Chapter 9

MORPHOLOGICAL AND HISTOLOGICAL CHANGES OF THYROID GLAND UNDER THE INFLUENCE OF THE SELECTED PLANT FOODS

Introduction

The effect of feeding of certain plant-foods of Indian origin on the morphological / histological status of thyroid gland has been presented in this chapter. In addition, the obtained results after feeding of those selected plant foods have been discussed in the light of available literature.

The mammalian thyroid gland consists of two distinct endocrine cell populations concerned with the synthesis of two different classes of hormones. Follicular cells secrete the metabolically active iodotyrosines, essential for normal growth and development where as the parafollicular cells or C cells are concerned with the production of calcitonin, a hormone that influence blood levels of calcium and phosphorous and bone cell metabolism (Capen and Martin, 1989).

A large number of agents in the environment, both naturally occurring and human made are known to interfere with the thyroid gland morphology and function, posing the danger of thyroid disease. Thyroid enlargement or goitre is the most prominent effect of these agents (Gaitan, 1989a). Naturally occurring agents like cyanogenic glucosides from cassava (Delange et al., 1982), bamboo shoots (Chandra et al., 2013) and flavonoids from millets (Gaitan et al., 1989) and glucosinolates from several commonly consumed cruciferous plants (Conn 1980) magnify the severity of goitre endimia.

Gaitan (1989) divides goitrogens into agents directly on the thyroid gland and those causing goitre by indirect action. The former group is subdivided into those inhibiting transport of iodine into thyroid (like thiocyanate, isothiocyanaate), those acting on the intra-thyroidal oxidation and organic binding process of iodine and / the coupling reaction (like phenolic compounds, and phthalate derivatives disulphides and goitrin) and those interfering with proteolysis, dehalogenation and hormone release (like iodine and lithium). Besides that an increase in the thyroid stimulating hormone (TSH) level is responsible for the development of goitre, as because the pituitary, sensing the lowered circulating levels of T_{3} and T_{4}, increased the secretion of TSH which resulatated in the morphologic evidence of flooicial cell stimulation in the long term studies, to maintain the normal activity of thyroid gland (Capen and Martin, 1989). Diet can increase TSH secretion in various ways: 1) low
iodine intake, 2) high goitrogen intake, especially in subjects living in iodine deficiency areas and 3) direct stimulation of anterior pituitary gland (Franceschi et al., 1990).

Hypertrophy and hyperplasia of thyroid gland was first observed by Chesney et al., (1928) following cabbage diet in rabbits. According to the changes in the thyroid gland morphology, goitrogen / anti-thyroid substances are classified into two groups, first group contain goitrogen / anti-thyroid substances that induce TSH stimulated diffuse goitre, composed of small follicles with activated tall columnar follicular epithelial cells and the second group contains goitrogen that induce colloid goitre composed of a mixture of colloid rich follicles with flat follicular cells and normal looking follicles with cuboidal follicular cells (Kanno et al., 1992). While Sharpless, (1939) reported the development of goitre after soyabean feeding showing the follicles were lined with low cuboidal cells and are filled with colloid. The colloid did not stain so darkly as the colloid in glands getting more iodine. The goitres had follicles lined with high cuboidal or columnar cells showing hypertrophy and hyperplasia. In the larger goitres there was a practically no colloid and epithelium had folded into the follicles so that there was a great increased in number of very small follicles. The first change appeared to be one of hypertrophy rather than hyperplasia. Iodine prevented this change and produces normal gland. De groot et al., (1991) reported thyroid changes characterised by signs of ‘morphological activation’ seen as an increase in the number of irregularly shaped follicles of small diameter, filled with less homogeneous colloid and lined by high epithelium of feeding Brussels sprout and the degree of the morphological activation was more marked in cooked Brussels sprout fed groups. Gaitan et al., (1993) also reported the colloid of iodine deficient thyroid gland takes more eosin than control and iodine sufficient thyroid. Decreased circulating levels of thyroid hormones in the blood results in increased release of thyroid stimulating hormone by the anterior pituitary gland. This, in turn, resulted in hypertrophy and hyperplasia of the thyroid (Paynter et al., 1988). Even they also reported after prolonged stimulation of the pituitary / thyroid axis, hyperplasia might progress to neoplasia. A dietary pattern of fresh vegetables led to an increased risk of follicular cancer (Markaki et al., 2003). The extra follicular growth (hyperplasia) of the thyroid parenchyma also resulted in the development of epithelial goitre begins with proliferation of thyrocytes of the follicular wall (sometimes called “clear cells” and erroneously identified with parafollicular cells or C-cells).

