental animals have been investigated. The weight of the thyroid gland was higher in all the treated groups of rats as compare to control after feeding of thiocyanate, catechin and a combination of catechin and thiocyanate respectively. Histopathologically, a severe degree of follicular hyperplasia of thyroids was found in thiocyanate-catechin treated rats, developing a morphological state of hypothyroidism. This abnormality was of lesser magnitude after individual exposure of thiocyanate and catechin in experimental animals. Enlargement of thyroid gland that occurred under the influence of those goitrogenic substances might be for the reduced synthesis of T4 and T3 that in turn stimulates the release of TSH through hypothalamo-pituitary axis by compensatory mechanism¹⁵ that causes further growth and development of thyroid follicles resulting enlargement of thyroid gland weight¹⁵. Thyroid peroxidase (TPO) is the heme-containing enzyme and its activity is restricted to the apical membrane, but most of the thyroid TPO is intracellular, being located in the perinuclear part of the endoplasmic reticulum^{29,30}. The biosynthesis of thyroid hormone from thyroglobulin is catalyzed by thyroid peroxidase (TPO). Thiocyanate competes with iodide (I) trapping as well as its oxidation and binding to thyroglobulin at the level of the thyroperoxidase enzyme³¹. Hence, excess SCN induces both release of iodine from the thyroid cell and ultimately its loss from the body through urine. As a consequence, SCN ultimately results in an I-deficient thyroid and there occurs a decrease in thyroid hormone synthesis²⁸. Some of the natural flavonoids inhibit TPO *in vitro*^{16, 32}. It seems to be competitive; since the enzyme V_{max} was unchanged and K_m for iodide was significantly increased, it likely is able to scavenge H_2O_2 , an essential TPO cofactor³². The findings of this study provide evidence that the effects are substantially larger after combined exposure of thiocyanate and catechin than the effects seen on only after one factor at a time. Present investigation further suggests that both thiocyanate and

catechin are the potential antithyroid agents and decrease thyroid peroxidase (TPO) activity *in vivo* and the inhibition is more if they act synergistically. Through their inhibitory activity on thyroid peroxidase resulting less synthesis of thyroid hormone, they caused elevated TSH level, which promoted thyroid gland growth as observed in this study. Inside the cells, thyroid hormone molecules can be modified on a cell-specific fashion through the deiodinase group of enzymes. These are homodimeric Type I integral membrane selenoproteins composed of a single N-terminal trans-membrane segment connected to a larger globular domain with a selenocysteine-containing active centre embedded in a thioredoxin-like fold. The iodothyronine deiodinases catalyze the removal of an iodine residue from the pro-hormone thyroxine (T4) molecule, thus producing either the active form triiodothyronine T3 i.e. activation or inactive metabolites reverse T3 i.e. inactivation. Type I deiodinase (DIO1) catalyzes both reactions. DIO1 is expressed predominantly in the liver, kidney, and thyroid gland, but it is also detected in other organs, such as the heart, anterior pituitary gland, and lactating mammary gland. Catechin and combined exposure of thiocyanate-catechin significantly reduced the activity of DIO1 in the thyroid gland, suggesting that polyphenol decreases the rate of conversion of T4 to T3. It has previously been reported that synthetic flavonoids such as EMD21388, green tea, black tea, and natural plant-derived flavonoids inhibit DIO1 activity *in vivo*^{15,33}. Reduced DIO1 activity was also noted in thiocyanate administered group however there was no earlier report in this regard. The Na^+/I^- symporter (NIS) is the plasma membrane glycoprotein that mediates active I^- uptake into the thyroid follicular cells. The Na^+ gradient that provides the driving force for I^- uptake is maintained by the $(\text{Na}^+-\text{K}^+)\text{-ATPase}$. Both NIS and the $(\text{Na}^+-\text{K}^+)\text{-ATPase}$ are located on the basolateral side of the follicular cells. Thiocyanate (SCN^-) is a potent inhibitor like ouabain and perchlorate of iodine transport, acting as a competitor but without being concentrated in the thyroid and not inhibiting TSH-mediated cAMP

