Synergic actions of polyphenols and cyanogens of peanut seed coat (Arachis hypogaea) on cytological, biochemical and functional changes in thyroid

Amar K Chandra1*, Chiranjit Mondal1, Sabyasachi Sinha1, Arijit Chakraborty1 & Elizabeth N Pearce2

1Endocrinology & Reproductive Physiology Laboratory, Department of Physiology, University of Calcutta, Kolkata-700 009, West Bengal, India
2Section of Endocrinology, Diabetes and Nutrition, Boston University School of Medicine, Boston, Massachusetts, USA

Received 27 January 2014; revised 15 March 2014

In animals, long-term feeding with peanut (Arachis hypogaea) seed coats causes hypertrophy and hyperplasia of the thyroid gland. However, to date there have been no detailed studies. Here, we explored the thyroidal effects of dietary peanut seed coats (PSC) in rats. The PSC has high levels of pro-goitrogenic substances including phenolic and other cyanogenic constituents. The PSC was mixed with a standard diet and fed to rats for 30 and 60 days, respectively. Animals fed with the PSC-supplemented diet showed a significant increase in urinary excretion of thiocyanate and iodine, thyroid enlargement, and hypertrophy and/or hyperplasia of thyroid follicles. In addition, there was inhibition of thyroid peroxidase (TPO) activity, 5'-deiodinase-I (DIO1) activity, and (Na\(^{+}\)-K\(^{+}\))-ATPase activity in the experimental groups of rats as compared to controls. Furthermore, the PSC fed animals exhibited decreased serum circulating total T4 and T3 levels, severe in the group treated for longer duration. These data indicate that PSC could be a novel disruptor of thyroid function, due to synergistic actions of phenolic as well as cyanogenic constituents.

Keywords: (Na\(^{+}\)-K\(^{+}\))-ATPase activity, 5'-Deiodinase-I (DIO1) activity, Dietary goitrogens, Flavonoids, Goitrogenic activity, Groundnuts, Thyroid peroxidase (TPO) activity

Peanuts or groundnuts (Arachis hypogaea), are consumed in many forms such as boiled peanuts, peanut oil, peanut butter, and roasted peanuts. They are also added as peanut meal to snack food, energy bars, and candy. Peanuts are rich in calories and contain many nutrients, minerals, antioxidants and vitamins. Peanut seed coat (PSC) is a brown-coloured wafer thin edible coat covering the whitish seed within the hard non-edible shell. It contains glycosides (arachidoside) and flavonoids responsible for goitrogenic activity. Though the antithyroidal/goitrogenic potential of PSC has been suspected for decades, its goitrogenic constituents and antithyroidal potential have not been fully described. PSC contains flavonoids and some phenolic acids. Presence of resveratrol in PSC has led to increased PSC content in human diets. In the present study, we quantified the goitrogenic content (cyanogenic glucosides, glucosinolates, thiocyanate, and total polyphenols) of PSC and also explored its goitrogenic/anti-thyroid potential as evidenced by thyroid gland morphology, histology, thyroid functional status, thyroid peroxidase (TPO) activity, 5'-deiodinase-I activity, and (Na\(^{+}\)-K\(^{+}\))-ATPase activity in Wistar rats after prolonged dietary exposure.

Materials and methods

Reagents—DTE, imidazole, Na\(_{2}\)ATP and Tris-HCl were procured from SRL Mumbai, India. Ouabains, propyl thiouracil, thyroxine, bovine serum albumin, MnCl\(_{2}\) and EDTA, were procured from Sigma Chemical Company, Steinheim, Germany while SDS was procured from LOBA Chemie Pvt. Ltd, Mumbai, India, and Dowex 50WX8 from Alfa Aesar.