In the present study the effect of feeding the selected cyanogenic and polyphenols/flavonoids containing plant foods on thyroid morphology and histological status have been evaluated to understand the goitrogenic / anti-thyroid effect of these substances on the thyroid gland.
**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 (Chandra *et al.*, 2006) by peanut seed coat, moringa leaves, spinach and sugarcane juice obtained from a local market in Kolkata. The animals were maintained with above mentioned regimen dividing into two sets- first set treated for 30 day and the second set treated for 60 day 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.

**Morphological and histological studies of thyroid gland**

Described in the methodology section.

**Results**

Weight of the thyroid gland is expressed as mg/100gm body weight. Thyroid gland weight of the peanut seed coat, drumstick, spinach and sugarcane juice fed group of rats were measured and compared with control (Table 3.). Weight of thyroid gland found highest in moringa leaves treated group followed by peanut seed coat, sugarcane juice and spinach.

Thyroid weight was increase significantly (*p*<0.05) in the peanut seed coat (PSC) fed rats in both the group treated for 30 day and 60 day as compared to control. The increase was more in 60 day treated group rats when compared to 30 day treated group.

In moringa leaves treated group rats showed significant increase (*p*<0.05) in thyroid weight and it was more evident in 60 day treated group fed with moringa leaves than 30 day treated group (*p*<0.05).

Significant increased in thyroid weight was observed in fresh spinach fed groups (*p*<0.05) when compared to control while the values were more in 60 day treated group fed with fresh spinach than 30 day treated group (*p*<0.05).

Similarly, thyroid weight was increase significantly (*p*<0.05) after the administration of sugarcane juice for 30 and 60 day when compared with the control groups, 60-day treated group showed a more increase in the thyroid weight.
Thyroid weight was increased significantly ($p<0.05$) after supplementation of selected plant foods (viz. peanut seed coat, moringa leaves, spinach, sugarcane juice) for 30 and 60 days when compared with the control groups, 60-day treatment showed more pronounced effect on thyroid weight.

### Table 3. Selected cyanogenic and polyphenols/flavonoids containing plant foods induced alterations on relative thyroid weight in albino rats

<table>
<thead>
<tr>
<th>Treated groups</th>
<th>Control</th>
<th>Peanut seed coat</th>
<th>Moringa leave</th>
<th>Spinach</th>
<th>Sugarcane juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated for 30 days</td>
<td>7.5 ± 0.50</td>
<td>9.5$^a$ ± 0.42</td>
<td>10.0$^a$ ± 0.18</td>
<td>8.50$^a$ ± 0.75</td>
<td>8.97$^a$ ± 0.18</td>
</tr>
<tr>
<td>Treated for 60 days</td>
<td>7.8 ± 0.38</td>
<td>10.5$^{b,c}$ ± 0.15</td>
<td>12.0$^{b,c}$ ± 0.27</td>
<td>10.06$^{b,c}$ ± 0.32</td>
<td>11.0$^{b,c}$ ± 0.10</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.

