Chapter III

IN VITRO THYROID PEROXIDASE INHIBITING ACTIVITY UNDER THE INFLUENCE OF THE SELECTED PLANTS

The influence of the cyanogenic plant foods on thyroid peroxidase activity is reviewed in this chapter and the effects of the selected plant foods on human thyroid tissue have been presented and discussed based on available literature.

Thyroid peroxidase (TPO) is a glyco-heamoprotein, located principally on the rough surfaced endoplasmic reticulum (Fawcett and McLeod 1963) and it is released in soluble form only after digestion of the membrane system with proteolytic enzymes (Hosoya and Morrison 1967). The molecular weight is about 60 kDa (Taurog 1970). Thyroid hormone synthesis is a sequential process, several tyrosine residues of thyroglobulin are first iodinated by thyroid peroxidase and then some of them are coupled to thyroid hormones by TPO (Taurog 1970; Pommier et al. 1973). Thyroid hormone formation requires the presence of peroxidase, \( \text{H}_2\text{O}_2 \), iodide, and acceptor protein at one anatomic locus in the cell. The peroxidase enzyme appears to be a protoporphyrin IX containing heme protein (Taurog 1970) with binding sites for both iodide and tyrosine (Degroot and Niepomniszcze 1977). The usual acceptor for iodide is thyroglobulin, which is iodinated within apical secretory vesicles at the cell border just prior to liberation into the colloid, or possibly after liberation into the colloid. The peroxidase is present in numerous cellular structures, but iodination activity occurs primarily, if not only, at the apical cell border (Degroot and Niepomniszcze 1977).

Michot et al. (1980) reported that iodide greatly stimulates the rate of bityrosine formation in the presence of thyroid peroxidase, suggesting that thyroid peroxidase contains a simple class of regulatory binding sites for iodide. SCN\(^-\) mimics iodide effects; maximal stimulatory effects were seen with about 0.5 mM thiocyanate and \( \text{Km} \) for SCN\(^-\) was equal to 0.1 mM. The effects of SCN\(^-\) and those of iodide were not additive. These results suggest that SCN\(^-\) binds to the same regulatory site as iodide but with a slightly lower affinity.

Thyrotrophin modulation of iodide uptake, \( \text{H}_2\text{O}_2 \) generation, thyroglobulin synthesis, and peroxidase enzyme level obviously are the main regulations and many of
these actions are thought to involve mediation of adenyl cyclase and subsequent activation of intracellular phosphokinases (Degroot and Niepomniszcze 1977). Many goitrogenic constituents exert a direct effect on the thyroid gland to disrupt the biosynthesis of thyroid hormones. Inhibition of the iodide trapping mechanism is caused by thiocyanate, perchlorate, pertechnetate anions similar to iodide. Blockage of organic binding of iodine and coupling of iodothyronines to form thyroxine (T₄) and triiodothyronine (T₃) is caused by sulfonamides, thiourea, methimazole, propyl thiouracil, they react with the oxidized iodine intermediate and are irreversibly inactivated themselves and also by thiocyanate, glucosinolates etc (Capen 1992). In vitro studies have indicated that anti-thyroid drugs have inhibitory effects on TPO activity (Taurog 1976).

Cabbage, cauliflower, mustard and radish contain glucosinolates, cyanogenic glucosides and thiocyanate - the naturally occurring goitrogenic / anti-thyroid constituents. Cyanogenic glucosides after ingestion are readily converted to thiocyanate by glucosidases and sulfur transferase enzyme present in animal tissues (Dahlberg et al. 1984).

Glucosinolates undergo a rearrangement to form isothiocyanate derivatives and thiocyanate too (Schone et al. 2001). These constituents affect hormone synthesis in thyroid gland either by inhibiting iodide uptake or interfering the activity of thyroid peroxidase i.e. by inhibiting the organification of iodide (I⁻ leads to I₂) or iodination of tyrosine in thyroglobulin and coupling reaction (Van Etten 1969; Taurog 1970; Virion 1980; Gaitan 1990; Stoewsand 1995).

The goitrogenic / anti thyroid potential of a plant not only depends on the relative concentrations of these constituents found in the plant but also on its processing as foods, so in the areas where these plant foods are used the common measures to reduce the goitrogenic potency are soaking, washing, boiling, cooking etc. (de Groot et al. 1991; Oke 1982). Moreover extreme differences in the glucosides contents of the plants belong to same family and taxonomy even within the same geographical areas owing to their genetic backgrounds and ecological factors have also been reported (Delange et al. 1982).
The information on the anti-thyroidal activity of the common plant foods of Indian origin is scanty. Therefore in the present study in vitro anti-thyroidal potency of fresh/uncooked and cooked edible parts of the plants viz. cabbage, cauliflower, mustard and radish were determined. Relative anti-thyroidal potency of those plants were also evaluated in terms of concentration necessary to produce 50% inhibition of the normal TPO activity i.e. IC₅₀ and PTU equivalence.

MATERIALS AND METHODS

Preparation of plant extracts

Described in the methodology section.

Measurement of anti-TPO activity

Described in the methodology section.