Animals and treatment—Ninety days old male adult Wistar strain albino rats weighing 170 ± 10 g were maintained following national guidelines and protocols as approved by the Institutional Animal Ethics Committee. All the animals were maintained in clean polypropylene made cages and kept in a temperature regulated environment at 22 °C ± 2 °C and relative humidity (40-60%) in the animal house with a constant 12 h L:D schedule. The animals were maintained on a standardized diet (wheat 70%, Bengal gram 20%, fish meal powder 5%, dry yeast...
powder 4%, refined sesame oil 0.75%, shark liver oil 0.25%) and water ad libitum. The experimental animals were divided into 4 groups of 10 animals each. The first two groups were fed with PSC diet for 30 and 60 days, respectively. Both the experimental groups were paired with respective control groups. Experimental rats in each group received two third normal laboratory diet and the remaining one-third of the diet was replaced by PSC procured from the local market of Kolkata Metropolitan city. Control rats were fed with normal laboratory standardized diet. At every 7th day feed consumption, corrected for wasted/unused feed, and the body weight of the animals were noted. In the final week of the treatment, animals of all the groups were placed in metabolic cages for 24 h for collection of urine in xylene for iodine and thiocyanate assay. The body weights of the rats were noted at the end of the experimental period also. The animals were sacrificed, depending upon the groups, respectively on the 30th and 60th days of the experiment.

All the animals were sacrificed 24 h after the last feeding (between 9-10 a.m. on the day of sacrifice to avoid any discrepancy that may cause diurnal variation) following standard protocols and ethical procedures. Samples of blood were collected from hepatic portal vein and serum was separated for hormone assay.

Measurement of cyanogenic glucosides—Cyanogenic glucosides were determined as per the procedure of Lambert et al. PSC weighing 10 mg to 1 g were hydrolysed using glucosidase (β-glucosidase, Sigma, USA); hydrocyanic acid (HCN), thus generated, was trapped in sodium hydroxide. Cyanide content of the trapped HCN was then quantitatively assayed in a spectrophotometer.

Measurement of thiocyanate—Thiocyanate was measured in PSC following Aldridge method as modified by Michajlovskij and Langer. For the purpose, PSC was extracted with thoroughly clean sand in water, and the water extract containing thiocyanate was treated with trichloroacetic acid, and then by saturated bromine water and arsenous trioxide, and finally allowed to react with pyridine-benzidine hydrochloride mixture. The colour intensity thus developed was determined by a spectrophotometer.

Measurement of glucosinolates—Glucosinolates were estimated following the method of Gmelin and Virtanen. Initially, a methanolic extract of PSC was prepared and evaporated in vacuo. The residue obtained was treated with lead acetate followed by hydrogen sulphide for precipitation of the excess lead ions present in it. The filtrate containing glucosinolate was further concentrated in vacuo and treated with myrosinase (thioglucosidase from Sigma, USA) to obtain thiocyanate. The thiocyanate was later assayed as previously done for thiocyanate estimation.

Measurement of total polyphenols—Total polyphenol content of PSC was determined following the method of Matthaus. In a 0.2 ml of PSC extract, 1 ml of Folin-ciocalteau (diluted 10 fold) and 0.8 ml of 2% Na₂CO₃ were added. The volume was further made to 10 ml adding water-methanol (4:6) as the diluting fluid and the absorbance was measured after 30 min at 740 nm using spectrophotometer. Using gallic acid standard solutions of known concentrations, the standard curve was prepared and the obtained results were expressed as mg gallic acid equivalent/g sample.

Measurement of urinary iodine and thiocyanate—The collected urine samples of the animals were digested in strong alkali followed by subsequent ashing in a muffle furnace at 600 °C, and iodide was assayed by its catalytic action on the reduction of ceric ion (Ce³⁺) to cerous ion (Ce³⁺) following the method of Karmarkar et al., maintaining internal quality control; urinary thiocyanate concentrations were assayed as done earlier following the established procedure.

Thyroid gland (weighing and histological study)—The rat thyroid glands were dissected and weighed immediately after sacrifice, and preserved in neutral buffered formalin (10%), and embedded in paraffin having melting point 55-58 °C. The relative weight of thyroid gland (mg) was described per 100 g body weight. Thyroid sections were prepared in microtome, preserved in clean slides and then stained with haematoxylin & eosin (HE) and observed under a light microscope.

