Chapter IVA

EFFECT OF FEEDING THE EDIBLE PARTS OF THE SELECTED PLANTS ON URINARY THIOCYANATE & IODINE LEVELS

Urinary thiocyanate reflects the consumption pattern of cyanogenic foods while urinary iodine reflects the consumption of iodine through diet. In this chapter available literature on urinary thiocyanate and iodine levels and their impact on thyroid status along with urinary iodine and thiocyanate ratio obtained after feeding the selected plant foods have been presented and discussed in the light of available literature.

Thiocyanate is largely of exogenous origin, i.e. it is derived through the ingestion of various glucosinolates that release thiocyanate upon hydrolysis (Van Etten 1969). The common source of these glucosinolates is plants belonging to the Brassicaceae family (Van Etten 1969; de Groot et al. 1991). Another important source of thiocyanate is the enzymatic detoxification of cyanide by the enzyme thiosulfate sulfur transferase or rhodonase. This enzyme is present in most mammalian tissues although its highest concentrations are found in liver, kidney, adrenals, thyroid and pancreas (Reinwein 1961). Cyanide intake in man is essentially due to ingestion of cyanogenic glucosides, which are present in several staple foods like cassava, bitter almonds, linseed and lima beans (Wood 1975). Besides that in smokers, tobacco smoke is also an important source of cyanide (Brunneman et al. 1977). Glucosinolates can be cleaved by the enzyme myrosinase. Any destruct of the plant cell brings the substrate and the enzyme together creating isothiocyanate, nitriles, oxazolidinethione, thiocyanate (Conn 1980). Some gastrointestinal microbes have a myrosinase like activity causing intestinal glucosinolates degradation (Oginsky et al. 1965). Isothiocyanates not only use the thiocyanate metabolic pathway but also form derivatives with thiourea like anti-thyroid effects (Gaitan 1990). Further more detoxification of nitrile also leads to the formation of thiocyanate (Van Etten 1969). Therefore, the amount of thiocyanate in the urine is a good indicator of the presence of glucosinolates in the food (Gaitan 1986b). Querido et al. (1974) further suggested that dietary supplies of cyanogenic glucosides and glucosinolates might be estimated from the daily urinary excretion and concentration of thiocyanate. Consumption of cyanogenic plants in relation to iodine intake has been considered as etiological factors for the persistence of iodine deficiency disorders in certain region of India (Chandra and Ray 2002; Marwaha et al. 2003).
Iodine plays a central role in the thyroid physiology, being both the constituent of thyroid hormone and a regulator of thyroid gland function (Taurog 1991; Visser 1996). The metabolic activity of the thyroid gland of healthy individual is disrupted by dietary iodine deficiency developing a number of disorders in human called iodine deficiency disorders (Hetzel 1987). Iodine is generally taken through food or water in a variety of chemical forms, most of it is broken down to iodide in the gut and absorbed into the blood stream in that form. Iodide present in the blood either is taken up by the thyroid and converted into thyroid hormone or is excreted in the urine. The iodide trapped by the thyroid, which may approach 100% of ingested iodine in the areas of iodine deficiency, is converted into the thyroid hormone (thyroxine and triiodothyronine) and secreted as hormone into the circulation. In target tissues (principally liver, kidney, muscle and developing brain) iodine is removed from the thyroid hormones and returned to the circulation for eventual excretion by the kidney. Iodine may also escape from the body in faeces and in breast milk. However, over 90% usually comes out in the urine, presumably as iodide (Dunn et al. 1993). Thus the iodine level in urine reflects the iodine nutritional status of the subject (Dunn et al. 1993).

Concentration of iodine and thiocyanate in urine is thus considered as an index of the dietary supplies of iodine and thiocyanate of the body. There is an interrelationship between the urinary thiocyanate and iodine concentration. The available literature suggests that consumption of large quantities of food containing thiocyanate precursors is not necessarily related with the development of goitre (enlargement of thyroid gland - the most important manifestation of iodine deficiency disorders) but the development of goitre is critically related to the balance between dietary supplies of iodine and thiocyanate. The dietary supplies of thiocyanate and iodine are determined from the urinary iodine and thiocyanate ratio (I/SCN i.e. μg/mg in dl); this ratio is higher than 7 under normal conditions. Endemic goitre develops when it reaches a critical threshold of about 3 and endemic cretinism develops when the ratio goes below 2 (Hennart et al. 1982). However, in the environment where the precursors of thiocyanate (isothiocyanate etc.) have anti-thyroid or thyroid peroxidase inhibitory activity, adequate iodine supplementation would fail to prevent IDD (Chandra and Ray 2002).

Therefore, urinary iodine and thiocyanate are the indicators to study / evaluate the consumption pattern of iodine and cyanogen present in food and water. In addition, these
two components are intimately related in the maintenance of thyroid functional status. Considering all these aspects in the present study urinary iodine and thiocyanate levels were estimated in experimental animals.

**MATERIALS AND METHODS**

**Animal maintenance and treatment**

Described in methodology section.

**Analysis of urine**

Described in the methodology section.

**RESULTS**

Urinary iodine and urinary thiocyanate levels in uncooked / fresh and cooked cabbage, cauliflower, mustard and radish fed group of rats respectively were measured and compared with the control (Table 8). Wide variations in both the urinary thiocyanate and iodine levels were observed after feeding fresh / uncooked and cooked plant foods.

![Fig 20. Selected cyanogenic plant foods induced alterations in urinary thiocyanate level in albino rats (Values are mean ± SD)]
Rats fed uncooked and cooked cabbage respectively showed significant increase in urinary thiocyanate concentration (p<0.001) compared to control. Thiocyanate excretion was significantly higher in uncooked cabbage fed group as compared to cooked cabbage fed group (p<0.001). Urinary iodine level was also increased significantly in the uncooked cabbage fed group (p<0.001). Uncooked cabbage fed group of rats showed higher excretion pattern of iodine in comparison to that of cooked cabbage fed group of rats (p<0.001).

