

Goitrogenic content of Indian cyanogenic plant foods & their *in vitro* anti-thyroidal activity

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Background & objectives: Consumption of cyanogenic foods has been considered as one of the etiological factors in certain instances for the persistence of endemic goitre. The present study was undertaken to study the cyanogenic glucosides, glucosinolates and thiocyanate content in edible portion of certain selected plant foods of Indian origin. Further *in vitro* anti-thyroidal activity using raw, boiled and cooked extracts of these plants with and without excess iodide was also studied.

Methods: Cyanogenic plant foods generally vegetables were collected from different areas of West Bengal and Tripura. Cassava was obtained from Meghalaya and Kerala and their cyanogenic glucosides, glucosinolates and thiocyanate were estimated. Thyroid peroxidase activity (TPO) of human thyroid was assayed from microsomal fraction following I_3^- from iodide. The anti-TPO activities of the plants were assayed after adding raw, boiled and cooked extracts in the assay medium with and without extra iodide. Relative antithyroidal potency of the plant extracts was also evaluated in terms of the concentration (IC_{50}) necessary to produce 50 per cent inhibition of TPO activity. PTU equivalence of the plant foods was also determined.

Results: Cabbage and cauliflower were rich in glucosinolates, bamboo shoot and cassava were rich in cyanogenic glucosides, mustard, turnip and radish were relatively rich in thiocyanate however all the constituents were present in each plant. Boiled extracts showed maximum inhibition of TPO activity followed by cooked and raw extracts. Excess iodide was found relatively effective for raw extract but less effective for boiled and cooked extracts in reversing anti-TPO activity. Inhibition constant (IC_{50}) was found highest with bamboo shoot and least with cabbage.

Interpretation & conclusion: Raw, boiled and cooked extracts of the plants showed anti-thyroidal activity *in vitro*. Excess iodide reversed the anti-TPO activity to same extent but could not neutralise it.

Key words Cyanogenic glucosides - dietary goitrogen - glucosinolates - iodine - thiocyanate - thyroid peroxidase

Cyanogenic glucosides, glucosinolates (thioglucosides) and thiocyanate are the goitrogenic/antithyroid constituents of cyanogenic plants that are often used as food by men and animals. Large differences in glucosides content of plants belonging to the same family and the same taxonomy and grown within the same geographical area owing to their genetic backgrounds and ecological factors have been reported¹. Cyanogenic constituents affect hormone synthesis in thyroid gland either by inhibiting iodide

uptake or interfering the activity of thyroid peroxidase (TPO), *i.e.* by inhibiting the organification of iodide (I^- leads to I_2^-), or iodination of tyrosine in thyroglobulin and coupling reaction²⁻⁶. The goitrogenic or antithyroid potential of a plant not only depends on the relative concentrations of cyanogenic constituents found in fresh plant but also on its processing as food, so in the areas where these plant foods are consumed, the common measures to reduce the goitrogenic potency include soaking, washing, boiling, cooking *etc.*^{7,8} and to

supplement adequate iodide as it ameliorates the antithyroidal activity^{9,10}.

The information on goitrogenic content of cyanogenic plant foods of Indian origin and their antithyroidal activity is scanty. Therefore, the present work was undertaken to measure goitrogenic content *viz.*, cyanogenic glucosides, glucosinolates and thiocyanate of certain cyanogenic plants used as foods and to evaluate their *in vitro* antithyroidal activity in raw, boiled and cooked extract with and without extra iodide.

Material & Methods

The study was conducted in the Endocrinology and Reproductive Physiology Research Laboratory of the Physiology Department, University of Calcutta, Kolkata during January 2001 to May 2003.

