Chapter 7

THYROID HORMONE PROFILES UNDER THE INFLUENCE OF THE SELECTED PLANT FOODS

Introduction

Normal thyroid hormones (THs) levels are important in growth, differentiation and metabolism for organism. Indeed, TH is required for the normal function of nearly all tissues, with major effects on oxygen consumption and metabolic rate (Petty et al., 1990). Disorders of the thyroid gland are among the most common endocrine maladies. Furthermore, endemic cretinism and goitre due to iodine deficiency remains a public health problem in developing countries at the advent of the third millennium. Thus the study of TH action has important biological and medical implications.

TH synthesis and secretion is exquisitely regulated by a negative-feedback system that involves the hypothalamus, pituitary and thyroid gland [hypothalamic/pituitary/thyroid (HPT) axis] (Shupnik et al., 1989). Thyrotropin releasing hormone (TRH) is a tripeptide (PyroGlu-His-Pro) synthesized in the paraventricular nucleus of the hypothalamus. It binds to TRH receptors in pituitary thyrotropes, a subpopulation of pituitary cells that secrete thyroid stimulating hormone (TSH). TRH stimulation leads to release and synthesis of new TSH in thyrotropes. TSH is a 28-kDa glycoprotein composed of α and β-subunits designated as glycoprotein hormone. Both TRH and TSH secretion are negatively regulated by TH. An important mechanism for the negative regulation of TSH may be the intra-pituitary conversion of circulating T₄ to T₃ by deiodinase. A number of thyroid genes, including Na⁺/I⁻ symporter (NIS), thyroglobulin (Tg) and thyroid peroxidase (TPO) are stimulated by TSH and promote the synthesis of TH. The THs, T₄ and the more potent T₃ are synthesized in the thyroid gland. Iodide is actively transported and concentrated into the thyroid by NIS (Smanik et al., 1996; Dai et al., 1996). The trapped iodide is oxidized by TPO in presence of hydrogen peroxide and incorporated into the tyrosine residues of a 660-kDa glycoprotein, Tg. This iodination of specific tyrosines located on Tg yields monoiiodinated and diiodinated residues (MIT, monoiodo-tyrosines; DIT, diiodo-tyrosines) that are enzymatically coupled to form T₄ and T₃. The iodinated Tg containing MIT, DIT, T₄, and T₃, then is stored as an extracellular storage polypeptide in the colloid within the lumen of thyroid follicular cells. The secretion of THs requires endocytosis of the stored iodinated Tg from the apical surface of the thyroid follicular cell (Taurog, 1996). The internalized Tg is incorporated in
phagolysosomes and undergoes proteolytic digestion, recapture of MIT and DIT, and release of T₄ and T₃ into the circulation via the basal surface. The majority of released TH is in the form of T₄, as total serum T₄ is 40-fold higher than serum T₃. Most pathways for the production of T₃ is via 5'-deiodination of the outer ring of T₄ by deiodinases and accounts for the majority of the circulating T₃. Type I deiodinase is found in peripheral tissues such as thyroid gland, liver and kidney and is responsible for the conversion of the majority of T₄ to T₃ in circulation.

The thyroid gland is capable of meeting physiologic demands for T₄ and T₃ up to a certain point. However, beyond that point, continuous stimulation may result in changes that could eventually lead to disease. Persistent elevation of TSH levels stimulates the thyroid gland to deplete its existing stores of THs. When the thyroid is not able to keep up with the demand, the follicular cells undergoes hypertrophy and division, leading to hyperplasia resulting nodular hyperplasia (Gavaret et al., 1980).

Certain natural or artificial compounds can also be goitrogenic by directly or indirectly interfering with the thyroid gland function. Prevalence of thyroid hypofunction can be caused or aggravated by thiocyanate or flavonoids generating food items. In India, vegetarianism is dominant and culturally preferred food habit. The number of items of plant foods consumed by the people of the country is very large and many of them are reported to have goitrogenic, like cyanogenic glucosides, glucosinolates, thiocyanate and flavonoids (Chanrda and De, 2010; Chandra et al., 2015).