ELISA of serum triiodothyronine (T3) and thyroxine (T4)—Prior to sacrificing the animals, blood samples were collected from the hepatic portal vein of each rat, and by centrifugation the serum was separated for T3 and T4 assay. All the serum samples were preserved at −50 °C prior to thyroid hormone assay. Serum total 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.
Assay of thyroid peroxidase (TPO) activity—All the thyroid glands collected from the sacrificed animals were pooled and a homogenate (10%) was prepared of thyroid tissue in sucrose solution (500 mM) and phosphate buffer (pH 7.2, 100 mM) at 4°C. Homogenization of the thyroid samples was conducted in a glass homogenizer of Potter-Elvehjem, Germany. The homogenate was centrifuged at 1000 g for 10 min and the low-speed supernatant was centrifuged further at 10000 g for 10 min at 4°C to get the mitochondrial fraction. The maximal peroxidase activity is normally found in the microsomal fraction obtained by centrifuging the post-mitochondrial supernatant at 105000 g for one h only. The precipitate was dissolved immediately after centrifugation in phosphate buffer; thyroid peroxidase activity was measured in a 1 ml quartz cuvette containing 0.9 µl acetate buffer (pH 5.2, 50 mM). An amount of 10 µl potassium iodide (1.7 mM), 20 µl microsomal fraction of thyroid tissue, and 20 µl freshly prepared hydrogen peroxide (0.3 mM) were added to initiate the reaction for assay of TPO activity (ΔOD/min/mg protein) in a spectrophotometer (UV-1240 Shimadzu) at wavelength of 353 nm. The pooled sample was assayed twice to avoid any error. The activity of thyroid peroxidase was measured following the Alexander method\textsuperscript{11}. The protein level of thyroid tissue was determined by the method of Lowry et al.\textsuperscript{12} using bovine serum albumin as a standard. The obtained results were expressed as change in optical density (ΔOD)/min/mg protein.

5'-Deiodinase I (5'-DI) assay—Iodothyronine 5'-deiodinase type I (DIO1) activity was determined by slightly modifying the method of Kodding et al.\textsuperscript{15}. In brief, 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 in a final volume of 400 ml was incubated for 30 min at 37°C. The monodeiodination reaction of T4 to T3 was terminated adding 800 µl ice-cold absolute ethanol (Bengal Chemicals, Kolkata) by shaking for 8 min at 4°C. The reactants were then centrifuged at 10500 g for 8 min at 4°C, and the supernatants in ethanol were collected for assay of T3 content. For all the 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 determined by ELISA. The activity of DIO1 was calculated as the difference between 0 and 30 min values and expressed in terms of 'p' moles T3 formed/mg protein. The pooled sample was also assayed in duplicate to minimize errors. The validity of this assay method was determined by pre-incubating the sample with PTU (propylthiouracil), a strong DIO1 inhibitor, that resulted in >50% inhibition of the enzymatic activity. Transformation of T4 to rT3 by DIO1, unlike T4 to T3 monodeiodination, cannot proceed under such simulated conditions, but rT3 formation can occur only under high pH and substrate concentrations.

Thyroidal Na\textsuperscript+-K\textsuperscript+-ATPase assay—Na\textsuperscript+-K\textsuperscript+-ATPase activity of thyroid was measured by the modified method of Esmann et al.\textsuperscript{14}. In nut-shell, 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 MgCl\textsubscript{2} and (ii) 1 mM ouabain (Sigma Chemical Co., St. Louis, MO 63178, USA) at pH 7.4 at 0°C for 60 min. The reaction initiated by adding 4 mM Tris-ATP at 37°C and was stopped by adding 0.1 mL of 20% SDS after 10 min. Inorganic phosphate (Pi) thus liberated was spectrophotometrically measured by recording the absorbance in a UV-mini1240 spectrophotometer, (Shimadzu, Japan) at 850 nm, as per Baginski et al.\textsuperscript{15}. The Na\textsuperscript+- K\textsuperscript+-ATPase activity was expressed as 'n' mols of (Pi) liberated per hour per mg protein extrapolated from a standard curve of potassium dihydrogen phosphate. The pooled sample was assayed in duplicate.

Protein estimation—The method of Lowry et al.\textsuperscript{12} was followed for protein estimation in the samples by using bovine serum albumin (BSA) as standard.