Table 8. Selected Cyanogenic plant foods induced alterations on urinary thiocyanate and iodine level in albino rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urinary Iodine</th>
<th>Urinary thiocyanate</th>
<th>Urinary Iodine thiocyanate ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmoles/L</td>
<td>µg/dl</td>
<td>µmoles/L</td>
</tr>
<tr>
<td>Control</td>
<td>32.80 ± 0.71</td>
<td>417.84±9.45</td>
<td>0.19±0.06</td>
</tr>
<tr>
<td>Cabbage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncooked/fresh</td>
<td>35.61 ± 1.21*</td>
<td>451.96±15.42**</td>
<td>1.37±0.12*</td>
</tr>
<tr>
<td>Cooked</td>
<td>33.57±0.9</td>
<td>426.07±11.37</td>
<td>1.01±0.08*</td>
</tr>
<tr>
<td>Cauliflower</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncooked/fresh</td>
<td>36.15 ± 1.15*</td>
<td>458.78±14.58*</td>
<td>1.45±0.17*</td>
</tr>
<tr>
<td>Cooked</td>
<td>34.81 ± 1.2*</td>
<td>441.81±15.25*</td>
<td>1.18±0.06*</td>
</tr>
<tr>
<td>Mustard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncooked/fresh</td>
<td>35.51 ± 1.76*</td>
<td>450.69±22.33*</td>
<td>1.67±0.24*</td>
</tr>
<tr>
<td>Cooked</td>
<td>33.47 ± 0.91</td>
<td>424.80±11.60</td>
<td>1.25±0.08*</td>
</tr>
<tr>
<td>Radish</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncooked/fresh</td>
<td>34.61 ± 1.65**</td>
<td>439.27±21.01**</td>
<td>1.07±0.1*</td>
</tr>
<tr>
<td>Cooked</td>
<td>32.87 ± 1.0</td>
<td>417.19±12.66</td>
<td>0.94±0.09*</td>
</tr>
</tbody>
</table>

Values are mean ± SD of ten observations.
* p<0.001, ** <p<0.01 when compared to control

There were a significant increase in urinary thiocyanate (p<0.001) and iodine levels (p<0.001) of cauliflower fed rats after feeding fresh / uncooked and cooked cauliflower as compared against their control values. The excretion pattern of both the
iodine and thiocyanate were found high in uncooked or fresh cauliflower fed group to that of cooked cauliflower fed group (p<0.001 for urinary thiocyanate and p<0.02 for urinary iodine).

Rats fed fresh mustard seeds showed higher urinary thiocyanate (p<0.001) and iodine (p<0.001) levels than the control. The values were higher in fresh / uncooked mustard fed group of rats in comparison to that of cooked mustard fed group (p<0.001 for urinary thiocyanate and p<0.001 for urinary iodine).

Urinary thiocyanate level of uncooked and cooked radish fed rats was increased significantly (p<0.001). Iodine excretion pattern of uncooked radish fed group was increased significantly (p<0.01) but the increase in cooked radish fed group was not significant. Both the values were higher in groups fed with uncooked radish (p<0.05 for urinary thiocyanate and p<0.01 for urinary iodine).

![Fig 21. Selected cyanogenic plant foods induced alterations in urinary iodine level in albino rats (Values are mean ± SD)](image)

Urinary iodine and thiocyanate ratio (µg/mg in dl) was measured in different groups of rats and the ratios were in the ranges from 47-415. The ratios were higher in
the control group (415.07 ± 165.58) but were decreased in fresh cabbage (57.4 ± 6.05), fresh cauliflower (55.26 ± 7.46), fresh mustard (47.47 ± 8.31) and fresh radish fed group (70.88 ± 8.75) as compared to control and their respective cooked counterpart fed groups. The urinary iodine thiocyanate ratio was more in all the cooked plant fed groups compared to their uncooked counter parts.

Urinary iodine level was highest in cauliflower fed group of rats while thiocyanate excretion pattern was highest in mustard fed group. Among all the experimental groups lowest urinary iodine / thiocyanate ratio was found in uncooked mustard fed rats and highest value was found in cooked radish fed rats.

DISCUSSION

The effect of feeding cabbage, cauliflower, mustard and radish on thyroid physiology was evaluated in the present study. As these plant foods are consumed both in the cooked and uncooked conditions, so attempt has been made to investigate is there any variation in the alteration of thyroid physiological status after feeding those plants in those conditions? Therefore, both the cooked and uncooked vegetables respectively, were mixed with the standard diet by replacing one-third portion of the diet and fed to rats and this dietary regimen was maintained for 60 days and the alteration in urinary iodine and thiocyanate levels were determined and compared with the control values.

Urinary thiocyanate level under the influence of plant foods

Urinary thiocyanate level was markedly increased in the cooked cabbage, cauliflower, mustard and radish fed group of rats. The highest level was observed in the mustard fed rats followed by cauliflower, cabbage and radish fed rats. Further increase in thiocyanate excretion level was noted after feeding those plant foods under uncooked condition indicating that the excretion of thiocyanate was more in uncooked plant foods fed groups in comparison to that of the same amount of cooked plant food fed groups though consumption of food was almost same in quantities.

The studied plant foods contain cyanogenic glucosides, glucosinolates and thiocyanate in significant proportion as discussed earlier. The concentration of thiocyanate was almost same in both the cooked and uncooked food obtained from the same plant. However, glucosinolates level was lower and cyanogenic glucosides content
was reduced markedly in the cooked foods as compared to their respective uncooked counterparts.

Therefore, the increase in urinary thiocyanate level after the feeding of those plant foods either in uncooked or in cooked conditions was due to the metabolism / conversion of the cyanogenic glucoside, glucosinolates into thiocyanate or the thiocyanate itself present in those plants.

After ingestion, cyanogenic glucosides and glucosinolates present mainly in foods are readily converted to active goitrogenic / anti-thyroid agents, viz. thiocyanate or isothiocyanate etc. by glucosidases and sulfur transferase enzymes present in the plant itself and animal tissues (Van Etten 1969). In the animal organism thiocyanate is also formed endogenously from cyanides, nitriles and sulfur-containing compounds and thus both the thiocyanate brought exogenously into the organism and the thiocyanate present in the uncooked autolysed foods and that formed endogenously in the body have to be taken into consideration (Gmelin and Virtanen 1960). Michajlovskij and Langer (1958) reported that ingestion of Brassica vegetables by animals and man causes a rise of thiocyanate ion in the 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 (Gaitan 1986b).