Cyanogenic glucosides are readily converted into the active goitrogenic agent thiocyanate by glucosidases and sulphur transferase enzymes present in plant and animal tissues (Ermans and Bourdoux, 1989). 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. 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 (Divi and Doerge, 1996). Iodination of tyrosyl residues in thyroglobulin and subsequent oxidative coupling yields T₄ and T₃. Thiocyanate and thiocyanate-like compounds inhibit thyroidal iodide-concentrating mechanisms (Bobek, 1992) and form insoluble iodinated thyroglobulin in the thyroid (Van Middlesworth, 1985). NIS-mediated iodide transport is also inhibited by the (Na⁺-K⁺)-ATPase inhibitor ouabain as well as by the competitive inhibitors thiocyanate and perchlorate (Spitzweg and Morris, 2002) and is stimulated by TSH.
There are reports that a number of plant foods, including cauliflower, cabbage, mustard, turnip and cassava relatively rich those substances may induce alterations in thyroid function as observed in vivo and in vitro studies (Chandra et al., 2004; Chandra et al., 2006). Peanut seed coat, moringa leaves, spinach and sugarcane juice also contains cyanogenic glucosides, glucosinolates, thiocyanate including polyphenols (flavonoids) – which are considered as naturally occurring goitrogenic / anti-thyroid substances. The effect of cyanogenic glucosides, glucosinolates, thiocyanate and polyphenols / flavonoids present in the studied plant-foods on thyroid hormone synthesizing enzyme activities viz. TPO activity, 5’-deiodinase I activity and activity (Na⁺-K⁺)-ATPase have already been discussed in the preceding chapters. However, no data are available on serum thyroid hormone profiles (T₄, T₃ and TSH) under the influence of those plant foods though all these are considered as more accurate and reliable to understand the functional status of thyroid gland.

The present investigation is thus undertaken to explore the status of thyroid gland after the exposure of those respective studied plants containing dietary goitrogen in different proportion by measuring i.e. thyroid hormone profiles (serum T₄, serum T₃ and TSH) that ultimately reflects the thyroid gland functional status.

**Materials and methods**

**Maintenance of animals**

Described in the methodology section.

**Animal treatment**

Seventy two adult rats weighing 150±10 gm were allocated control and experimental groups. In the treatment schedule, rats were equally divided into nine groups each considered of eight rats per group. One group was kept as respective controls and fed normal laboratory diet whereas experimental rats in each group received a normal laboratory diet with one-third of the diet replaced by peanut seed coat, moringa leaves, spinach and sugarcane juice (Chandra et al, 2006) collect from a local market in Kolkata. The animals were maintained with above mentioned regimen dividing into two sets- i.e. 30- day set and 60- day set respectively. Feed consumption, corrected for wasted feed and body weight were measured every seven days. Animal sacrifice and all other experimental procedures were same as described earlier.

**Measurement of total serum thyroxine (T4), triiodothyronine (T3) and thyroid stimulating hormone (TSH) levels**

Described in the methodology section.
Results

Table 2: Effect of selected plant-foods on total serum thyroxine (T4) triiodothyronine (T3) and thyroid stimulating hormone (TSH) levels in experimental animals treated for 30 days and 60 days respectively