Selection of materials: Cyanogenic plant foods generally used as vegetable *viz.*, cauliflower (*Brassica oleracea* var., *botrytis*), cabbage (*Brassica oleracea* var. *capitata*), mustard seeds (*Brassica juncea*), turnip (*Brassica rapa*), radish (*Raphanus sativus*), bamboo shoot (*Bambusa arundinacea*) and cassava (*Manihot*) were selected for the present study. Samples of these plants were collected at random from different areas of West Bengal and Tripura while cassava was collected from Meghalaya and Kerala states.

Measurement of cyanogenic glucosides: Cyanogenic glucosides were measured following the method of Lambert *et al.*¹¹. Edible parts of fresh plants varying from 10mg to 1g were hydrolysed by the enzyme glucosidase (β -glucosidase, Sigma, USA) and the hydrocyanic acid thus liberated was trapped in sodium hydroxide. Cyanide content of trapped hydrocyanic acid was then determined quantitatively.

Measurement of glucosinolates: The enzyme myrosinase (thioglucosidase) reacts with glucosinolates present in plant foods and generates thiocyanate. Following this principle glucosinolate was measured by the procedure of Gmelin and Virtanen¹². Methanolic extract of fresh edible part of respective plant was prepared and then evaporated *in vacuo*. The residue was treated with lead acetate followed by H₂S to precipitate the excess lead ions. The filtrate containing glucosinolate was again concentrated *in vacuo* and

treated with myrosinase (thioglucosidase from Sigma, USA) to obtain thiocyanate. Thiocyanate was then estimated by the method of Aldridge¹³ as modified by Michajlovskij and Langer¹⁴.

Measurement of thiocyanate: The plant foods were extracted with clean sand and water and refluxed subsequently. The extract containing thiocyanate was treated with trichloroacetic acid, followed by saturated bromine water and arsenous trioxide and allowed to react with pyridine-benzidine hydrochloride mixture. The intensity of colour thus formed was measured by using spectrophotometer (UV-1240 Shimadzu, Japan) following the method of Aldridge¹³ as modified by Michajlovskij and Langer¹⁴.

Assay of thyroid peroxidase (TPO) activity: A 10 per cent homogenate was prepared using human thyroid tissues collected from the ENT Department, S.S.K.M. Hospital, Kolkata; in 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 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 h. Immediately after centrifugation the precipitate was solubilized in phosphate buffer. Thyroid peroxidase activity was measured by the method of Alexander¹⁵. The tissue protein level was determined by the method of Lowry *et al.*¹⁶ using bovine serum albumin as standard. The results are expressed as change in optical density (Δ OD)/min/mg protein.

Assay of anti-TPO activity of plant foods: Edible part of each fresh plant (raw, boiled and cooked) was homogenized in assay buffer (5 mg plant tissue in 5 ml phosphate buffer) and centrifuged at 700 g for 10 min. After centrifugation fixed amounts of aliquot of the supernatant of raw, boiled and cooked plant were added separately in a 1ml cuvette containing acetate buffer, potassium iodide, microsomal fraction of thyroid tissue and hydrogen peroxide was added to start the reaction. The TPO activity (Δ OD/min/mg protein) was measured following the procedure of Gaitan *et al.*¹⁷.

Anti-TPO activities of the plant extracts were also studied in presence of excess potassium iodide. For this

purpose in the cuvette maintaining the same concentration of assay buffer, plant extract (raw, boiled and cooked) and H_2O_2 , the concentration of potassium iodide was increased (until highest activity obtained) and change in optical density (ΔOD)/min/mg protein was recorded.

Assay of IC_{50} : The activity of raw plant extracts was evaluated in terms of the concentration necessary to produce 50 per cent inhibition (IC_{50}) of TPO activity to evaluate their relative antithyroidal potency. The effect was also studied at different concentrations ranging from 10 to 150 μg fresh plant to determine the concentration required to produce IC_{50} of TPO activity. To compare the relative anti-TPO activity of the studied plants against a known antagonist, IC_{50} of 6-n-propyl-2-thiouracil (PTU from Sigma, USA) was determined.