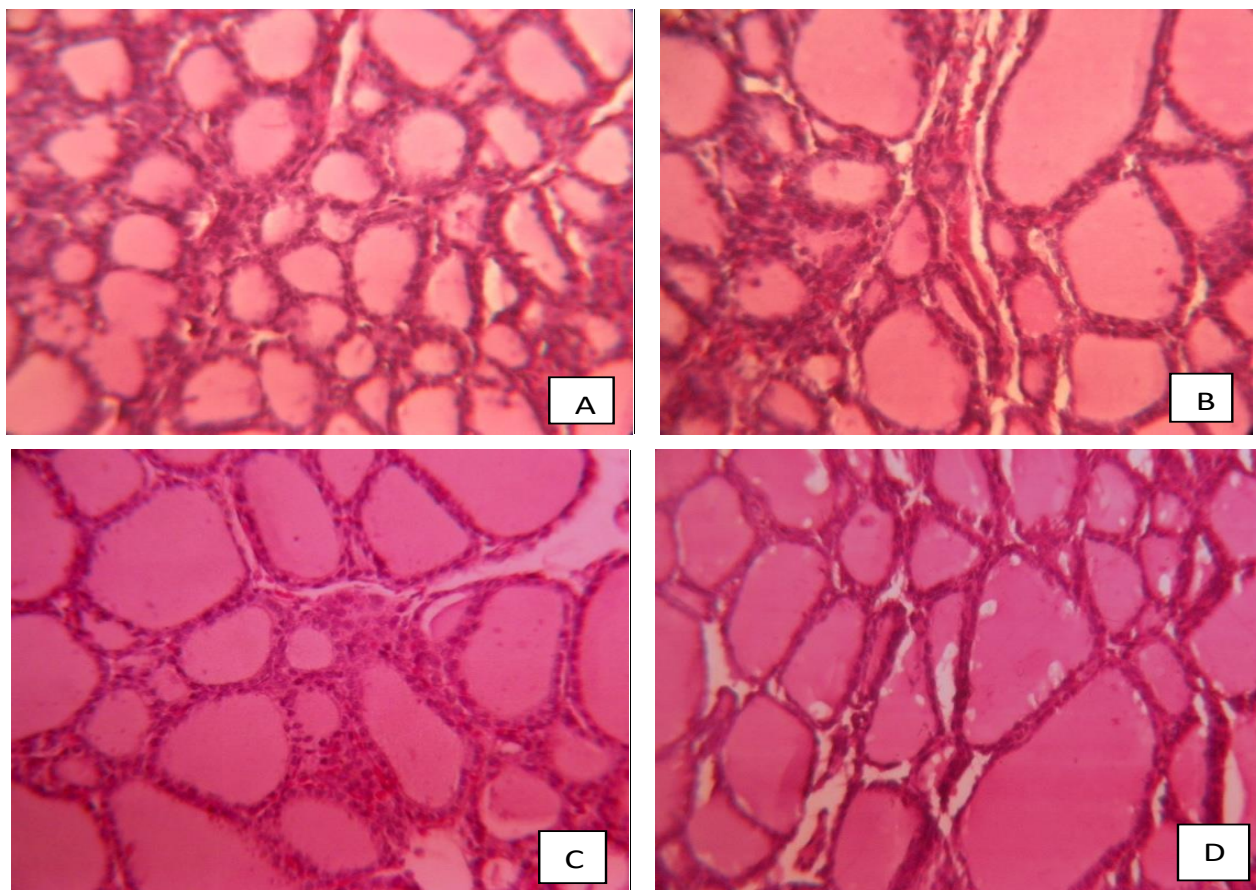


Plate 1. Photomicrographs of paraffin-embedded H&E-stained rat thyroid sections. (A) Rat thyroid section (400X) from control animals; thyroid follicles were lined by low cuboidal epithelial cells filled with colloid and all the follicles were almost equal and regular in size. (B) Rat thyroid section (400X) from treated with catechin for 30 days; there was an increase in the number of irregularly-shaped follicles filled with depleted colloid, and including hypertrophied and hyperplastic follicular epithelial cells. (C) Rat thyroid section (400X) from animals treated with thiocyanate (SCN) for 30 days; thyroid follicles were more irregular and larger with hypertrophy and hyperplasia of follicular epithelial cells. (D) Rat thyroid section (400X) from animals treated with thiocyanate (SCN) + catechin for 30 days; thyroid follicles were more irregular and larger with hypertrophy and hyperplasia of follicular epithelial cells. The number of small follicles is decreased.