Statistical analysis—Obtained results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was first carried out to test differences across the mean values of all groups. If the differences between-group 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

Goitrogen content—The goitrogen content of PSC, measured in mg/kg wet weight, was as follows: Free thiocyanate, 150±10.3; Cyanogenic glucosides, 2.4±0.7; and glucosinolates, 1.3±0.4. Total polyphenol content was 38.50±4.6 mg of Gallic acid eq/gm dry weight.
Body weight and food consumption—The body weight of the control animals increased progressively throughout the period of investigation, with a net body weight gain of +11.75 g and +21.0 g at 30 days and 60 days, respectively (Table 1). However, the net body weight gain of animals fed with PSC was only +8.5 g at 30 days and +18 g at 60 days. Total food consumption by weight was similar in all the groups including control animals. In the 30-day groups, average food consumption of the control rats was 21 g/day and that of the rats consuming PSC was 22.5 g/day ($P > 0.05$). Similarly, in the 60-day groups, average food consumption of the control rats and PSC fed rats was 21.5 g/day and 23.25 g/day ($P > 0.05$), respectively (Fig. 1).

Thyroid weight—The weight of the thyroid glands significantly increased ($P < 0.05$) after PSC administration in a time dependent-manner in both the 30-day and 60-day groups as compared to their respective control groups. Changes in the thyroid gland weight were noticed between the 30 -days and 60- days PSC exposed groups also (Fig. 2).

Thyroid peroxidase assay—The PSC treatment did influence the thyroid peroxidase activity (Fig. 3A). TPO activity was decreased significantly ($P < 0.05$) after PSC administration for 30 and 60 days compared to the respective control groups. The 60-day treatment caused a more pronounced decrease ($P < 0.05$) in the enzyme activity than the 30-day treated group.

Thyroidal 5’-deiodinase I assay—PSC exhibited a statistically significant ($P < 0.05$) inhibition against DIO1 activity in a time dependent manner. Significant changes ($P < 0.05$) was also found between the 30-day and 60-day treated groups (Fig. 3B).

Table 1—Changes in body weight (g) of experimental animals after feeding Peanut seed coat (PSC) for 30 and 60 days

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Body weight gain (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Control for 30 days</td>
<td>174.0±6.25</td>
<td>185.75±8.50</td>
</tr>
<tr>
<td>PSC for 30 days</td>
<td>180.50±6.5</td>
<td>189.0±5.50</td>
</tr>
<tr>
<td>Control for 60 days</td>
<td>173.50±7.0</td>
<td>194.50±7.25</td>
</tr>
<tr>
<td>PSC for 60 days</td>
<td>179.5±6.5</td>
<td>197.50±6.50</td>
</tr>
</tbody>
</table>

$^a$Data are presented as the mean ± SD, n = 8. ANOVA test followed by Tukey’s post hoc test was performed; $^b$Significantly different by ANOVA at $P < 0.05$ when compared to control.
Thyroidal (Na\(^{+}\)-K\(^{+}\))-ATPase assay—Similarly, (Na\(^{+}\)-K\(^{+}\))-ATPase activity was also found significantly \((P < 0.05)\) decreased after administration of PSC for 30 days and 60 days compared to controls. However, changes between the 30-day and 60-day PSC fed groups were not significant (Fig. 3C).

Thyroid hormone level—The serum T3 and T4 levels were found significantly \((P < 0.05)\) decreased in animals administered with PSC as compared to controls. Further, significant changes \((P < 0.05)\) were also noticed between the treated groups depending on the duration of the treatment. (Fig. 4)

Urinary iodine and thiocyanate concentration—Urinary excretion of iodine had also significantly \((P < 0.05)\) increased in the PSC-fed groups compared to controls. The increase in the 60-day treated group was significant \((P < 0.05)\) than the 30-day treated group (Fig. 5). Similarly, rats fed with PSC showed significant \((P < 0.05)\) increase in urinary thiocyanate concentration as compared to their respective controls. Thiocyanate excretion was higher in the group fed PSC for a longer duration (Fig. 5).