Results

Goitrogen content: The moisture content of the plants varied from 59 to 95 per cent except mustard seeds (8.5%). Based on relative concentrations of cyanogenic glucosides, glucosinolates and thiocyanate, the plants were grouped and the anti-TPO activities of raw, boiled and cooked extracts without (not adding extra iodide) and with extra iodide were determined.

Anti-TPO activity of cabbage and cauliflower: These two plants were rich in glucosinolates followed by thiocyanate and cyanogenic glucosides (Table I). The activity of the enzyme was reduced in presence of the raw cauliflower (74.75%) and cabbage (65% reduction) extract; further reduction observed after boiled extract (90.12 and 79.44%) and for cooked extract the values were almost same that of raw extract (75 and 66.05%). In presence of extra iodide recovery in TPO activity was maximum with raw extract (only 20.04 and 15% reduction), moderate for boiled extract and remain almost unchanged for cooked extract of the plants (Table II). TPO activity of control (with optimum potassium iodide) in absence of any plant extract was 1.62 ± 0.054 (ΔOD min/mg protein).

Anti-TPO activity of bamboo shoot and cassava: Both these plants were rich in cyanogenic glucosides followed by thiocyanate and glucosinolates. However, concentrations of all three were almost double in bamboo shoots than cassava (Table I). Consistent with these raw, boiled and cooked extracts of these plants showed TPO

Table I. Distribution of cyanogenic glucosides, glucosinolates and thiocyanate content in selected fresh plant foods

Plant foods	Cyanogenic glucosides	Glucosinolates	Thiocyanate
<i>Brassica family</i>			
Cauliflower (<i>Brassica oleracea</i> var. <i>botrytis</i>)	1.82 \pm 0.4	17.28 \pm 1.6	5.04 \pm 0.5
Cabbage (<i>Brassica oleracea</i> var. <i>capitata</i>)	1.6 \pm 0.3	15.7 \pm 1.3	11.6 \pm 1.7
Mustard (<i>Brassica juncea</i>)	0.24 \pm 0.01	4.0 \pm 0.3	50.5 \pm 2.9
Turnip (<i>Brassica rapa</i>)	1.3 \pm 0.5	4.6 \pm 0.8	20.1 \pm 0.9
Radish (<i>Raphanus sativus</i>)	1.28 \pm 0.4	2.64 \pm 0.2	13.28 \pm 0.9
<i>Non-Brassica family</i>			
Bamboo shoot (<i>Bambusa arundinacea</i>)	551.05 \pm 72	9.57 \pm 0.5	24.31 \pm 5.2
Cassava (<i>Manihot</i>)	304.31 \pm 41	4.32 \pm 0.8	12.95 \pm 2

Values are mean \pm SD of 6 observations, expressed in terms of mg/kg wet weight

inhibition and thus anti-TPO potency of bamboo shoot was almost twice to that of cassava (reduction in bamboo shoot raw 84.69%, boiled 85% and cooked 84.38%, cassava raw 69.88%, boiled 70.86% and cooked 70.25%). Extra iodide had reversed anti-TPO activity of cassava and bamboo shoot but it was more effective for raw and cooked extracts (Table II).

Anti-TPO activity of mustard, turnip and radish: These plants contain maximum thiocyanate followed by glucosinolates and cyanogenic glucosides (Table I). Maximum inhibition of TPO activity was found with boiled extract (88.65, 81.85, and 79.75%) followed by cooked extract (81.79, 74.57 and 75.25%) and raw extract (79.69, 70.99 and 59.57%). Extra iodide had reversed the anti-TPO activity of raw extract of these plants; but recovery after boiled and cooked extract was less than the raw extract (Table II).