Enhanced level of plasma thiocyanate following the feeding of Brassica plants has been observed (Bobek 1992). Dahlberg et al. (1984) also reported that increased serum levels coincided with an increased thiocyanate excretion through urine. Rapeseed meal treatment with significant glucosinolates content caused increase in serum thiocyanate level irrespective of glucosinolates content of the feed (Schone et al. 1994). An increase in thiocyanate concentration in urine could be found in different species after feeding rapeseed meal (solvent extracted), rapeseed press cake or ground rapeseed (Schone et al. 2001). Lakshmy et al. (1995) found a significant increase in the excretion of thiocyanate by following one-third supplementation of cabbage. Mustard meal supplementation also caused increased serum thiocyanate level (Tripathi et al. 2001). de Groot et al. (1991) reported increased thiocyanate concentration in a dose related manner in blood plasma and urine after feeding Brussels sprouts. Similarly the dose response was more pronounced in urine than in plasma. The thioglucosides / glucosinolates derivatives converted to thiocyanate in the blood followed by its
appearance in the urine (Michajlovskij and Langer 1959). Langer (1961) also reported feeding of Brassica plants to guinea pigs caused increased SCN ion in serum. Langer (1964) also showed that following a dose of allyl isothiocyanate there was an increase in serum level of thiocyanate. Moreover dietary cyanide causes significant increase in serum and urinary thiocyanate excretion (Tewe and Maner 1985). Tewe et al. (1984) also reported significant correlation between cyanide intake and serum thiocyanate level. Barret et al. (1978) reported that linamarin, the principal cyanogenic glucosides in cassava causes increased excretion of thiocyanate ion. The cyanide given alone or to animals pre-treated with thiosulfate is extensively converted to thiocyanate in mice (Frankenberg and Sorbo 1975). A study of the distribution of C\textsuperscript{14}-labelled cyanide was carried out in rats exposed to a regular intake of cyanide in the diet for 3 weeks. Most of this was in the contents of the stomach, of which over 80% was in the form of thiocyanate. When a small amount of S\textsubscript{14}CN\textsuperscript{-} was given by mouth to rats with elevated plasma thiocyanate levels, most of the activity was excreted in the urine and only small amounts were found in the faeces. This indicated the existence of a gastrointestinal circulation of thiocyanate, in which a substantial amount of this substance secreted into the stomach contents of the rat was reabsorbed by the intestine into the body fluid to be partly excreted in the urine (Okoh and Pitt 1982). Chandra et al. (2004c) also reported bamboo shoot (rich in cyanogenic glucosides) supplementation in albino rats causes a marked increase in urinary thiocyanate level.

In the case of cooked food the urinary thiocyanate level was higher than the control but lower than the uncooked plant foods or in other words the urinary thiocyanate level was more in the uncooked plant fed groups. de Groot et al. (1991) reported that increase in serum and urinary thiocyanate levels were consistently smaller with the cooked foods than with the uncooked Brussels sprouts. During heating significant loss in the amount of cyanogenic glucosides and a moderate loss in glucosinolates was observed as evidenced by the concentration of those in cooked food in comparison to that of uncooked / fresh foods. The differences in urinary level thiocyanate between fresh and cooked radish fed groups might be due to the reduction in the concentration of thiocyanate precursors during cooking. Thus in spite of the consumption of same amount of foods (uncooked or cooked) the overall concentration of those in the uncooked plant food was more than their cooked counterparts.
Therefore the increase in urinary thiocyanate level as observed in this study was due to presence of glucosinolates, thiocyanate and cyanogenic glucosides in the studied plant foods. The urinary thiocyanate level was almost consistent with the consumption of the different plant foods containing goitrogenic principles. The present observations are also consistent with the earlier observations and it also suggests that these plants are metabolized after ingestion and liberate thiocyanate mostly in the animal body.

**Urinary iodine level under the influence of plant foods**

Urinary iodine level was increased in the fresh / uncooked cabbage, cauliflower, mustard and radish supplemented groups; however, the values were higher with uncooked plant foods than with their cooked counterparts. Maximum iodine excretion value was observed in fresh cauliflower fed group followed by fresh cabbage, mustard and radish fed groups. In case of cooked cauliflower fed group the values were significant in comparison to control. A tendency for increased urinary iodine level was found in all the selected plant fed groups.

All the studied plants contain glucosinolates, thiocyanate and cyanogenic glucosides in significant proportion in uncooked / fresh and cooked conditions. Glucosinolates and cyanogenic glucosides content were reduced markedly during cooking while thiocyanate remained almost unaltered after cooking as discussed earlier.

A report by Beyssen et al. (1999) showed that drugs inducing hypothyroidism, also increases excretion of urinary iodine when dietary iodine was restricted. Schone et al. (2001) further reported that spot urine and faeces samples from sows eating rape seed press cake diet containing a significant amount of glucosinolates, had increased iodine concentration. Moreover they reported glucosinolates and their degradation compounds resulting in higher renal and intestinal iodine excretion. Tewe and Maner (1980) observed that dietary cyanide supplementation increased urinary iodine excretion. A study of Lakshmy et al. (1995) showed increased excretion of iodine in rats following cabbage supplementation in diets. Moreover thiocyanate or thiocyanate like compounds primarily inhibits iodide concentrating mechanism of thyroid gland an also increases the excretion of iodine through urine (Gaitan 1990). Prolonged consumption of bamboo shoot also increases urinary iodine excretion level in albino rats (Chandra et al. 2004c).
Therefore, the increased urinary excretion of iodine after feeding uncooked / fresh and cooked cabbage, cauliflower, mustard and radish was due to the glucosides or other goitrogenic / anti-thyroid constituents present in the cyanogenic foodstuff which were metabolised to produce thiocyanate mostly. The liberated thiocyanate in turn had increased the excretion of iodine through urine by removing more iodine from the thyroid gland by replacing thiocyanate within it. Present findings are consistent with the earlier observations. In case of cooked food supplementation in the diet, excretion of iodine through urine was less compared to uncooked / fresh plant foods because of thiocyanate, glucosinolates and cyanogenic glucosides content of the uncooked plant foods were more than cooked foods that caused extra removal of iodine from the gland.