### Table 4. Morphometric / Histometric analysis of thyroid gland of the experimental animals under the influence of selected plant foods fed for 30 days and 60 days

<table>
<thead>
<tr>
<th>Treated groups</th>
<th>Control</th>
<th>Peanut seed coat</th>
<th>Moringa leave</th>
<th>Spinach</th>
<th>Sugarcane juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cellular area (mm$^2$)</td>
<td>1.38±0.02</td>
<td>a. 1.55$^*$ ± 0.05</td>
<td>a. 1.50$^*$±0.06</td>
<td>a. 1.46$^*$±0.04</td>
<td>a. 1.79$^*$±0.03</td>
</tr>
<tr>
<td>a. 1.96$^*$±0.03</td>
<td>b. 1.86$^*$±0.04</td>
<td>b. 2.42$^*$±0.02</td>
<td>b. 2.03$^*$±0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total colloid area (mm$^2$)</td>
<td>1.26±0.05</td>
<td>a. 1.40$^*$ ±0.03</td>
<td>a. 1.39$^*$±0.07</td>
<td>a. 1.31$^*$±0.03</td>
<td>a. 1.67$^*$±0.04</td>
</tr>
<tr>
<td>a. 1.80$^*$±0.02</td>
<td>b. 1.74$^*$±0.05</td>
<td>b. 2.25$^*$±0.05</td>
<td>b. 1.89$^*$±0.01</td>
<td></td>
<td></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. Values are asterisk are significantly different by ANOVA at $p < 0.05$ when compared to control for 30 days (a) and for 60 days (b).
Table 5. Semi-quantitative assessment of thyroid gland of the experimental animals under the influence of selected plant foods fed for 30 days and 60 days

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Peanut coat</th>
<th>Moringa leave</th>
<th>Spinach</th>
<th>Sugarcane juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small size follicles (%)</td>
<td>36.0</td>
<td>a. 42.0</td>
<td>a. 32.0</td>
<td>a. 45.0</td>
<td>a. 46.0</td>
</tr>
<tr>
<td>Medium size follicles (%)</td>
<td>39.0</td>
<td>a. 41.0</td>
<td>a. 41.0</td>
<td>a. 34.0</td>
<td>a. 30.0</td>
</tr>
<tr>
<td>Large size follicles (%)</td>
<td>25.0</td>
<td>a. 17.0</td>
<td>a. 27.0</td>
<td>a. 21.0</td>
<td>a. 24.0</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD, n = 8. Small follicle= Follicular diameter of 5-10µm; Medium= Follicular diameter of 11-15µm, Large follicle= Follicular diameter of 16-20µm. (a) and (b) denoted as treated for 30 days and 60 days respectively.

The data of morphometric / histometric analysis of thyroid gland of the control and different plant foods fed groups are depicted in Table 4. that showed increase in the areas of both the follicular cells as well as colloid following respective plant foods treatment. Semiquantitative assessment of thyroid follicles of control and selected plant foods treated rats (Table 5.) showed that in the treated groups, the relative number (%) of large, medium and small sized follicles were increased. The control as well as 30 days treated group was shown mostly to be made up of small and medium sized follicle with a relatively few large follicle while the large and medium sized follicles increased in 60 days selected plant foods treated groups, the numbers seemed to be dependent on the duration of treatment. But the more changes were found in moringa leaves treated group followed by peanut seed coat, sugarcane juice and spinach that indicates a development of plant foods induced cytomorphological modifications.
Plate-I. Photomicrographs of paraffin-embedded H&E-stained rat thyroid sections. (A) Rat thyroid section (400X) of control animals. (B) Rat thyroid section (400X) of treated with peanut seed coat (30 days). (C) Rat thyroid section (400X) of treated animals with peanut seed coat (60 days). [F – follicle, C – colloid]
Plate-II. Photomicrographs of paraffin-embedded H&E-stained rat thyroid sections. (A) Rat thyroid section (400X) of control animals. (B) Rat thyroid section (400X) of treated with fresh moringa leaves (30 days). (C) Rat thyroid section (400X) of treated animals with fresh moringa leaves (60 days). [F – follicle, C - colloid]
Plate-III. Photomicrographs of paraffin-embedded H&E-stained rat thyroid sections. (A) Rat thyroid section (400X) of control animals. (B) Rat thyroid section (400X) of treated with fresh spinach (30 days). (C) Rat thyroid section (400X) of treated animals with fresh spinach (60 days). [F – follicle, C - colloid]
Plate-IV. Photomicrographs of paraffin-embedded H&E-stained rat thyroid sections. (A) Rat thyroid section (400X) of control animals. (B) Rat thyroid section (400X) of treated with sugarcane juice (30 days). (C) Rat thyroid section (400X) of treated animals with sugarcane juice (60 days). [F – follicle, C - colloid]
Histological assessments performed on thyroid sections from treated with different plant foods. The histological changes that occurred in the thyroid gland in the selected cyanogenic and polyphenol/s flavonoids containing plants fed groups and control group of rats are shown in the Plate I, II, III and IV. In general, cyanogenic and polyphenols/flavonoids containing plants fed groups, thyroid gland were with high cuboidal and low columnar cells showing hypertrophy and hyperplasia filled with less homogeneous colloid and some follicles were invaded by epithelial cells. In control group the follicles were lined with low cuboidal cells and filled with colloid. All the follicles were almost uniform in size. Changes in the histology of thyroid glands after the feeding of cyanogenic and polyphenols / flavonoids containing plants for 30 days were similar in nature though slight variations observed in the number and size of the follicular cells that surrounded the colloid. In addition colloid stained more densely with eosin in selected plants (viz. peanut seed coat, moringa leaves, spinach and sugarcane juice) fed group of rats as compared to control group of rats. However, thyroid gland sections of selected plants viz. peanut seed coat, moringa leaves, spinach and sugarcane juice treated groups showed the presence of marked hypertrophy and or hyperplastic changes of thyroid follicular epithelial cells. In the 60 day treatment group, colloid took up more eosin than the 30 day treated groups and control.