Histological studies of thyroid—Histological assessments performed on thyroid sections from the different groups 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 PSC-fed rats there was an increase in the

![Fig. 4](image-url) — Serum T3 (---) and serum T4 (-----) levels of PSC-fed animals for 30 days and 60 days, respectively. Each line denotes mean± SD, \(n = 8\). Differences across means of different groups were determined by ANOVA test followed by Tukey’s post hoc test. T4 values: \(^{*}\)Control 30 days vs. PSC-fed animals for 30 days \((P < 0.05)\); \(^{b}\)Control 60 days vs. PSC-fed animals for 60 days \((P < 0.05)\); \(^{c}\)PSC treated 30 days vs. PSC-fed animals for 60 days \((P < 0.05)\), T3 values: \(^{a}\)Control 30 days vs. PSC-fed animals for 30 days \((P < 0.05)\); \(^{b}\)Control 60 days vs. PSC-fed animals for 60 days \((P < 0.05)\); \(^{c}\)PSC treated 30 days vs. PSC-fed animals for 60 days \((P < 0.05)\).

![Fig. 5](image-url) — Urinary excretion of thiocyanate (-----) and iodine (-----) of PSC-fed animals for 30 days and 60 days. Each denotes mean± SD, \(n = 8\). Differences across means of different groups were determined by ANOVA test followed by Tukey’s post hoc test. Urinary iodine: \(^{a}\)Control 30 days vs. PSC-fed animals for 30 days \((P < 0.05)\); \(^{b}\)Control 60 days vs. PSC-fed animals for 60 days \((P < 0.05)\); \(^{c}\)PSC treated 30 days vs. PSC-fed animals for 60 days \((P < 0.05)\). Urinary thiocyanate: \(^{a}\)Control 30 days vs. PSC-fed animals for 30 days \((P < 0.05)\); \(^{b}\)Control 60 days vs. PSC-fed animals for 60 days \((P < 0.05)\); \(^{c}\)PSC treated 30 days vs. PSC-fed animals for 60 days \((P < 0.05)\).

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 peanut seed coat (PSC) (30 days); there was an increase in the number of irregularly-shaped small follicles filled with relatively less colloid, and including hypertrophied and hyperplastic follicular epithelial cells. (C) Rat thyroid section (400X) from animals treated with PSC (60 days); thyroid follicles were more irregular and larger with hypertrophy and hyperplasia of follicular epithelial cells. The number of small follicles is decreased.
number of irregularly-shaped small follicles filled with relatively less colloid, and including hypertrophied and hyperplastic follicular epithelial cells. No neoplastic changes of thyroid follicular cells were observed.

Discussion

Variety of environmental substances, including foods, can induce goitre in rats\textsuperscript{16}. Regular consumption of cyanogenic foods containing cyanogenic glucosides, glucosinolates and thiocyanate affect thyroid physiology and may lead to the development of endemic goitre, especially in iodine deficient environments\textsuperscript{17}. We and others have previously 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 studies\textsuperscript{16-18}. Bamboo shoot consumption, even in the presence of adequate iodine, may cause endemic goiter\textsuperscript{16,19}. The content of the goitrogenic substances in the same foods grown in different regions is not uniform; the same foodstuff may show large differences in goitrogen content from region to region due to genetic and ecologic factors\textsuperscript{17}. Tea extracts, both green and black, have also shown potent anti-thyroidal activity possibly due to the presence of flavonoids\textsuperscript{20,21}.

Peanuts are enriched with nutrients that are beneficial to human health. However, peanut seed coat (PSC) contains a variety of dietary goitrogens, including cyanogenic glycosides, glucosinolates, thiocyanate as well as polyphenolic flavonoids. These goitrogenic substances may act in conjunction with iodine deficiency to cause endemic goitre and associated disorders\textsuperscript{22}. However, information regarding the systemic quantification of different goitrogenic/anti-thyroid components of PSC, from Indian region in particular is limited and that in fact prompted this study.

Cyanogenic glucosides are widely distributed in the plant kingdom; more than 2500 different plant species and about 120 different glucosinolates have been reported\textsuperscript{23}. The glucosinolates are a class of organic compounds (water soluble anions) that contain sulphur, nitrogen and a group derived from glucose\textsuperscript{24}. Cyanogenic glucosides are phytoanticipins which readily convert into the active goitrogenic agent thiocyanate by glucosidases and sulphur transferase enzymes present in plant and animal tissues\textsuperscript{23,24}. Their function in plants is dependent on activation by β-glucosidases, which release toxic volatile HCN as well as ketones or aldehydes in order to fend off herbivore and pathogen attack\textsuperscript{25}. Glucosinolates undergo a rearrangement to form isothiocyanate derivatives; isothiocyanates react spontaneously with amino groups to form thiourea that interferes with organification of iodide and formation of thyroid hormone. This action cannot be antagonised by iodide\textsuperscript{26}. In experimental animals, it has been demonstrated that a high consumption of flavonoids diminishes thyroid iodide uptake and thyroid peroxidase (TPO) activity; however, inhibitory potencies have differed across studies\textsuperscript{27}.