Assay of IC₅₀ and PTU equivalence

Described in the methodology section.

RESULTS

Inhibitory effects of fresh/uncooked and cooked plants viz. cabbage, cauliflower, mustard and radish on thyroid peroxidase activity were measured and compared with control (Table 6). Varying degrees of inhibition in TPO activity was shown by the extracts of the studied plants; even the effects of fresh and cooked plant extracts were not uniform.

TPO activity of control as observed in this study in absence of any plant extract was 1.62 ± 0.05 (OD/min/mg protein). The activity of the enzyme was reduced in presence of uncooked cabbage extract (65%), further reduction was observed after cooked extract (66%) as compared to control. Fresh cauliflower extract had reduced TPO activity about 74% while the reduction was 75% with its cooked counterpart. Fresh/uncooked mustard had reduced TPO activity about 79% while the inhibition was about 81.69% with cooked extract as compared to control. The inhibition in the activity of this enzyme with fresh/uncooked radish was 59.57% and cooked radish was 75.25%.
Table 6. *In vitro* thyroid peroxidase activity (ΔOD /min/mg protein) of raw and cooked plant extracts of the selected plant foods

<table>
<thead>
<tr>
<th>Plants</th>
<th>Uncooked/ fresh</th>
<th>Cooked</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>1.62 ± 0.05</td>
<td>0.550 ± 0.018</td>
</tr>
<tr>
<td>Cabbage</td>
<td>0.562 ± 0.016</td>
<td>(65%)</td>
</tr>
<tr>
<td></td>
<td>0.550 ± 0.018</td>
<td>(66.05%)</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>0.409 ± 0.014</td>
<td>(74.75%)</td>
</tr>
<tr>
<td></td>
<td>0.405 ± 0.001</td>
<td>(75%)</td>
</tr>
<tr>
<td>Mustard</td>
<td>0.329 ± 0.019</td>
<td>(79.69%)</td>
</tr>
<tr>
<td></td>
<td>0.295 ± 0.013</td>
<td>(81.79%)</td>
</tr>
<tr>
<td>Radish</td>
<td>0.655 ± 0.02</td>
<td>(59.57%)</td>
</tr>
<tr>
<td></td>
<td>0.401 ± 0.042</td>
<td>(75.25%)</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 6 observations.
Percent inhibition of TPO activity against control is given in the parentheses. Figures in the parenthesis are percentage.

[The activity of the thyroid peroxidase (25 μl microsomal fraction of thyroid tissue) was assayed in control and in presence of 50 μl plant extract (containing 50 μg plant) in 1ml cuvette containing 50 mM Acetate buffer (pH 5.2), 1.7 mM KI and 0.33 mM H₂O₂ added last to start the reaction]

Table 7. Concentrations of fresh and cooked cyanogenic plant foods producing 50% inhibition (IC₅₀) and PTU equivalence of thyroid peroxidase activity

<table>
<thead>
<tr>
<th>Plants</th>
<th>IC₅₀ (μg) Uncooked</th>
<th>PTU Equivalence Uncooked</th>
<th>PTU Equivalence Cooked</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTU</td>
<td>0.9 ± 0.2</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Cabbage</td>
<td>66.25 ± 1.42</td>
<td>25.0 ± 0.59</td>
<td>1.36</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>51.25 ± 0.52</td>
<td>22.5 ± 1.21</td>
<td>1.76</td>
</tr>
<tr>
<td>Mustard</td>
<td>45.0 ± 0.67</td>
<td>27.5 ± 0.87</td>
<td>2.0</td>
</tr>
<tr>
<td>Radish</td>
<td>51.88 ± 1.2</td>
<td>31.25 ± 0.95</td>
<td>1.73</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 6 observations.
IC₅₀ = Inhibition Constant ₅₀
PTU= 6-n-Propyl-2- thiouracil
Among all the selected plants, mustard extracts had shown maximum inhibition in both uncooked and cooked conditions.

The relative anti-TPO potency of the studied plants was determined by estimating the amount of plant food or PTU capable of producing 50% inhibition of TPO activity using both the fresh / uncooked and cooked plant extracts (Table 7). The IC$_{50}$ was highest in mustard seed followed by cauliflower, radish and cabbage for uncooked extracts while IC$_{50}$ was highest with cooked cauliflower followed by cooked cabbage, mustard and radish extracts. This observation was confirmed by PTU equivalence of the studied plants. PTU equivalence was highest in mustard among the uncooked plant foods while cauliflower had highest PTU equivalence among the cooked plant foods.

**DISCUSSION**

Inhibition of thyroid peroxidase activity of human thyroid tissue collected from hospital sources was measured in presence of cabbage, cauliflower, mustard and radish using both the fresh and cooked extracts of each of the selected plant foods.
Glucosinolates, cyanogenic glucosides and thiocyanate have been demonstrated as goitrogenic principles of those selected plant foods belong to cyanogenic origin (Van Etten 1969). Goitrogenic / anti-thyroidal potential of plant foods depend not only on the nature and the relative concentration of these goitrogenic constituents present in them but also on their processing as food or the iodine nutritional status of the body (Oke 1982).