Discussion

A considerable proportion of the vegetables in human diet are derived from the various plants. These vegetables include peanut seed coat, moringa leaves, spinach and sugarcane juice which are commonly consumed that contain significant proportion of cyanogenic glucosides, glucosinolates, thiocyanate and polyphenols (flavonoids). After ingestion of selected plant foods, cyanogenic glucosides and glucosinolates are degraded markedly and produce thiocyanate like active goitrogenic components.

In present study the effect of prolonged feeding of peanut seed coat, moringa leaves, spinach and sugarcane replacing a portion of the normal diet respectively on thyroid gland morphology as evaluated by measuring thyroid weight, morphometric / histometric, semiquantitative assessment of thyroid follicles and histological changes of the gland in all the plant-fed groups and compared with control.

Morphological changes of thyroid gland under the influence of plants foods

Weight of the thyroid glands was increased significantly after feeding the studied plant foods but the increase was more profound in 60 days treated groups than that of the 30-
day treated groups and control. The increase in weight of thyroid glands in the treated animals might be for the presence of cyanogenic glucosides, glucosinolates, thiocyanate and polyphenols/flavonoids present in those plants.

Glucosinolates and their breakdown products cause morphological and functional changes in thyroid gland (Spiegel et al., 1993). Heaney and Fenwick, (1995) found that the excessive amounts of glucosinolates in animal feed might affect thyroid. Rapeseed meal with high glucosinolates content induced strong increase of thyroid weight in pigs and poultry animals (Schone et al., 1994). The goitrogenic effect of iodine deficiency might be enhanced by the consumption of glucosinolates in cabbage, as well as by thiocyanate formed in the body from cyanogenic glucosides present plant foods (Montgomari, 1965). A number of plant foods, including cauliflower, cabbage, mustard, turnip, and cassava, containing goitrogenic substances inducing alterations in thyroidal structure and function was observed in in vivo and in vitro studies (Gaitan, 1990; Chandra et al., 2006). Chandra et al., (2015) showed that a significant increase in thyroid weight after supplementation of one third peanut seed coat (PSC) in diets and this increase in thyroid weight might be due to increased secretion of TSH in the PSC fed group. Moreover, many glucosinolates breakdown products after degradation may affect thyroid primarily by increasing weight of thyroid gland (Schone et al., 2001; Arstila et al., 1969).

Therefore in the present study the increase in thyroid weight might be for the presence of glucosinolates, cyanogenic glucosides and thiocyanate in the selected plant foods (viz. peanut seed coat, moringa leaves, spinach and sugarcane juice) and also because of degradation of these goitrogenic / anti-thyroid constituents to various breakdown products that acted on thyroid gland through TSH to increase weight.