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum total T3 (ng/mL)</th>
<th>Serum total T4 (µg/dl)</th>
<th>TSH (µIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A. 1.15 ± 0.030</td>
<td>A. 6.10 ± 0.46</td>
<td>A. 0.142 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>B. 1.05 ± 0.035</td>
<td>B. 5.90 ± 0.42</td>
<td>B. 0.151 ± 0.006</td>
</tr>
<tr>
<td>Peanut seed coat</td>
<td>A. 0.60 ± 0.020</td>
<td>A. 5.06 ± 0.28</td>
<td>A. 0.500 ± 0.023</td>
</tr>
<tr>
<td></td>
<td>B. 0.40 ± 0.019</td>
<td>B. 4.38 ± 0.36</td>
<td>B. 0.833 ± 0.056</td>
</tr>
<tr>
<td>Moringa leaves</td>
<td>A. 0.40 ± 0.017</td>
<td>A. 4.80 ± 0.32</td>
<td>A. 1.200 ± 0.046</td>
</tr>
<tr>
<td></td>
<td>B. 0.38 ± 0.020</td>
<td>B. 4.23 ± 0.49</td>
<td>B. 1.360 ± 0.031</td>
</tr>
<tr>
<td>Spinach</td>
<td>A. 0.74 ± 0.025</td>
<td>A. 5.60 ± 0.28</td>
<td>A. 0.206 ± 0.042</td>
</tr>
<tr>
<td></td>
<td>B. 0.62 ± 0.022</td>
<td>B. 5.00 ± 0.25</td>
<td>B. 0.480 ± 0.026</td>
</tr>
<tr>
<td>Sugarcane juice</td>
<td>A. 0.72 ± 0.036</td>
<td>A. 5.30 ± 0.41</td>
<td>A. 0.458 ± 0.037</td>
</tr>
<tr>
<td></td>
<td>B. 0.60 ± 0.029</td>
<td>B. 4.82 ± 0.28</td>
<td>B. 0.666 ± 0.054</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD, n = 8 One-way analysis of variance (ANOVA) test followed by Tukey’s post hoc test was to determine differences across means of different groups. Mean values are significantly different by ANOVA at \( p < 0.05 \). \(^a\) control 30 day versus treated 30 day group; \(^b\) control 60 day versus treated 60 day group; \(^c\) treated 30 day versus treated 60 day group. (A) and (B) treated for 30-day and 60-day respectively.
Fig 13. Selected plant foods induced alteration in serum thyroxin (T₄) level and total serum triiodothyronine (T₃) level and serum thyroid stimulating hormone (TSH) level in experimental animals for 30 days and 60 days respectively. Each bar denotes mean ± SD, n = 8, One-way analysis of variance (ANOVA) test followed by Tukey’s post hoc test was done to determine differences across means of different groups. Mean values are significantly different by ANOVA at p < 0.05. a control 30 day versus treated 30 day group; b control 60 day versus treated 60 day group; c treated 30 day versus treated 60 day group.
In consistent with the altered activities of thyroid hormone synthesizing enzymes, serum thyroid hormone profile also modulated upon the exposure to studies plant foods. Serum total thyroxine (T\(_4\)) levels of the experimental rats were found to be lower than their respective control groups. Serum total triiodothyronine (T\(_3\)) levels also decreased in treated groups, i.e., in a synchronized fashion with that of enzyme activities.

There was a significant (\(p<0.05\)) decrease in serum total thyroxine (T\(_4\)) and triiodothyronine (T\(_3\)) levels in the peanut seed coat (PSC) fed rats in both the groups treated for 30 day and 60 days as compared to control. The decrease was more in 60 day treated group rats as compared to 30 day treated group.

Significant reduction in serum total thyroxine (T\(_4\)) and triiodothyronine (T\(_3\)) levels were also found in moringa leaves treated groups for 30 day and 60 days as compared to control (\(p<0.05\)). As expected the reduction was more profound in 60 day treated group fed with moringa leaves than 30 day treated group (\(p<0.05\)).

Similarly, rats fed with fresh spinach showed a significant decrease (\(p<0.05\)) in serum total thyroxine (T\(_4\)) and triiodothyronine (T\(_3\)) levels as compared to control. Decrease in serum total thyroxine (T\(_4\)) and triiodothyronine (T\(_3\)) levels were more evident in 60 day treated group fed with fresh spinach than 30 day treated group (\(p<0.05\)).

Consistent with those serum total thyroxine (T\(_4\)) and triiodothyronine (T\(_3\)) levels were also decreased significantly (\(p<0.05\)) after ingestion sugarcane juice for 30 and 60 day respectively as compared with the control groups, with the 60-day treatment causing a more decrease in the enzyme activity. Among all the studied plant foods, the experiment group treated with moringa leaves had shown maximum reduction of serum total thyroxine (T\(_4\)) and triiodothyronine (T\(_3\)) levels followed by peanut seed coat, sugarcane juice and spinach treated groups for 30-days and 60 days respectively.

Finally, a considerable increase in thyroid stimulating hormone (TSH) levels in serum was observed in the studied plant fed groups of experimental animals in a duration dependent fashion as compared to the control group and marked enhancement thyroid stimulating hormone (TSH) level was observed in moringa leaves treated group followed by peanut seed coat, sugarcane juice and spinach treated groups indicating considerable modulations in the hypothalamo-pituitary-thyroid (HPT) axis after prolonged exposure of studied plant foods.

**Discussion**

In this study the effect of prolonged exposure (30-day and 60-day respectively) of peanut seed coat, moringa leaves, sugarcane juice and spinach by replacing a portion of
normal diet on different aspect thyroid physiology has been evaluated. After the treatment period hormonal profile (T₃, T₄ and TSH) were assayed and compared with the control group maintained with laboratory standardized normal diet.