Urinary iodine and thiocyanate ratio was found higher in the group of rats fed with cooked cyanogenic plants in comparison to their uncooked / fresh counterparts.

Glucosinolates and cyanogenic glucosides present in the studied plant foods were metabolised after consumption to thiocyanate and thereby causing enhanced excretion of thiocyanate. Excretion of iodine that considered as a marker of iodine nutritional status was supposed to be regulated by the thiocyanate concentration in the body that in turn depended on the consumption pattern of cyanogenic plant foods containing cyanogenic goitrogens. This study also suggested that the iodine retaining capacity of the thyroid / body was dependent to an extent on the consumption pattern of the cyanogenic plant foods.
The effect of consumption of cruciferous plants on the morphological status of thyroid gland is presented in this chapter. In addition, the obtained results after feeding the selected plant foods have been discussed in the light of available literature.

The mammalian thyroid gland is composed of two distinct endocrine cell populations concerned with the synthesis of two different classes of hormones. Follicular cells secrete the metabolically active iodothyronines, essential for normal growth and development where as the C or parafollicular cells are concerned with the production of calcitonin, a hormone that influences blood levels of calcium and phosphorus, 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 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), flavonoids from millets (Gaitan et al. 1989) and glucosinolates from several commonly consumed cruciferous plants (Conn 1980; Tookey et al. 1980) magnify the severity of goitre endemia.

Chesney et al. (1928) first reported greatly enlarged thyroids in rabbits fed with cabbages as a major part of diet. Hercus and Purves (1936) also showed cabbage and related Brassica seeds caused enlargement of thyroid glands in rats. Later investigators were able to produce consistent thyroid enlargement in different animals and named as “Brassica seed goitre”. Extensive studies have been made on the effect of various articles of the diet on the induction of goitre in animals. Certain plants such as turnip (Hercus and Purves 1936), rapeseed (Kennedy and Purves 1941), soyabeans (Mc Carrison 1933) and peanut (Mc Carrison 1933) have been reported to cause thyroid enlargement. It is often suggested that the effect of these foods may be attributable to goitrogenic compounds contained within them (Astwood et al. 1949).
Abnormal thyroid growth can be classified into two main categories: a) those cases due to excess of thyroid stimulators extrinsic to the gland; b) situations in which an intrinsic alteration in the gland occurs: Extrinsic (excess thyroid stimulation) i) Iodide deficiency with elevated TSH, ii) Goitrogens, iii) Graves' immunoglobulins, iv) Thyroid stimulating factors produced by tumors, v) Dishormonogenesis with hypothyroidism and Intrinsic (normal TSH) i) Increased sensitivity to TSH (iodine depletion), ii) Altered autoregulation, iii) Abnormal TSH receptor and iv) Other biochemical abnormalities (Pisarev et al. 1980).

The basic mechanisms acting in the transformation of a normal thyroid gland into a toxic or nontoxic goitre are summarized as: 1) Any goitre arises from multiplication of follicular epithelial cells forming new follicles. 2) In the follicular epithelium there are cell families with much higher than average growth potential. 3) Cells of an individual follicle are not identical but heterogeneous. 4) Each follicular cell has a certain level of autonomy of growth and of function (Gerber et al. 1988).

Gaitan (1989a) divides goitrogens into agents acting directly on the thyroid gland and those causing goitre by indirect action. The former group is subdivided into those inhibiting transport of iodine into the thyroid (like thiocyanate, isothiocyanate), those acting on the intra-thyroidal oxidation and organic binding process of iodine and/ the coupling reaction (like phenolic compounds, some phthalate derivatives disulphides and goitrin) and those interfering with proteolysis, dehalogenation and hormone release (like iodine and lithium). Besides that an increase in TSH level is responsible for the development of goitre, as because the pituitary, sensing the lowered circulating levels of T3 and T4, increased the secretion of thyroid stimulating hormone which resulted in the morphologic evidence of follicular 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, goitrogens / anti-thyroid substances are classified into two groups, first group contains goitrogens / 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 goitrogens 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. 1990). 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 practically no colloid and the epithelium had folded into the follicles so that there was a great increase 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 on feeding Brussels sprouts and the degree of 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 result 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 and cooked vegetables led to an increased risk of follicular cancer (Markaki 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 the parafollicular cells or C-cells) (Aleshin 1981).

In the present study the effect of feeding the selected cyanogenic 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

Animal maintenance and treatment

Described in the methodology section.

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 uncooked and cooked cabbage, cauliflower, mustard and radish fed group of rats were measured and compared with control (Table 9). Variations in the weight of the thyroid gland were observed under the influence of uncooked and cooked vegetables.

Table 9. Selected cyanogenic plant foods induced alterations on thyroid status in albino rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Thyroid weight mg/100 gm body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.93 ± 0.4</td>
</tr>
<tr>
<td>Cabbage</td>
<td></td>
</tr>
<tr>
<td>Uncooked/ fresh</td>
<td>12.19 ± 0.84*</td>
</tr>
<tr>
<td>Cooked</td>
<td>10.95 ± 0.62*</td>
</tr>
<tr>
<td>Cauliflower</td>
<td></td>
</tr>
<tr>
<td>Uncooked/ fresh</td>
<td>12.44 ± 0.35*</td>
</tr>
<tr>
<td>Cooked</td>
<td>10.61 ± 0.3*</td>
</tr>
<tr>
<td>Mustard</td>
<td></td>
</tr>
<tr>
<td>Uncooked/ fresh</td>
<td>11.46 ± 0.84*</td>
</tr>
<tr>
<td>Cooked</td>
<td>10.72 ± 0.42*</td>
</tr>
<tr>
<td>Radish</td>
<td></td>
</tr>
<tr>
<td>Uncooked / fresh</td>
<td>11.18 ± 0.51*</td>
</tr>
<tr>
<td>Cooked</td>
<td>9.69 ± 0.62**</td>
</tr>
</tbody>
</table>

Values are mean ± SD of ten observations.

* p<0.001, ** p<0.01 when compared to control.
There was a significant increase in thyroid weight (P<0.001) in both the uncooked / fresh and cooked cabbage fed rats as compared to control. The increase was more in uncooked cabbage fed group than cooked cabbage fed group (p<0.001).