production or $(\text{Na}^+ - \text{K}^+) - \text{ATPase}$ activity^{34, 35, 36}. In the present investigation, $(\text{Na}^+ - \text{K}^+) - \text{ATPase}$ activity was decreased after thiocyanate exposure and increased after catechin exposure; however after the combined exposure of thiocyanate-catechin, the activity of this enzyme found less than normal showing the predominance of thiocyanate over catechin. In this study, thiocyanate, catechin and thiocyanate-catechin induced hypothyroidism presumably results from an elevated circulating thyroid-stimulating hormone (TSH) concentrations in response to the inhibition of thyroid hormone synthesis. This has been evidenced by high circulating level of TSH and corresponding decrease in T4 level. Urinary iodine excretion is a good marker of the very recent dietary intake of iodine and therefore, is the index of choice for evaluating the degree of iodine deficiency and of its correction. Urinary iodine reflects iodine nutritional status because 90% of ingested iodine is excreted in the urine³⁷. In the present study the urinary excretion of iodine was markedly high in thiocyanate and thiocyanate-catechin treated group of rats but no alteration was found in control

and catechin treated group of rats because thiocyanate when present in excess concentration, also stimulates the efflux of iodide from thyroid gland^{28,38,39} that results in increased excretion of iodine through urine⁴⁰. This study consolidates earlier findings that the iodine-retaining capacity of the thyroid gland/body depends on the concentration of thiocyanate as well as the amount of iodine ingested⁹. Serum T3 and T4 levels found significantly lower in the treated groups as compared to controls. Decreased circulating hormone levels were associated with decreased thyroid hormone synthesis due to inhibition of TPO activity of the thyroid gland under the influence of thiocyanate and catechin. We can explain this result by the fact that thiocyanate has an inhibitory effect on T4 5'-monodeiodination (DI), as demonstrated by Langer⁴¹. It is known that flavonoids cause inhibition *in vitro* TPO activity^{16,17} and scavenge H_2O_2 ³². Inhibition of both iodide uptake and thyroidal iodide efflux due to excess thiocyanate⁴² and flavonoids for its anti TPO activity and deiodination might have decreased the synthesis of thyroid hormones. This result can be

explained by a feedback to the hypothalamo-pituitary-thyroid axis, indicating the low levels of T4 and T3 in the blood; hence, more thyroid-stimulating hormone (TSH) was released in order to increase the production of these hormones. Consequently, hypothyroidism and hypertrophy of the thyroid gland were evidenced. Thyroid histology was also altered in the treated groups. Thyroid follicles in treated rats were lined with follicular epithelial cells containing less colloid, irregular in size and shape whereas in control, thyroid follicles were almost regular in shape and of normal in size, filled with relatively more homogeneous colloid, lined by flat cuboidal epithelial cells. The number of thyroid follicles was greater in treated groups, indicating hypertrophy and hyperplasia of the follicular epithelium under the influence of thiocyanate and catechin. The severity was more with thiocyanate than in catechin and found highest in thiocyanate-catechin administered group. The findings of this study clearly provide evidence that when thiocyanate or catechin are administered separately in equal concentration the decrease of T4 and T3 hormone levels found more with thiocyanate than that of catechin. However the concomitant exposures of thiocyanate-catechin in similar concentration further decreased in T3 and T4 level than their individual exposure. All these indicate that the thiocyanate does not antagonise the action of catechin on thyroid when they act synergistically rather their action found potentiated.

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