Relative anti-TPO potency: The relative anti-TPO potency of studied plants and PTU was determined by

Table II. *In vitro* TPO activity ($\Delta OD/ \text{min/mg}$ protein) of raw, boiled and cooked plant extracts without and with extra iodide

Plants	Raw extract		Boiled extract		Cooked extract	
	Without extra KI	With extra KI	Without extra KI	With extra KI	Without extra KI	With extra KI
Control	1.62 ± 0.054	-	-	-	-	-
Cauliflower	0.409 ± 0.014 (74.75)	1.295 ± 0.024 (20.04)	0.160 ± 0.007 (90.12)	0.569 ± 0.02 (64.88)	0.405 ± 0.001 (75)	0.405 ± 0.006 (75)
Cabbage	0.562 ± 0.016 (65)	1.368 ± 0.012 (15)	0.333 ± 0.024 (79.44)	0.655 ± 0.011 (59.57)	0.550 ± 0.018 (66.05)	0.545 ± 0.017 (66.36)
Bamboo shoot	0.248 ± 0.02 (84.69)	0.562 ± 0.164 (65.31)	0.243 ± 0.024 (85)	0.655 ± 0.013 (59.17)	0.253 ± 0.018 (84.38)	0.896 ± 0.037 (44.69)
Cassava	0.488 ± 0.009 (69.88)	1.063 ± 0.01 (34.38)	0.472 ± 0.04 (70.86)	0.792 ± 0.023 (51.11)	0.482 ± 0.024 (70.25)	0.884 ± 0.017 (45.43)
Mustard	0.329 ± 0.019 (79.69)	1.062 ± 0.005 (34.44)	0.281 ± 0.015 (88.65)	0.483 ± 0.037 (70.19)	0.295 ± 0.013 (81.79)	0.881 ± 0.017 (45.62)
Turnip	0.470 ± 0.033 (70.99)	1.362 ± 0.005 (15.93)	0.294 ± 0.014 (81.85)	0.487 ± 0.008 (69.94)	0.412 ± 0.018 (74.57)	0.479 ± 0.019 (70.43)
Radish	0.655 ± 0.02 (59.57)	1.458 ± 0.008 (10)	0.328 ± 0.018 (79.75)	0.473 ± 0.02 (70.80)	0.401 ± 0.042 (75.25)	0.407 ± 0.04 (74.88)

KI, potassium iodide; TPO, thyroid peroxidase

With extra KI indicated addition of excess iodide in the incubation medium than control and others

Values are mean \pm SD of 6 observations

Per cent inhibition of TPO activity against control is given in the parentheses

estimating the amount of plant food or PTU capable of producing 50 per cent inhibition (IC_{50}) of TPO activity (Table III). The IC_{50} was highest in bamboo shoot, followed by cassava, mustard, cauliflower, radish, turnip and cabbage. This observation was confirmed by PTU equivalence of the studied plants.

Discussion

Many vegetables containing cyanogenic constituents are often consumed but the information on the systemic quantification of different goitrogenic/anti-thyroid components of these vegetables of Indian origin is scanty. Thiocyanate content of cauliflower, cabbage, cassava (tapioca), mustard, radish and turnip was measured by

earlier workers^{18,19} while cyanogenic glucosides were studied in bamboo shoot²⁰ and cassava²¹. Marked variations were noted in the observations apparently for differences in genetic backgrounds and ecological factors and also for presentations of data. Cyanogenic glucosides, glucosinolates and thiocyanate are known as goitrogenic principles of cyanogenic plants. Goitrogenic/antithyroidal potential of a plant food depends not only on the nature and the relative concentration for these goitrogenic principles present in it but also on how it is processed as food or the iodine nutritional status of the body.