Similarly, rats fed with uncooked and cooked cauliflower showed significant increase in thyroid weight (P<0.001). Increase in thyroid weight was more evident in uncooked / fresh cauliflower fed group than cooked cauliflower fed group (p<0.001).

Mustard fed both the groups (i.e. uncooked and cooked) showed increased thyroid weight (P<0.001) as compared to control. Increase in thyroid weight was more with uncooked mustard than cooked one (p<0.01).

![Bar chart showing thyroid weight in albino rats fed with different plant foods](image)

**Fig 22. Selected cyanogenic plant foods induced alterations on thyroid weight in albino rats (Values are mean ± SD)**

Significant increase in thyroid weight (P<0.001) was observed in uncooked radish fed and cooked radish fed groups (p<0.01) when compared to control while the values were more in uncooked radish fed group than cooked radish fed group (p<0.001).
Among the studied uncooked plant foods, highest increase in thyroid weight was found in cauliflower fed group of rats while among the cooked plant foods the maximum increase was with cabbage fed group of rats.

The histological changes that occurred in the thyroid gland in the selected cyanogenic plants fed groups and control group of rats are shown in the Plate I, II, III and IV. In general, in cyanogenic plants fed groups thyroid glands were lined with high cuboidal and low columnar cells showing hypertrophy and hyperplasia filled with less homogeneous colloid and some follicles were invaded by epithelial cells. There was a great increase in the number of small follicles while 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 different cyanogenic plant foods in uncooked and cooked conditions 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 cyanogenic plant fed groups of rats as compared to control group of rats.

DISCUSSION
A considerable proportion of the vegetables in the human diet are derived from the cruciferous plants. These vegetables include cabbage, cauliflower, mustard seeds and radish contain glucosinolates and thiocyanate in significant proportion and cyanogenic glucosides in small amount. After cooking these vegetables, cyanogenic glucosides and glucosinolates are degraded markedly but thiocyanate remains almost same.

In the present study the effect of prolonged feeding of cabbage, cauliflower, mustard and radish in uncooked / fresh and cooked conditions replacing a portion of the normal diet respectively on thyroid morphology was evaluated by measuring thyroid weight and histological changes of the thyroid gland in all the experimental groups and compared with control.

**Morphological changes of thyroid gland under the influence of plant foods**
Weight of the thyroid glands was increased significantly after feeding the selected plant foods but the increase was more profound in uncooked / fresh plant food fed groups than that of the respective cooked plant food fed groups. The increase in weight of thyroid
Plate I – Photomicrographs of thyroid gland in control, fresh / uncooked & cooked cabbage fed rats (HE, 200X)
Plate II – Photomicrographs of thyroid gland in control, fresh / uncooked & cooked cauliflower fed rats (HE, 200X)
Plate III – Photomicrographs of thyroid gland in control, fresh / uncooked & cooked mustard fed rats (HE, 200X)
Plate IV — Photomicrographs of thyroid gland in control, fresh / uncooked & cooked radish fed rats (HE, 200X)
Plate I – Photomicrographs of thyroid gland in control, fresh / uncooked & cooked cabbage fed rats (HE, 200X)
Plate II – Photomicrographs of thyroid gland in control, fresh / uncooked & cooked cauliflower fed rats (HE, 200X)
Plate III – Photomicrographs of thyroid gland in control, fresh / uncooked & cooked mustard fed rats (HE, 200X)
Plate IV – Photomicrographs of thyroid gland in control, fresh / uncooked & cooked radish fed rats (HE, 200X)
glands in the experimental animals might be due to the presence of glucosinolates, thiocyanate and cyanogenic glucosides present in those plants.

Glucosinolates and their breakdown products cause morphological and functional changes in thyroid gland (Spiegel et al. 1993). 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 in the plant foods (Montgomery 1965). Bobek (1992) observed that the enhanced level of plasma thiocyanate following feeding with Brassica plants increased proportionally the goitrogenic action. 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). Vermorel et al. (1987) reported when rapeseed diets were given as powder or as mash, thyroid gland was 3-4 times as big as control. Tripathy et al. (2001) showed that mustard meal supplementation had increased thyroid weight. Lakshmy et al. (1995) also reported a significant increase in thyroid weight after one-third cabbage supplementation in diets. Moreover, many glucosinolates break down products after degradation may affect thyroid primarily by increasing weight of thyroid gland (Schone et al. 2001; Arstila et al. 1969). While Nordfeldt et al. (1954) found that feeding 10% and 20% rapeseed meal containing glucosinolates to growing pigs caused enlargement of thyroid but when the meal was fed after it had been extracted with warm water, thyroids were essentially normal. Weight of thyroid glands of the birds increased with rapeseed oil meal diets (Paik 1991). Inclusion of heat-treated rapeseed meal in the diet caused the thyroid glands to enlarge in broiler cockerels suggesting its goitrogenic activity (Chiasson et al. 1979). Thyroid gland weights were significantly higher in growing pigs fed rapeseed meal than in the group fed control (sunflower seed meal) (Svetina et al. 2003). Despite high iodine supplementation, glucosinolates load through rapeseed meal decreased growth, feed intake, iodine store of the thyroid and serum concentration of thyroid hormone and resulted in goitre formation (Schone et al. 1997). de Groot et al. (1991) reported marked and consistent weight increase of thyroid gland on feeding brussel sprouts and the increase was more in uncooked plant fed rats.

Therefore in the present study the increase in thyroid weight might be due to presence of glucosinolates, thiocyanate and cyanogenic glucosides in the plant foods and
also because of degradation of these goitrogenic / anti-thyroid constituents to various breakdown products that acted on thyroid gland to increase weight. As cooking causes reduction in glucosinolates and cyanogenic glucosides keeping thiocyanate almost same or slightly increased thus the effect was more pronounced in uncooked plant foods fed group of rats compared to their respective cooked counterparts.

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

Histological changes that occurred in the thyroid glands after feeding the selected plants in uncooked / fresh and cooked conditions respectively were evaluated and compared with control. Alteration in thyroid histology was noted in cabbage, cauliflower, mustard and radish fed group of rats but the variations after supplementation of a particular plant either in fresh or cooked condition were more or less similar in nature.