In our study, we observed increased weight of the thyroid gland in PSC-fed groups, and higher increase in rats fed PSC for 60 days compared to the 30-day group or controls. This increase might be due to increased secretion of TSH in the PSC fed group, although TSH was not measured. A high incidence of thyroid enlargement in rats was found in earlier studies of rats fed goitrogenic substances other than PSC\textsuperscript{18,28,29}. Urinary excretion of thiocyanate is a marker for exposure to cyanogenic foods\textsuperscript{7,18}. Thus, the increased concentration of thiocyanate in the urine of PSC-fed rats indicates that these goitrogens were metabolized to thiocyanate. The concentration of thiocyanate in urine was higher in the groups fed PSC for a longer duration revealing a cumulative effect due to chronic ingestion. Previous studies also reported that the presence of dietary cyanogenic glucosides/glucosinolates causes increased urinary excretion of iodine\textsuperscript{30,31}. Urinary iodine excretion too showed marked increase after PSC administration. Urinary iodine reflects iodine nutritional status because 90% of ingested iodine is excreted in the urine\textsuperscript{32}. The free thiocyanate released by the metabolism of different cyanogenic constituents present in PSC might have prevented the uptake of iodide by the thyroid gland and stimulated the efflux of iodide from thyroid, resulting in increased urinary iodine concentrations. This study confirms 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 ingested\textsuperscript{30}.

TPO is the heme-containing enzyme found in the apical membrane of thyroid follicular cells that catalyzes the reactions required for thyroid hormone synthesis. Iodination of tyrosyl residues in thyroglobulin and subsequent oxidative coupling
yields T4 and T3. Thiocyanate and thiocyanate-like compounds inhibit thyroidal iodide-concentrating mechanisms and form insoluble iodinated thyroglobulin in the thyroid. A high concentration of thiocyanate is also responsible for inhibition of TPO-catalysed oxidation (I → I2). This study demonstrates that administration of PSC decreases the activity of thyroid peroxidase in a time-dependent fashion. More pronounced inhibition in TPO activity was found after chronic exposure. Some of the natural flavonoids inhibit TPO in vitro. Flavonoids from Kalanchoe brasilienis, a popular medicinal plant used in Brazil, have been reported to cause competitive in vitro TPO inhibition, competitive, since the enzyme Vmax remians unchanged while Km for iodide significantly increases, and thereby scavenges H2O2, an essential TPO cofactor. Available literature shows that PSC is rich in dietary flavonoids, a popular medicinal plant derived from P. sativum, which have been shown to cause significant inhibition of TPO activity in vitro and in vivo. This study further demonstrates that the TPO-inhibiting activity of PSC is due to not only its cyanogenic glucosides but also the polyphenolic constituents.

The enzyme 5’-iodotyrosine deiodinase (Type 1 deiodinase, or DIO1) is responsible for converting T4 to T3, the biologically active hormone. The DIO1 can catalyse both, the activation of T4 by outer ring deiodination, and the inactivation of T4 by inner ring deiodination, to produce rT3. DIO1 is expressed predominantly in the liver, kidney and thyroid gland. It is also detected in other organs, such as the heart, anterior pituitary gland and lactating mammary gland. It functions as a homodimer of a 27-kDa subunit encoded by 2.1-kb messenger RNA (mRNA). PSC exposure significantly reduced the activity of DIO1 in the thyroid gland, which is also dependent on the duration of exposure for the cumulative effects of the polyphenolic constituents present in the seed-coat suggesting that polyphenol decreased 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 vitro.

