Effect of bamboo shoot, *Bambusa arundinacea* (Retz.) Willd. on thyroid status under conditions of varying iodine intake in rats

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Young shoots or sprouts of common bamboos are used as food in third world countries. Evidence suggests the presence of cyanogenic glucoside like anti-thyroidal substance in bamboo shoots (BS) but effect of prolonged BS consumption on thyroid status under conditions of varying iodine nutriture remains unexplored. The study was undertaken to evaluate goitrogenic content, *in vitro* anti thyroid peroxidase (TPO) activity and *in vivo* anti thyroid potential of BS with and without extra iodide. Fresh BS contains high cyanogenic glucoside (551 mg/kg), followed by thiocyanate (24 mg/kg) and glucosinolate (9.57 mg/kg). *In vitro* inhibition in TPO activity was found with raw, raw boiled and cooked extracts. Inhibition constant (IC50) and PTU equivalence of fresh BS were 27.5 ± 0.77 μg and 3.27 respectively. Extra iodide in the incubation media reduced TPO inhibition induced by BS but could not cancel it. Thyroid weight, TPO activity and total serum thyroid hormone levels of BS fed animals for 45 and 90 days respectively were determined and compared with controls. Significant increase in thyroid weight as well as higher excretion of thiocyanate and iodine along with marked decrease in thyroid peroxidase activity, T4 and T3 levels were observed in BS fed group. Chronic BS consumption gradually developed a state of hypothyroidism. Extra iodide had reduced the anti-thyroidal effect of BS to an extent but could not cancel it because of excessive cyanogenic glucoside, glucosinolate and thiocyanate present in it.

**Keywords:** Bamboo shoot, Cyanogenic glucosides, Glucosinolates, Thiocyanate, Iodide, Thyroid peroxidase, Thyroid hormones

**IPC Code:** Int. Cl. A61K35

The young shoots or sprouts of common bamboos, *Bambusa arundinacea* (Retz.) Willd., family Graminaceae are used as staple food as well as pickle and chutney in third world countries including India. Varying amounts of cyanogenic glucosides have been reported from different species of bamboo shoots (BS). The cyanogenic glucoside present in BS is taxiphyllin [2-(b-D-glucopyranosyloxy)-2-(4-hydroxyphenyl) acetonitrile]. Regular consumption of cyanogenic glucosides, glucosinolates and thiocyanate, the goitrogenic/anti-thyroid constituents of cyanogenic foods, affect thyroid physiology and may develop endemic goitre in long run. These dietary goitrogens disrupt the biosynthesis of thyroid hormones in several ways that include inhibition of the iodide trapping mechanism, blockage of organic binding of iodine to thyroglobulin and coupling reaction of thyroglobulin. The effect of regular consumption of BS on thyroid functions or information about its goitrogenic/anti-thyroid potential under conditions of varying iodide intake is scanty.

In the present study, the goitrogenic contents of fresh BS and *in vitro* thyroid peroxidase (TPO) activity of human thyroid tissue adding raw, raw boiled and cooked extracts under different iodide concentrations were measured. The influence of BS feeding with and without iodide supplementation in rats for different durations on thyroid status have also been evaluated determining urinary iodine and thiocyanate concentrations, thyroid weight, *in vivo* TPO activity and thyroid hormone profiles.

**Materials and Methods**

**Collection of BS**—Fresh bamboo shoots were collected from the local markets for the measurement
of goitrogenic constituents, assay of in vitro TPO activity and also for feeding the experimental animals.

Animals—Wistar rats (80) weighing 80 ± 5 g were allocated to control and experimental groups of ten each. Animals were caged in unheated well-ventilated stainless steel cages and maintained in the laboratory on standardized normal diet (20% protein) 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.

Animal treatment—Control rats were fed normal laboratory diet. BS fed group rats were fed normal laboratory diet replacing one-third portion of the diet with fresh BS. In potassium iodide (KI) supplemented group, rats were fed normal laboratory diet with extra iodide 12-14μg/rat/day. In BS fed potassium iodide (KI) supplemented group, rats were fed both BS (1/3 portion of diet) and extra iodide 12-14μg/rat/day. The animals were maintained with above-mentioned regimen for 45 days and 90 days respectively dividing into two sets. Based on alteration of body weight, the first set of rats, treated for 45 days were considered as growing rats, while the second set of rats treated for 90 days were considered as mature rats.