Marked alteration in thyroid gland histology was observed in the studied plants fed groups in comparison to that of control because selected plants contain cyanogenic glucosides, glucosinolates and thiocyanate; the follicles were reduced in size and surrounded by cuboidal or columnar epithelial cells showing hypertrophy and hyperplasia. In addition, there was a increase in the number of very small follicles without any colloid. Sharpless (1939) also reported the development of goitre after feeding soyabean in rats with similar type of observations. Kanno et al. (1990) classified the goitrogens into two types - goitrogen induced TSH stimulated diffuse goitre and goitrogen induced TSH independent colloid goitre. The histological changes as observed in the present study after feeding cyanogenic plant foods were the prototype of goitrogen induced TSH stimulated diffuse goitre. No remarkable alterations were noted in the histology of thyroid gland after feeding of a particular fresh / uncooked and cooked plant food. However, there were variations in histology of thyroid gland after feeding different types of plant foods. Schone (1993) reported strongly enlarged thyroid gland contained only little iodine associated with hypothyroidism in pigs fed rapeseed meal. The epithelium of the thyroid gland was cuboidal or columnar and the follicular area was moderately enlarged in pigs fed rapeseed meal (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 and reduction of lumenal 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.

Histological observations showed the hypertrophy and hyperplasia of the follicular epithelial cells that surround mostly the small follicles containing less colloid stained deeply with eosin indicating the presence of inadequate iodine in both uncooked and cooked cyanogenic plant fed groups, which resembles the prototype of goitrogen induced TSH stimulated diffuse goitre. Glucosinolates and their break down products, cyanogenic glucosides and thiocyanate itself present in the studied plants were responsible for the histological alterations of the thyroid gland in the studied plant fed groups of rats.

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

EFFECT OF FEEDING THE EDIBLE PARTS OF THE SELECTED PLANTS ON THYROID PEROXIDASE ACTIVITY & THYROID HORMONE PROFILES

The important markers to evaluate the functional status of thyroid gland are thyroid peroxidase activity and thyroid hormone profiles i.e. circulating levels of total thyroxine and triiodothyronine in the serum in addition to thyroid gland morphology. The effect of cruciferous plants on thyroid functional status has been reviewed and results available after feeding the most commonly used selected cyanogenic plant foods have been discussed in this chapter.

Thyroid peroxidase is the key enzyme in thyroid hormone biosynthesis. It is frequently been referred to as a microsomal enzyme (Hosoya and Morrison 1967). For thyroid hormone biosynthesis iodide enters the thyroid follicular cells as inorganic iodide and transformed through a series of metabolic steps into thyroid hormones. Thyroid peroxidase regulates the organification of iodine i.e. conversion of iodide to iodine, iodination of tyrosine residues of thyroglobulin i.e. the formation of mono iodothyronine (MIT) and di-iodothyronine (DIT) and finally coupling reaction i.e. binding between MIT with DIT and DIT with DIT to form tri-iodothyronine (T₃) and thyroxine (T₄) respectively attached to thyroglobulin in the thyroid follicular cell (Pommier et al. 1973; Sugawara 1985). Proteolysis of thyroglobulin results into the release of free thyronines and iodothyronines, release of iodine from iodothyronine and re utilization of the liberated iodide within the thyroid (Sugawara 1985).

Follicular cells of the thyroid are designed for hormone synthesis and secretion. T₃ and T₄ are the predominant circulating thyroid hormones synthesized and secreted by follicular cells in vertebrates. 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 thyroid hormones including T₃, T₄ and TSH, are commonly used as reliable indicators of the thyroid function in humans and experimental animals. Changes in the serum concentration of these hormones can reflect disturbances in their glandular synthesis / secretion as well as disorders in their extra-thyroidal peripheral metabolism. Thyroid hormones are metabolized in peripheral tissues (by
deiodination, conjugation, deamination and decarboxylation) and alterations in their metabolism may significantly influence the function of thyroid hormone metabolites at the 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 disease (Gaitan 1990).

Naturally occurring agents such as cyanogenic glucosides present in several staple foods like cassava, bamboo shoot, sweet potatoes, lima beans etc; flavonoids from millets, soyabean and glucosinolates are often present in vegetables of cruciferous family (Conn 1980; Gaitan 1990) interfere with thyroid gland function. After ingestion, cyanogenic glucosides can be readily converted to thiocyanate by widespread glucosidases and sulfur transferase enzymes (Dahlberg et al. 1984). Glucosinolates also undergo a rearrangement to form isothiocyanate derivatives and in some instances thiocyanate (Schone et al. 2001).

Transport of iodine for the active concentration of inorganic iodide in the thyroid gland is prevented by environmental goitrogens. Thiocyanate, isothiocyanate interfere with this process. Since thiocyanate (SCN\(^-\)) has a molecular volume and charge similar to iodide, it competes with iodide for transport into the thyroid cell (Maloof and Soodak 1959; Greer et al. 1966; Van Middlesworth 1985; Ermans and Bourdoux 1989; Gaitan 1990; Capen 1992; Knudsen et al. 2002; Laurberg et al. 2002). Besides that thiocyanate also inhibits tyrosine iodination (Raben 1949; Greer et al. 1966; Virion 1980; Van Middlesworth 1985). Moreover, thiocyanate inhibits the incorporation of iodide into thyroglobulin by competing with iodide at the thyroid peroxidase level (Maloof and Soodak 1959; Langer and Michajlovsjik 1972). Thiocyanate also inhibits the iodide oxidation (I\(^-\) leads to I\(_2\)) by thyroid peroxidase enzyme depending upon the binding of SCN\(^-\) to the substrate site with lower affinities (Virion et al. 1980).

Cyanogenic glucosides release cyanide after ingestion. The cyanide is then detoxified to thiocyanate and thiocyanate causes displacement of iodide from the thyroid gland and blocks organic binding of iodine (Greer et al. 1966; Hershman et al. 1985). Supplementation of cassava, bamboo shoot and other cyanogenic glucosides rich plant

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foods causes reduction in thyroid hormone profiles and also inhibits thyroid peroxidase activity in various animals (Chandra et al. 2004c; Hershman et al. 1985).