Active transport of iodide into the thyroid gland is a crucial and rate-limiting step in the biosynthesis of thyroid hormones which, in turn, play an important role in the processes of cell growth and proliferation. NIS co-transport two sodium ions along with one iodide ion, with the transmembrane sodium gradient serving as the driving force for iodide uptake. This sodium gradient, providing the energy for this transfer, is generated by the ouabain-sensitive (Na+–K+)-ATPase. NIS-mediated iodide transport is, therefore, inhibited by the (Na+–K+)-ATPase inhibitor ouabain as well as by the competitive inhibitors thiocyanate and perchlorate and is stimulated by TSH. PSC contain glucosinolates which are metabolised and converted to thiocyanate, isothiocyanate and nitrile-like active goitrogenic substances. Earlier studies have demonstrated that (Na+–K+)-ATPase activity could be competitively inhibited by isothiocyanates that are bound exclusively to the —SH group of the cysteine residue. The effects of flavonoids on proteins and enzymes such as topoisomerases (Na+–K+)-ATPases, heat shock proteins, cell cycle proteins and tyrosine kinases are well known. Therefore, it can be assumed that iodine influx will be decreased by flavonoid exposure. They also found that the efflux of iodide was increased by all flavonoids with the exception of myricetin. This may help to explain the overall decrease in intrathyroidal iodide accumulation after exposure to flavonoids. This is also evidenced by deeply stained eosinophilic colloid in follicles, indicating the presence of less iodine. Other mechanisms, such as the interference of flavonoids with NIS mRNA half-life, may also be relevant. An interesting finding in this study was the decreased activity of (Na+–K+)-ATPase in a dose-dependent manner. In the present study, decreased (Na+–K+)-ATPase activity may have contributed both to development of structural thyroid changes as well as hypothyroidism.

Another finding is the decreased serum T3 and T4 levels in the treated groups as compared to controls. The severity was more in the group after chronic exposure possibly for the cumulative effect of the goitrogenic constituents present in PSC. 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 goitrogens present in PSC. As discussed earlier, it is also known that the flavonoids cause in vitro TPO activity inhibition and scavenge H2O2, an essential TPO cofactor. Inhibition of both iodide uptake and thyroidal iodide efflux due to excess thiocyanate and flavonoids might be another reason for the decreased synthesis of thyroid hormones.

Thyroid histology was also altered in the treated groups. Thyroid follicles in treated rats were lined with follicular epithelial cells containing less colloid, were reduced in size, were irregularly shaped, and
stained deeply with eosin. Whereas in the controls, thyroid follicles were almost regular in shape and normal in size, filled with relatively more homogeneous colloid, and lined by flat cuboidal epithelial cells. The number of thyroid follicles was greater in the treated groups, indicating hypertrophy and hyperplasia of the follicular epithelium under the influence of cyanogenic constituents. The deeply-stained cosinophilic colloid in the follicles of the treated animals indicate that the entry of iodine into the follicles for synthesis of thyroid hormones was reduced. After prolonged stimulation of the pituitary-thyroid axis by dietary goitrogen, hyperplasia may progress to neoplasia. Cessation of exposure prior to the induction of neoplasia results in return to the normal state. There was no occurrence of thyroid neoplastic cells in chronic PSC exposed animals indicating that after the withdrawal of the treatment, thyroid gland would get back to normal state.

Conclusion
This study suggests that chronic exposure of peanut seed coat (PSC) rich in polyphenols and cyanogens synergistically inhibits the activity of thyroid hormone synthesizing enzymes (TPO, DIO1 and Na\(^+\)-K\(^+-\)ATPase) which, in turn, decrease T3 and T4 synthesis and bring about cytomorphological changes. It results in enlargement of the thyroid gland and hypothyroidism. In addition, the possible interaction between consumption of PSC and low intake of dietary iodine may constitute an important finding that requires further study in human populations.

Acknowledgment
The financial assistance by Rajiv Gandhi National Fellowship Scheme, University Grants Commission (UGC), New Delhi to the second author Chiranjit Mondal is gratefully acknowledged.

References
21 Chandra AK, De N & Choudhury SR, Effect of different doses of un-fractionated green and black tea extracts on thyroid physiology. Hum Exp Toxicol, 8 (2011) 884.
29 Sharpless GR, Pearsons J & Prato GS, Production of goiter in rats with raw and treated soy bean flour. J Nutr, 17 (1939) 545.
41 Spitzweg C & Morris J C, Sodium iodide symporter (NIS) and thyroid, Hormones, 1 (2002) 22.