Raw extract of all the plants reduced TPO activity from 60 to 85 per cent. Cyanogenic glucosides are readily

Table III. Concentration of fresh cyanogenic plant foods producing 50 per cent inhibition (IC_{50}) of thyroid peroxidase activity

Plant foods	IC_{50} (μ g)	PTU equivalence
PTU	0.9 \pm 0.2	100
Cauliflower	51.25 \pm 0.52	1.76
Cabbage	66.25 \pm 1.42	1.36
Bamboo shoot	27.5 \pm 0.77	3.27
Cassava	42.5 \pm 1.35	2.12
Mustard	45.00 \pm 0.67	2
Turnip	60.00 \pm 1.10	1.5
Radish	51.88 \pm 1.2	1.73

IC_{50} , inhibition constant₅₀
 Values are mean \pm SD of 6 observations
 PTU-6-n-propyl-2-thiouracil

converted into active goitrogenic agent thiocyanate by glucosidases and sulphur transferase enzymes present in the plant or in the animal tissues²². Thiocyanate or thiocyanate like compounds primarily inhibit iodide-concentrating mechanism of the thyroid¹⁰, at high concentration thiocyanate inhibits the incorporation of iodide into thyroglobulin by competing with iodide at the thyroid peroxidase level²³ and forming insoluble iodinated thyroglobulin in thyroid²⁴. High concentration of thiocyanate is also responsible for inhibition of TPO catalysed oxidation (I^- leads to I_2)⁶ while glucosinolates undergo a rearrangement to form isothiocyanate derivatives⁴. Isothiocyanate reacts spontaneously with amino groups to form thiourea that interferes in thyroid gland with organification of iodide and formation of thyroid hormone and this action cannot be antagonised by the iodide²³. Thus, the *in vitro* inhibition of TPO activity of raw extract seen in the present study appeared to be mediated through thiocyanate or isothiocyanate like anti-thyroid derivatives.

In the present study boiled extract of most of the plants studied reduced the TPO activity further (from 70 to 90%). Cooked extracts of plants showed less anti-TPO activity than their boiled counterparts and nearly equal activity like their respective raw extracts. Anti-TPO activity of cooked extract of most of the plants was less than their boiled extract because of discarding the liquor containing the active anti-thyroid compounds that might have produced through hydrolysis during boiling of the plant. Therefore cooking had beneficial effects against anti-thyroidal activity of these plants as has been

observed in *in vivo* studies with cooked brussel sprouts showing less harmful effect on thyroid functions²⁵.

The potent anti-thyroidal effect of cyanogenic foods is known to be enhanced by iodine deficiency²⁶. The goitrogenic action of thiocyanate or thiocyanate like compounds can be overcome by iodide administration^{9,10,27}. In our study addition of excess iodide showed different degrees of reversing effect against *in vitro* TPO inhibition. Reversing effect was encouraging for raw extracts of the plants except for bamboo shoot because of its higher concentration of goitrogenic constituents. Recovery was less for both boiled and cooked extracts because of conversion of cyanogenic constituents into thiocyanate completely or the cyanogenic glucosides and glucosinolates present in the plants were converted into more active anti-thyroid substances and their action was not antagonized by excess iodide. Feed containing high glucosinolates are shown to have goitrogenic effect, in swine and poultry²⁸; supplementation of iodide may reduce the anti-thyroid activity but cannot neutralise it.

Relative anti-thyroid potency of raw extract of bamboo shoot was found to be maximum followed by cassava, mustard, cauliflower, radish, turnip and cabbage as studied by their respective IC_{50} and PTU equivalence. TPO-catalysed organification of iodide depends on thiocyanate concentration or isothiocyanate like compounds in the medium^{6,10,23}. Anti-TPO activity of the plant extracts as observed in the present study was not consistent with goitrogenic content present in fresh plants. This may be because of differences in moisture contents of the plants or for the differences in conversion of inactive precursors to active constituents during mastication, boiling, cooking *etc.*, from plant to plant.

In conclusion, the results showed that the cyanogenic plants had *in vitro* anti-thyroidal activity. Boiled extracts showed highest anti-TPO potency followed by cooked and raw extracts. Excess iodide though reversed the anti-TPO activity of the plant foods to a certain extent but could not neutralise it.

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