Feed consumption, corrected for feed wasted and body weight were measured every seven days. During the last week of the treatment animals of each group were kept in metabolic cages for 24 hrs to collect the urine over xylene for the analysis of iodine and thiocyanate. At the end of the experimental period body weights of the rats were recorded and the animals were sacrificed of the 45th and 90th days of experiment following ethical procedure. Just before sacrifice, blood samples were collected from each rat from the portal vein under ether anaesthesia and the serum was separated for the assay of T4 and T3 and 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 1,05,000 g for one hour. After centrifugation at 1,05,000 g, the precipitate was solubilized in phosphate buffer. Thyroid peroxidase activity was measured following the method of Michajlovskij and Langer as modified by Trichloroacetic acid was added to urine sample, mixed and centrifuged. The supernatant saturated bromine water was added and 4% arsenic trioxide (As2O3) was then added to oxidise bromine present in the sample. After that benzidine hydrochloride and pyridine mixture were added and the colour developed gradually. After 30 min optical density was measured in a 1ml cuvette, 0.9ml of sodium acetate buffer (pH 5.250mM), 10μl KI

Measurement of dietary goitrogen in Bamboo shoot—Cyanogenic gluicosides: It was measured following the method of Lambert et al11. Plant tissues were hydrolysed by the enzyme glucosidase (β-glucosidase) and the hydrocyanic acid thus liberated was trapped in sodium hydroxide. Cyanide content of trapped hydrocyanic acid was then determined quantitatively.

Glucosinolates/thiogluicosides: The enzyme thioglucosidase reacts with thioglucosides present in plant producing thiocyanate. Following this principle thioglucoside was measured as per Gmelin and Virtanen12. The thiocyanate thus produced was estimated by the method of Aldridge as modified by Michajlovskij and Langer13.

Thiocyanate: The plant food was extracted with clean sand and water and refluxed subsequently. The extract containing thiocyanate was then allowed to react with benzidine hydrochloride and the intensity of colour thus formed was measured photometrically following the method of Michajlovskij and Langer14.

Analysis of urine—Estimation of iodine: It was measured by dry ashing following the method of Karimark et al15. In this method iodine content in urine sample was estimated by drying urine at 600°C in presence of potassium carbonate and the iodine present in the ash was measured by ceric-arsenite system.

Estimation of thiocyanate: It was measured following the method of Aldridge as modified by Michajlovskij and Langer13. Trichloroacetic acid was added to urine sample, mixed and centrifuged. To the supernatant saturated bromine water was added and 4% arsenic trioxide (As2O3) was then added to oxidise bromine present in the sample. After that benzidine hydrochloride and pyridine mixture were added and the colour developed gradually. After 30 min optical density was measured at the wavelength of 525 nm.

Measurement of thyroid peroxidase activity—A 10% homogenate was prepared using thyroid tissues in phosphate buffer (pH 7.2, 100mM) and sucrose solution (500 mM) at 4°C. Homogenisation was carried out in a Potter-Elvehjem glass homogeniser for 45-60 sec at 2000 rpm and about 15 strokes min−1. The homogenate was centrifuged at 1000 g for 10 min and 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 1,05,000 g for one hour. After centrifugation at 1,05,000 g, the precipitate was solubilized in phosphate buffer. Thyroid peroxidase activity was measured following the method of Alexander16. For performing the kinetic assay, in a 1ml cuvette, 0.9ml of sodium acetate buffer (pH 5.250mM), 10μl KI
(1.7mM) and 20μl microsomal fraction of thyroid tissue containing 0.03-0.04 mg protein were added and the reaction was started by the addition of 20μl freshly prepared H₂O₂ (0.5 mM) according to laboratory standardization. The thyroid tissue protein level was determined by the method of Lowry et al.¹⁷ using bovine serum albumin as standard. The results are expressed as ΔOD /min/mg protein.

**In vitro inhibitory effect of bamboo shoot on thyroid peroxidase activity**—To evaluate *in vitro* anti-TPO activity of BS human thyroid tissue was collected from ENT Department, S.S.K.M. Hospital, Kolkata. Edible part of fresh plant (both raw and cooked respectively) was homogenised in assay buffer (5 mg plant tissue in 5ml, pH 7.2, 100mM phosphate buffer) and then centrifuged at 700 g for 10 min. After centrifugation, 50μl of aliquot of the supernatant of raw (not boiled and boiled respectively) and cooked plants were added separately in a 1ml cuvette containing acetate buffer (pH 5.2, 50mM), potassium iodide (1.7 mM) and microsomal fraction of thyroid tissue. Freshly prepared hydrogen peroxide (0.3 mM) was added lastly to start the reaction to assay the TPO activity (ΔOD /min/mg protein) under the influence of respective plant extract following the procedure of Gaitan et al.¹⁸.