Moreover, glucosinolates are iodine antagonist, it also prevents iodination of thyroglobulin (Schone et al. 2001). Glucosinolates derivative isothiocyanate also causes general inhibition of iodine uptake by the thyroid (Stoewsand 1995) by competitive displacement of iodine by isothiocyanate (Gaitan 1990; Spiegel et al. 1993). Another degradation product goitrin causes inhibition of iodide oxidation (Gaitan et al. 1983; Kohler 1989). Presence of thiocyanate and its natural source like cabbage in diet lowers the circulating level of thyroxine (T₄) and triiodothyronine (T₃) in rats (Lakshmy et al. 1995). Glucosinolates and thiocyanate in the body lowers the circulating level of thyroxine and triiodothyronine in experimental animals, which were reported by earlier workers (Phillbrick et al. 1979; Rao and Lakshmy 1995). Besides that rapeseed meal, containing high glucosinolates level also causes profound changes in the thyroid hormone profiles (Vermorel et al. 1987; Papas et al. 1979).

Physiological perturbations alone such as the feeding of an iodine-deficient diet, partial thyroidectomy, natural goitrogens in certain foods, and transplantation of TSH-secreting pituitary tumors in rodents also can disrupt thyroid hormone economy (Capen and Martin 1989).

Therefore in the present study in vivo thyroid peroxidase (TPO) activity and serum T₃ and T₄ levels were determined to evaluate the functional status of thyroid gland after supplementing uncooked / fresh and cooked cabbage, cauliflower, mustard and radish respectively in the diet, as these plants contain a significant proportion of glucosinolates, thiocyanate and cyanogenic glucosides as constituents of goitrogenic / anti thyroid principles.

**MATERIALS AND METHODS**

**Animal maintenance and treatment**

Described in the methodology section.

**Assay of thyroid peroxidase activity**

Described in the methodology section.
Assay of serum total Triiodothyronine (T₃) and thyroxine (T₄) levels

Described in the methodology section.

RESULTS

Thyroid peroxidase activity (expressed in ΔOD/min/mg protein), serum total T₃ levels (ng/dl) and total thyroxine (μg/dl) were measured in control and experimental rats supplemented with one-third portion of their normal diet by uncooked and cooked cabbage, cauliflower, radish and mustard respectively and the obtained results were compared with respective control (Table 10). Wide variations in TPO activity, serum T₃ and T₄ levels were observed after feeding the uncooked and cooked selected plant foods to experimental animals.

Table 10. Selected plant foods induced alteration in thyroid peroxidase activity and total thyroid hormone levels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TPO activity ΔOD/ min/ mg protein</th>
<th>T₃ ng/dl</th>
<th>T₄ μg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.34 ± 0.26</td>
<td>143.12 ± 1.49</td>
<td>4.21 ± 0.28</td>
</tr>
<tr>
<td>Cabbage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncooked</td>
<td>1.56 ± 0.22*</td>
<td>109.43 ± 1.06*</td>
<td>3.37 ± 0.15*</td>
</tr>
<tr>
<td>Cooked</td>
<td>2.27 ± 0.17*</td>
<td>111.22 ± 2.07*</td>
<td>3.46 ± 0.17*</td>
</tr>
<tr>
<td>Cauliflower</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncooked</td>
<td>1.68 ± 0.25*</td>
<td>100.83 ± 1.14*</td>
<td>3.5 ± 0.18*</td>
</tr>
<tr>
<td>Cooked</td>
<td>2.54 ± 0.36*</td>
<td>105.71 ± 1.74*</td>
<td>3.54 ± 0.11*</td>
</tr>
<tr>
<td>Mustard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncooked</td>
<td>1.3 ± 0.21*</td>
<td>115.83 ± 1.71*</td>
<td>3.56 ± 0.23*</td>
</tr>
<tr>
<td>Cooked</td>
<td>2.92 ± 0.13*</td>
<td>120.23 ± 1.35*</td>
<td>3.71 ± 0.17*</td>
</tr>
<tr>
<td>Radish</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncooked</td>
<td>2.25 ± 0.14*</td>
<td>117.03 ± 1.85*</td>
<td>3.6 ± 0.07*</td>
</tr>
<tr>
<td>Cooked</td>
<td>2.82 ± 0.14*</td>
<td>119.76 ± 1.36*</td>
<td>3.68 ± 0.12*</td>
</tr>
</tbody>
</table>

Values are mean ± SD of ten observations.
* p<0.001 when compared to control

There were significant reduction in TPO activity (p<0.001), serum T₃ (p<0.001) and T₄ levels (p<0.001) in both the uncooked and cooked cabbage fed rats as compared
to control rats. In between the two cabbage fed groups, the reduction was more in uncooked cabbage fed group (TPO p<0.001, T3 p<0.02 and for T4 the values were non-significant).

Similarly, rats fed uncooked and cooked cauliflower also showed significant reduction in TPO activity (p<0.001), serum T3 (p<0.001) and T4 (p<0.001) levels when compared to control. TPO activity was reduced significantly in uncooked cauliflower fed rats compared to cooked cauliflower fed rats (p<0.001); no significant variation was found in serum T4 level in between uncooked and cooked cauliflower fed rats however, serum T3 level was reduced significantly (p<0.001) in uncooked cauliflower fed group than cooked cauliflower fed group.

Mustard (both uncooked and cooked) fed groups showed reduced TPO activity (p<0.001), lower serum T3 (p<0.001) and T4 (p<0.001) levels as compared to control. Reduction in TPO activity (p<0.001), T3 (p<0.001) and T4 (the values were non-significant) levels were more with uncooked mustard than its cooked counterpart.

Fig 23. Selected plant foods induced alteration in thyroid peroxidase activity
[Values are mean ± SD]
Fig 24. Selected plant foods induced alteration in serum triiodothyronine (T3) level
[Values are mean ± SD]

Significant reduction in TPO activity, serum T3 and T4 levels were found in both the uncooked and cooked radish fed groups respectively (p<0.001) as compared to control. The reduction was more profound in uncooked radish fed group than cooked radish fed group (for TPO p<0.001, for T3 p<0.02 and for T4 the values were non significant).