Follicular cells of the thyroid gland are designed for the synthesis of thyroid hormone and their release also. T₃ and T₄ are the predominant circulating thyroid hormones are synthesized and secreted by follicular cells in man and animals. T₃ is considered a biologically active thyroid hormone and most of the circulating T₃ is generated by extra thyroidal deiodination of T₄, taking place mainly in the liver. However, T₄ is synthesized only in the follicular cells of the thyroid (Kelly, 2000). Serum levels of the thyroid hormone including T₃, T₄ and TSH are commonly used as reliable marker of the thyroid gland function in human and experimental animals. Changes in serum concentration of these hormones can reflect disturbances in their glandular synthesis / secretion as well as disorders in their hypothalamo-pituitary-thyroid regulation or in their extra-thyroidal peripheral metabolism. Thyroid hormones are metabolised in peripheral tissues (by deiodination, conjugation and decarboxylation) and alterations in their metabolism may significantly influence the function of thyroid hormone metabolites at cellular level (Kelly, 2000).

A large number of the agents in the environment, both naturally occurring and human made are known to interfere with thyroid gland morphology and function, posing the danger of thyroid diseases (Gaitan, 1990).

Serum total T₃ level was decreased significantly after peanut seed coat, moringa leaves, sugarcane juice and spinach fed groups of animals. Among all the studied plant foods, maximum reduction was found in moringa leaves fed group followed by peanut seed coat, sugarcane juice and spinach fed groups. In consistent with their, reduction in serum T₄ level was also noticed in peanut seed coat, moringa leaves, sugarcane juice and spinach fed rats as compared to control.

Reduced activities of the enzymes (viz. TPO, Na⁺–K⁺-ATPase and 5’-deiodinase I) activities might be responsible for decreased in thyroid hormone levels because the enzyme regulate the synthesis of thyroid hormone in thyroid gland (Tourogl, 1970; Pommier et al., 1977; Chandra and De 2013).

It has been discussed earlier that cyanogenic glucosides, glucosinolates and thiocyanate present in the foodstuff and their degradation products like thiocyanate, isothiocyanate, goitrin and polyphenols are responsible for the inhibition of thyroidal enzymes activities (Greer et al., 1966; Virion, 1980; Kohler, 1989; Gaitan, 1990; Orgiazzi and Millot, 1994; Schone et al., 2001). The metabolic product of transformation of cyanide is
thiocyanate, which competes with iodide at the Na⁺/I⁻ symporter level in the thyroid gland, consequently inhibiting the synthesis and clearance of thyroid hormones (Dohan et al., 2000). The Na⁺ gradient that provides the driving force for I⁻ uptake is maintained by the (Na⁺-K⁺)-ATPase. Both NIS and the (Na⁺-K⁺)-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 production or (Na⁺-K⁺)-ATPase activity (Taurag, 1978; Hill et al., 1989; Spitzweg and Morris, 2002). Glucosinolates arise from rapeseed meal decreased serum concentration of thyroid hormones and resulted in goitre formation in cattle’s (Schone et al., 1994). At high concentration of thiocyanate, iodide efflux is greatly accelerated and thiocyanate ion inhibits the unidirectional clearance of iodide in the gland (Mitchell and O’Rourke, 1960). Thiocyanate has also shown to raise the rate constant for exit of iodide but only slightly reduce the rate of entry (Van Middlesworth, 1958; Wollman, 1962). Gaitan, (1990) found that thiocyanate or thiocyanate like compounds primarily inhibit the iodide concentrating mechanism of thyroid. Thiocyanate is a mono valent anion having the molecular size corresponding to that of iodine and it is concentrated in thyroid gland and inhibits the normal metabolism of iodine (Greenspan, 1978). Capen, (1992) reported that thiocyanate causes inhibition of iodide trapping mechanism. Bourdoux et al., (1978) found that when thiocyanate intake is high, an adaptation is obtained only at the expense of drop of plasma T₄ level with a very high increase of plasma TSH level. Lakshmy and Rao, (1995) reported a partial suppression of thyroid gland function by thiocyanate as evidenced by a decrease in circulating T₄ concentration. A study by Phillbrick et al., (1979) reported that thiocyanate treated mature animals showed decreased plasma T₄ level. Lakshmy and Rao, (1995) further showed that the addition of thiocyanate in food and deprived of potassium iodide brought down circulating levels of T₄ significantly in rats. A study with crushed cabbage hydrolysed containing glucosinolates caused marked decrease in serum T₃ and T₄ concentrations analogous with methimazole, a well-known antithyroid drug (Heary et al., 1992).