Anti-TPO activities of the plant extracts in the above-mentioned conditions were also studied in presence of excess potassium iodide. For the purpose, in the cuvette maintaining the same concentration of assay buffer, plant extract (raw, raw boiled and cooked) and H₂O₂, the concentration of potassium iodide was increased and it was found highest after adding 20μl of KI (3.4mM) and ΔOD /min/mg protein was recorded.

**Assay of IC₅₀ and PTU equivalence**¹⁸.—The activity of raw plant extract was also evaluated in terms of the concentration necessary to produce 50% inhibition (IC₅₀) of thyroid peroxidase activity. The effect of raw plant extract was studied at different concentrations ranging from 10μg to 150μg original fresh plant to determine the concentration required to produce IC₅₀ of thyroid peroxidase activity. The TPO activity under the influence of the plant at a particular concentration, as a percentage of inhibition of the control value was plotted against the concentration of the original plant extract, and the concentration at which the 50% inhibition occurred (IC₅₀) was determined from the plot. The IC₅₀ value of plant given is mean ± SD of 6 observations. To compare the relative anti TPO activity of the studied plant against a known antagonist, IC₅₀ of 6-n-propyl-2-thiouracil (PTU obtained from Sigma Chemical Co.) was determined.

**Assay of total circulating thyroxine and triiodothyronine using ELISA Kit**—Total serum thyroxine and triiodothyronine were measured using ELISA Kits supplied by Lilac Chemicals according to manufacturer’s instructions.

**Statistical analysis**—All data were statistically analyzed and presented in the table as mean ± SD. Comparison among the groups was performed by ANOVA and the level of significance was expressed at P<0.05.

**Results**

**Goitrogen content**—Goitrogen content in fresh edible portion of BS was measured. Cyanogenic glucosides in high concentration (551.05 ± 72 mg/kg weight), glucosinolates (9.57 ± 0.57 mg/kg weight) and free thiocyanate (24.3 ± 5.2 mg/kg weight) were found in the plant.

**In vitro inhibitory effect of bamboo shoot on thyroid peroxidase activity**—TPO activity of control (10μl KI, 1.7mM) in absence of any plant extract was 1.62 ± 0.054 ΔOD /min/mg protein. Addition of extra iodide (20μl, 3.4 mM) in the incubation medium did not show any further improvement in the enzyme activity. *In vitro* inhibitory effect of edible part of young BS on thyroid peroxidase activity was determined after application of raw, raw boiled and cooked extracts respectively with and without extra KI in the incubation medium. In absence of extra iodide *in vitro* inhibitory effect of BS on thyroid peroxidase activity was found almost equal with all the three different extracts (84.69, 85, 84.38% inhibition respectively in respect of control value). After adding extra iodide in the medium (20μl KI, 3.4mM), inhibition of TPO activity by BS was measured under the above-mentioned conditions and found that extra iodide had reversed the inhibitory activity maximum with cooked extract (44.69%), moderate with raw boiled extract (59.17%) and minimum with raw extract (65.31%) (Table 1).

**Relative anti TPO potency**—The relative anti TPO potency of studied plant and PTU was determined by estimating the amount of plant food or PTU capable of producing 50% inhibition (IC₅₀) of TPO activity. IC₅₀ and PTU equivalence of BS were 27.5 ± 0.77 μg and 3.27 respectively.
Urinary thiocyanate, urinary iodine as well as thyroid weight, TPO activity and serum levels of total T4, T3 in control, BS fed, KI supplemented and BS fed plus KI supplemented groups of rats treated for 45 days and 90 days respectively were measured and results are presented in Table 2.

Due to non-availability of rat TSH kits, TSH level was not measured in the present study.

**Discussion**

The carbohydrate, protein, fat, minerals and moisture content of BS are 5.7, 3.9, 0.5, 1.1 and 88.8 respectively when the values are expressed in g/100g edible portion of BS.

BS contains the goitrogenic substances cyanogenic glucosides, glucosinolates and thiocyanate. Goitrogenic/anti-thyroid potential of dietary goitrogens viz. cyanogenic glucosides, glucosinolates and thiocyanate is well established. Bagchi and Ganguly measured the HCN content in BS of different varieties of Indian origin in its different portion and found the level from 0.098-0.800 g/100 g of BS after pulping and soaking in water for 2 hr.