**Histological changes under the influence of plant foods**

Histological changes that occurred in the thyroid glands after feeding different plant foods viz. peanut seed coat, moringa leaves, spinach and sugarcane juice containing different concentration of cyanogenic and polyphenolic constituents respectively were evaluated and compared with control. In addition, comparison was made between 30 day and 60 day feeding group with respective plant foods; however, the histological variation were more or less similar though severity was more in the group treated for longer duration. Marked alteration in thyroid gland histology was observed in the studied plants fed groups in comparison to that of control because selected plants contain glucosinolates, cyanogenic glucosides and thiocyanate including polyphenols (flavonoids). In all the treated groups, the
thyroid follicles were enlarged due to increase in colloidal area that surrounded by hyperplastic and hyperplasia follicular epithelial cells. In addition, in the plant-fed groups the numbers of small follicles were more containing less colloid. Sharpless, (1939) reported the development of goitre after feeding of soya-bean containing isoflavon in rats with similar type of changes in comparison to that of the control. Kanno et al., (1990) classified the goitrogen into two types – i.e. the goitrogens that stimulate TSH secretion resulting diffuse goitre and the goitrogens causes colloid goitre independent of TSH action. The histological changes as observed in the present study after feeding cyanogenic and polyphenols containing plant foods were the prototype of goitrogen induced TSH stimulated diffuse goitre. There were variations in histology of thyroid gland after feeding different types of plant foods because of the differences in cyanogenic constituents and polyphenol content in different plant foods. In pigs fed rapeseed meal containing glucosinolates; the epithelium of the thyroid gland was cuboidal or columnar and the follicular area was moderately enlarged (Svetina et al., 2003). Thyroid histology of animals fed VTO (D-5-vinyl-2-thiooxazolidone) or DRSM (detoxified rapeseed meal) showed smaller follicles lined by tall cells with reduced luminal colloid, these changes were more severe in animals fed VTO (Redmond and Tuffery, 1981). They also postulated that both VTO and isothiocyanate might decrease thyroid hormone production that in turn stimulates TRH and TSH release leading to glandular hyperplasia and hypertrophy. Chandra and De, (2012) also reported that proliferative histological changes that occur in rats thyroid after the administration of catechin (polyphenols) due to compensatory function of the thyroid for decreased thyroid hormones and subsequent stimulation by the increased TSH level.

In normal thyroid tissue, abnormal cellular proliferation remains inhibited (Dumont et al., 1992; Wynford, 1993). Activation of the proliferation of thyroid follicular cells (following iodine deficiency) is known as a cause in goiter development. Recent studies demonstrated that the activation of AMPc signal (cyclic adenosine monophosphate) in thyrocytes mediates the development of mitotic activity (Roger et al., 1988). The induction of AMPc cascade demonstrated the increase proliferation activity of thyroid epithelial cells in mice in vivo (Ledent et al., 1992) and in vitro (Ivan et al., 1997). It is assumed that the adenosine A2 receptor, which activates adenylyl cyclase via coupling to the stimulating G protein (Gs), has shown to promote constitutive activation of the cAMP cascade when transfected into various cell types. Thyroid-specific expression of the A2 adenosine receptor transgene also promoted gland hyperplasia and severe hyperthyroidism causing premature death of the animals.
Histological observations showed that in the plant fed group there occurred hypertrophy and hyperplasia of the follicular epithelial cells that surrounded mostly the small follicles containing less colloid stained deeply with eosin indicating the presence of inadequate iodine, which resembles the prototype of goitrogen induced TSH stimulated diffuse goitre. Glucosinolates and their breakdown products, cyanogenic glucosides and thiocyanate itself present in the studied plants were responsible for histological alterations of the thyroid gland of rats.

This study further revealed that in spite of adequate iodine intake as reflected by urinary iodine level, the thyroid gland gets little or less iodine as evidenced by relatively eosinophilic colloid due to interference of cyanogenic constituents present in the selected plant foods on iodine concentrating mechanism of thyroid gland.