It has been reported that a number of plant foods, including cauliflower, cabbage, mustard, turnip and cassava, containing goitrogenic substances may induce alterations in thyroid function as observed in in vivo and in vitro studies (Gaitan, 1986; Venturi et al., 2000, Chandra et al., 2006). Vermorel et al., (1987) also reported that when rapeseed diets were given as powder or mash causes a 30% to 50% decrease in the plasma T₄ level. Plasma T₃ concentration was reported to reduce after rapeseed meal supplementation (Papas et al.,
The levels of thyroxine (T₄) and triiodothyronine (T₃) in the serum of rats fed low-glucosinolates meals indicated normal function of the thyroid, whereas those in rats fed high glucosinolates meals revealed hypothyroid state of the animals (Chandra et al., 2006).

In addition to thiocyanate, thiooxazolidone another breakdown or intermediate product of glucosinolates also inhibits the synthesis of thyroid hormone (Gmelin and Virtanen, 1960). Another intermediate product of glucosinolates, goitrin administration decreases the synthesis of thyroid hormones in the thyroid (Ermans and Bourdoux, 1989).

Bamboo shoots rich in cyanogenic glucosides when supplemented to albino rats causes reduction in thyroid hormone profiles (Chandra et al. 2004c, Chandra et al. 2013). Cyanogenic glucosides are readily converted into the active goitrogenic agent thiocyanate by glucosidases and sulphur transferase enzymes present in plant and animal tissues (Ermans et al., 1989). Regular consumption of cyanogenic foods containing cyanogenic glucosides, glucosinolates, and thiocyanate affect thyroid gland physiology and may lead to the development of endemic goitre, especially in iodine deficient environments (Delange et al., 1982).

In addition to cyanogenic constituents, polyphenols are the other goitrogenic constituents of the studied plants. It is known that flavonoids cause inhibition in vitro TPO activity (Gmelin and Vitranen, 1960) and scavenge H₂O₂ (Ferreira et al., 2002). Inhibition of both iodide uptake and thyroidal iodide efflux due to excess thiocyanate (Moller and Poulton, 1993) and flavonoids for its anti TPO and anti-deiodinase activity might have decreased the synthesis of thyroid hormones. The low levels of T₄ and T₃ in the blood through feedback to the hypothalamo-pituitary-thyroid axis release more thyroid-stimulating hormone (TSH) in order to increase the production of thyroid hormones and enlarged the thyroid gland producing goitre.

Decreased circulating levels of serum T₃ and T₄ and increased TSH levels in peanut seed coat, moringa leaves, spinach and sugarcane juice fed animals as observed in this study were for the interference of cyanogenic glucosides, glucosinolates, thiocyanate or their degradation products like goitrin, isothiocyanate, nitril, thiocyanate and thiooxazolidone etc. and polyphenols/flavonoids formed by the enzymatic degradation in the body that reduced thyroid hormone synthesis by interfering the activities of thyroid hormone synthesizing enzymes viz. thyroid peroxidase (TPO) activity, 5’-deiodinase- I activity, (Na⁺-K⁺)-ATPase activity at different cellular levels in addition to their inhibition on iodide uptake in thyroid gland or stimulating iodide efflux or suppressing the normal metabolism of iodide.
The inhibitory effect was more marked in moringa leaves followed by peanut seed coat, sugarcane juice, and spinach fed rats because of higher concentration of goitrogenic constituents. Inhibition of the activity of (Na⁺-K⁺)-ATPase was associated with decreased concentration of iodide / iodine in the thyroid gland, decreased activity of TPO associated with less of iodide oxidation, organification of iodine and coupling reaction resulting less T₃ and T₄ synthesis and reduced deiodinase activity caused less conversion of T₃ to T₄ as reflected by reduced thyroid hormone levels in blood under the influence of selected plant foods.

Overall results show that under the influence of the studied plants, both the T₃ and T₄ levels were reduced while the TSH level was enhanced developing a relative biochemical hypothyroid condition. Maximum reduction in T₃ and T₄ level found after feeding moringa leaves followed by peanut seed coat, sugarcane juice and spinach; these variation found proportionate with the concentration of respective goitrogenic substances present in them.