However, the anti-thyroidal activity of BS of Indian origin under varying intake of iodide is not available.

To assess the direct effect of BS on thyroid physiology, in vitro TPO activity was measured using human thyroid tissue with raw, raw boiled and cooked aqueous extracts and found almost equal inhibition. Anti-thyroid potentiality of fresh BS was studied against a known antagonist PTU and showed its

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**Table 1—In vitro TPO activity (ΔOΔ/min/mg protein) of raw, raw boiled and cooked plant extracts without and with extra iodide**

<table>
<thead>
<tr>
<th>Plants</th>
<th>Raw extract Without extra KI</th>
<th>Raw extract With extra KI</th>
<th>Raw boiled extract Without extra KI</th>
<th>Raw boiled extract With extra KI</th>
<th>Cooked extract Without extra KI</th>
<th>Cooked extract With extra KI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bamboo shoot</td>
<td>0.248±0.02</td>
<td>0.562±0.164</td>
<td>0.243±0.024</td>
<td>0.655±0.013</td>
<td>0.253±0.018</td>
<td>0.896±0.037</td>
</tr>
</tbody>
</table>

KI: Potassium iodide
TPO: Thyroid peroxidase
Without extra iodide - 10μl KI (1.7mM)
With extra iodide- 20μl KI (3.4 mM)

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**Table 2—Bamboo shoot induced alteration on urinary thiocyanate and iodine and thyroid status under varying iodide intake in albino rats for (A) 45 days and (B) 90 days**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urinary thiocyanate m moles/l</th>
<th>Urinary iodine μ moles/l</th>
<th>Thyroid weight mg/100g body weight</th>
<th>TPO activity ΔOΔ/min/mg protein</th>
<th>Serum T4 μg/dl</th>
<th>Serum T3 ng/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A 0.16 ± 0.01</td>
<td>24.94 ± 1.06</td>
<td>7.26 ± 0.15</td>
<td>3.45 ± 0.38</td>
<td>3.90 ± 0.06</td>
<td>123.63 ± 3.94</td>
</tr>
<tr>
<td></td>
<td>B 0.19 ± 0.05</td>
<td>32.06 ± 0.73</td>
<td>8.94 ± 0.94</td>
<td>5.34 ± 0.27</td>
<td>4.21 ± 0.28</td>
<td>143.12 ± 1.49</td>
</tr>
<tr>
<td>Bamboo shoot fed</td>
<td>A* 1.24 ± 0.12</td>
<td>35.4 ± 2.18</td>
<td>10.83 ± 0.2</td>
<td>2.62 ± 0.21</td>
<td>3.5 ± 0.11</td>
<td>107.59 ± 1.50</td>
</tr>
<tr>
<td></td>
<td>B** 2.02±0.13</td>
<td>41.12±1.11</td>
<td>13.07±0.96</td>
<td>0.39±0.08</td>
<td>3.39±0.09</td>
<td>94.42±0.84</td>
</tr>
<tr>
<td>KI supplemented</td>
<td>A* 0.17 ± 0.01</td>
<td>178.48 ± 1.7</td>
<td>5.85 ± 0.21</td>
<td>7.51 ± 0.31</td>
<td>4.14±0.09</td>
<td>134.52±3.04</td>
</tr>
<tr>
<td></td>
<td>B** 0.21 ± 0.09</td>
<td>187.66±1.37</td>
<td>7.69 ± 0.32</td>
<td>9.96±1.74</td>
<td>4.75±0.29</td>
<td>152.19±1.84</td>
</tr>
<tr>
<td>Bamboo shoot fed KI</td>
<td>A* 1.58 ± 0.17</td>
<td>190.61±0.38</td>
<td>9.5 ± 0.41</td>
<td>3.1±0.18</td>
<td>3.73±0.08</td>
<td>123.53±1.39</td>
</tr>
<tr>
<td></td>
<td>B** 2.65±0.16</td>
<td>197.03±0.93</td>
<td>11.56±0.93</td>
<td>2.04±0.29</td>
<td>3.69±0.11</td>
<td>126.67±1.09</td>
</tr>
</tbody>
</table>

Values bearing superscripts are significantly different by ANOVA at P<0.05.
* when compared with (A) 45 days control
** when compared with (B) 90 days control
relative potency. Raw boiled and cooked extracts showed inhibition indicating that anti-thyroid potential of BS was not decreased on heating. Heating inactivates glucosidases but in contact with fresh glucosidases, glucosides again decomposed producing active anti-thyroid compounds because glucosides are heat stable. No discrepancy was noted in protein content in the extract under different conditions indicating that the protein present in BS in no way related with anti-thyroidal activity.