In uncooked plants maximum inhibition of TPO activity was found with mustard followed by cauliflower, cabbage and radish. In cooked plants highest inhibition was noted with mustard followed by radish, cauliflower and cabbage. The anti TPO potency was found higher in cooked foods in comparison to that of uncooked counterparts.

Aqueous extract of uncooked plants viz. cabbage, cauliflower, mustard and radish had reduced TPO activity from 60-80 percent. All these food plants contain significant concentrations of glucosinolates, thiocyanate and cyanogenic glucosides as constituents of goitrogenic principles.

Cyanogenic glucosides present in the plant food are readily converted into active goitrogenic agent thiocyanate by glucosidases and sulfur transferase enzyme present in the plant itself or in the animal tissues (Montgomery 1969). Thiocyanate at high concentration inhibits the incorporation of iodide into thyroglobulin by competition with iodide at thyroid peroxidase level (Ermans and Bourdoux 1989) and forming insoluble iodinated thyroglobulin in the thyroid gland (Van Middlesworth 1985). High concentration of thiocyanate is also responsible for the inhibition of TPO catalysed oxidation ($I$ leads to $I_2$) (Virion 1980). In addition thiocyanate in low concentration causes an acceleration of the exit rate of thyroidal iodide (Ermans 1981). Dahlberg et al. (1984) also reported potent anti-thyroid effect of thiocyanate.

But glucosinolates present in the foodstuff undergo a rearrangement to form isothiocyanate derivatives (Van Etten 1969). Isothiocyanates react spontaneously with amino groups to form thiourea like ant-thyroid compounds that interfere in the thyroid gland with organification of iodide and formation of thyroid hormones and their actions cannot be antagonized by iodine (Ermans and Bourdoux 1989). Isothiocyanate arises from enzymatic degradation of glucosinolates also possess intrinsic antithyroid activity \textit{in vitro} by inhibition of iodide uptake and organification of iodine (Gaitan et al. 1983). Moreover, glucosinolates are iodine antagonist. The presence of glucosinolates changes
the action of TPO (Schone et al. 2001). According to in vitro assay of Kohler et al. (1988) the enzyme oxidizes glucosinolates degradation products and thus uses elemental iodine that is reduced to iodide, which cannot be taken up by the thyroglobulinin.

Therefore in vitro inhibition of TPO activity of fresh / uncooked extracts of these selected plants was mediated through thiocyanate or isothiocyanate like anti-thyroid derivatives and glucosinolates that directly interfere with the activity of thyroid peroxidase.

The studied plants are characteristically eaten mostly after being cooked into various types of dishes. Therefore the anti-thyroidal activities of the cooked food extracts were also found important and determined. Cooked extracts of all the studied plants had reduced the TPO activity further from their uncooked / fresh counterparts indicating that after cooking the in vitro TPO inhibiting activity of the plant foods were increased than their uncooked counterparts. In other words TPO inhibiting potency of cooked plant foods were more than uncooked / fresh foods.

It has already been discussed that during cooking cyanogenic glucosides further degrade into thiocyanate and glucosinolates are also degraded into isothiocyanates and thiocyanate derivatives causing the reduction in the concentration of glucosinolates and cyanogenic glucosides in the cooked foods. Thiocyanate concentration of cooked food remains almost same because of conversion of cyanogenic glucosides and glucosinolates into thiocyanate present in the food might have been detoxified to sulfate and other byproducts. Moreover, isothiocyanate may form thiourea like strong anti-thyroid compounds on heating having potent inhibitory activity on thyroid peroxidase in cooked food than their uncooked counterparts. Heating of the plants inactivates glucosidases that convert cyanogenic glucosides to thiocyanate but in contact with fresh glucosidases present in plant and animal tissue, glucosides are again decomposed to produce active antithyroid compounds because glucosides are heat stable (Montgomery 1969). Cooking of the plant also partially inactivate myrosinase or thioglucosidase that convert the glucosinolates to thiocyanate or isothiocyanates (de Groot et al. 1991). So the uncooked vegetable contained considerably less intact glucosinolates than did its cooked product, probably as a result of more extensive enzymatic degradation in the uncooked product (de Groot et al. 1991). Thus the presence of heat stable glucosides and more intact
glucosinolates in cooked plants than its fresh or uncooked counterpart may cause greater inhibition of thyroid peroxidase activity in vitro.

Anti-TPO potency of fresh / uncooked mustard was highest followed by cauliflower, radish and cabbage. While in cooked food highest inhibition was with cauliflower followed by cabbage, mustard and radish. Anti-TPO activity of the plant extracts as observed in the present study was not consistent with their goitrogenic content in fresh and cooked plant foods because of differences in the moisture contents of the plants or for the differences in conversion of inactive precursors to active goitrogenic / anti-thyroid constituents during mastication, boiling, cooking etc. that varied from plant to plant.

The overall results showed that the aqueous extracts of studied cyanogenic plants had in vitro inhibitory effects on thyroid peroxidase activity. Cooked extracts of most of the plants showed more inhibition than their fresh or uncooked extracts because of the presence of more active anti-thyroidal substances produced on heating of the plants.