The potent anti-thyroidal effect of cyanogenic foods is enhanced by iodine deficiency. The goitrogenic action of thiocyanate or thiocyanate like compounds can be overcome by iodine administration. Therefore, in vitro TPO activity of the BS extracts were also determined in presence of extra iodide and found that the extra iodide had reduced the anti TPO activity but could not cancel it.

After ingestion, cyanogenic glucosides and glucosinolates present in foods are readily converted to active goitrogenic/anti-thyroid agents, viz. thiocyanate or isothiocyanates etc. by glucosidases and sulphur transferase enzymes present in plant itself and animal tissues and can give rise to thiocyanate in blood followed by its appearance in the urine. Thus the amount of thiocyanate in the urine is a good indicator of the presence of goitrogen in foods. In the present study, thiocyanate concentrations in urine in BS fed groups were found high. Prolonged treatment had shown more profound effect because it had increased more thiocyanate in urine. Thiocyanate or thiocyanate like compounds primarily inhibit iodide-concentrating mechanism of thyroid gland and also increase the excretion of iodine through urine. Present findings are consistent with earlier observations further suggesting that BS rich in glucosides undergo hydrolysis to thiocyanate that had increased the excretion of iodine through urine probably by removing more iodine from the thyroid and replacing thiocyanate in thyroid gland.

The thyroid gland weight was increased significantly after BS feeding, mediated through its goitrogenic constituents. Diet rich in thiocyanate, and glucosinolates increase both the size and weight of thyroid gland. On the other hand, excess iodide decreases thyroid enlargement induced by anti-thyroid drugs without changing serum levels of TSH and thyroid hormones. Supplementation of iodide reduces the anti-thyroid effect of BS to an extent but it could not cancel it because the effect of thiocyanate may be neutralised by adequate iodine but the activity of excess thiocyanate and glucosinolate derivatives viz. isothiocyanates, goitrin etc. cannot be neutralised by iodine supplementation. Glucosinolates undergo rearrangement to form isothiocyanate derivatives, which may react spontaneously with amino groups forming thiourea like derivatives that interfere in the thyroid gland with organification of iodine and the formation of active thyroid hormone by interfering TPO activity and their action usually may not be antagonised by iodine. In moderately iodide intake groups or in KI non-supplemented groups the effect of BS on thyroid weight was more pronounced. Even adequate iodide supplementation could not bring back it in a normal physiological state and this observation is consistent with those of earlier findings. The results showed that prolonged consumption had more deleterious effect on thyroid morphology as evidenced by more weight gain of thyroid gland.

Thiocyanate being the same molecular size as that of iodide inhibits the incorporation of iodide into thyroglobulin by competing with iodide at the thyroid peroxidase level. Depending upon the binding of SCN to the substrate site with lower affinities, thiocyanate inhibits the iodide oxidation (I leads to I) by thyroid peroxidase enzyme. In the present study, specific TPO activity in in vitro experiment under control condition was quite different from that of control as observed in animal experiment. The probable discrepancy was for the variation in the thyroid tissue. In in vitro experiment human thyroid tissue that was used obtained from the hospital sources while in the latter thyroid tissue obtained from albino rats maintained with normal diet. In BS treated groups TPO activity was markedly reduced because cyanogenic glucosides are readily converted to thiocyanate or thiourea like derivatives produced from glucosinolate. Prolonged BS feeding reduced TPO activity further indicating that chronic consumption of this plant had more pronounced effect.

Thiocyanate in foods deprived of potassium iodide brought down the circulating levels of T4 and T3 in rats. Intake of BS decreased the serum total T4 and T3 levels significantly. Reduced TPO activity may be responsible for decreasing thyroid hormone levels because it regulates the synthesis of thyroid hormone. Inhibition of iodide uptake in thyroid gland by thiocyanate may be another reason of this reduction of hormonal profile.
The overall results suggest that chronic BS consumption gradually develops a state of biochemical as well as morphological hypothyroidism even in presence of adequate iodide. The etiological factors underlying this phenomenon are excessive cyanogenic glucosides, glucosinolates and thiocyanate of BS. Supplementation of iodide had reduced the goitrogenic/anti-thyroidal effect of BS but could not cancel it.

Acknowledgement

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