Among all the uncooked plant fed groups highest inhibition in TPO activity was found in uncooked mustard fed rats while among the cooked foods the maximum inhibition was found with cabbage. Serum T4 level was reduced maximally in both the uncooked and cooked cabbage fed group of rats in comparison to any other plants fed rats irrespective of cooked or uncooked conditions but maximum reduction in serum T3 level was found after feeding cauliflower (both uncooked and cooked condition) in comparison to other foods.
In general, thyroid peroxidase activity was inhibited, serum T₃ and T₄ levels were reduced in cabbage, cauliflower, mustard and radish fed group of rats in various proportion however, the reduction was more profound in each case with uncooked plants than their cooked counter parts.

**DISCUSSION**

The effect of chronic consumption of cabbage, cauliflower, mustard and radish either in uncooked or cooked conditions respectively by replacing one third portion of the normal diet on thyroid physiology was evaluated in the present study. The dietary regimen was continued for 60 days and after the treatment period thyroid peroxidase activity, serum total T₄ and T₃ levels were determined and compared with the control group maintained with laboratory standardized normal diet.
**TPO activity under the influence of plant foods**

Thyroid peroxidase activity (TPO) was reduced markedly in cooked cabbage, cauliflower, mustard and radish fed groups. Highest inhibition was noted with cabbage followed by cauliflower, radish and mustard. Further inhibition in TPO activity was noted when these foods were fed fresh/uncooked condition indicating that *in vivo* TPO inhibition under the influence of these plant foods was more with fresh/uncooked plant than that of cooked one.

All these foods contain cyanogenic glycosides, glucosinolates and thiocyanate as was discussed earlier. The concentration of glucosinolates was slightly lower in cooked foods in comparison to that of respective fresh food; while thiocyanate concentration was found almost same or slightly higher in cooked foods than uncooked plant foods but the concentration of cyanogenic glucosides were reduced markedly in the cooked plants than that of fresh or uncooked respective plants.

Raben (1949) showed that at high concentration thiocyanate inhibits the incorporation of iodide into thyroglobulin. Dahlberg *et al.* (1984) reported the potent anti-thyroid activity of thiocyanate. Van Middlesworth (1985) found that thiocyanate increases the formation of an essentially insoluble iodinated thyroglobulin within the thyroid in iodine depleted condition, however, only a small fraction of iodination may occur through this route. Maloof and Soodak (1959), Langer and Michajlovskij (1972) showed that iodide and thiocyanate compete at the level of thyroid peroxidase at its binding sites. The extensive study by Virion (1980) further revealed that thiocyanate inhibits the iodide oxidation i.e. conversion of $I^-$ leads to $I_2$ by inhibiting TPO. Iodination of tyrosine residue of thyroglobulin is also inhibited under the influence of excess thiocyanate that reacts on thyroid peroxidase. Conversely thiocyanate stimulates the coupling reaction i.e. the formation of $T_3$ and $T_4$ from MIT and DIT. Orgiazzi and Millot (1994) reported that thyroid peroxidase is the target of thiocyanate. It blocks the iodination of tyrosine residues and coupling of iodotyrosines into iodothyronines. Greer *et al.* (1966) found that thiocyanate has an inhibitory effect on iodination of thyroglobulin. Thiocyanate at high concentration also inhibits the incorporation of iodide into thyroglobulin by competitions with iodide at the thyroid peroxidase level (Ermans and Bourdoux 1989), thiocyanate or thiocyanate like compounds primarily inhibit iodide-concentrating mechanism of thyroid (Capen 1992). Therefore, the inhibition of
TPO under the influence of these plant foods was probably due to the interference in TPO activity by the indigenous thiocyanate present in those plants itself and also due to the conversion of cyanogenic glycosides and glucosinolates to thiocyanate in presence of widespread glucosidases, sulfur transferases and myrosinase present in the different animal tissues (Reinwein 1961; Conn 1980).

Inhibition of thyroid peroxidase activity also might be due to the presence of glucosinolates in the foodstuff, because in presence of glucosinolates the action of TPO changes (Schone et al. 2001). In vitro studies of Kohler et al. (1988) demonstrates that TPO oxidizes oxazolidinethiones and probably further degradation products of glucosinolates and thus uses elemental iodine. Iodine is thus reduced to iodide which cannot be taken up by the thyroglobulin and thus interfere with the activity of thyroid peroxidase. Glucosinolates degradation product - goitrin inhibits oxidation of iodide (Kohler 1989). The thionamide like anti-thyroid effects of goitrin have also been confirmed in in vitro studies evidenced by marked inhibition of thyroid peroxidase and iodide organification (Gaitan et al. 1983; Gaitan 1986a). Another degradation product of glucosinolates, isothiocyanate also interferes with the organification of iodine thereby affecting thyroid peroxidase activity (Gaitan 1989a). Therefore, the inhibition of thyroid peroxidase activity might be also due to the direct action of glucosinolates or its degradation products like isothiocyanate, goitrin etc. present in the studied plants.

Moreover, cyanogenic glucosides release cyanide after ingestion. The cyanide is detoxified to thiocyanate and the thiocyanate blocks the organic binding of iodine thereby affecting thyroid peroxidase activity (Greer et al. 1966). A study by Gaitan (1990) also suggests that cyanogenic glycosides act on the thyroid mainly by their rapid conversion to thiocyanate. Moreover, Chandra et al. (2004c) also reported marked inhibition in TPO activity after chronic consumption of bamboo shoot rich in cyanogenic glucosides. Soyabean of Indian origin also inhibited thyroid peroxidase activity severely in albino rats (Mukhopadhyay et al. 2004).

Inhibition of thyroid peroxidase activity was more in fresh uncooked plants than their respective cooked counter parts though contrary observation was noted in in vitro studies on human thyroid tissues (Chandra et al. 2004a). This observation is consistent with Fragu et al. (1979) who reported that the effects of PTU on TPO in in vivo and in vitro studies were not uniform.
Finally, the inhibition of thyroid peroxidase activity as found in the present study in cabbage, cauliflower, mustard and radish fed animals was due to the presence of cyanogenic glucosides, glucosinolates and thiocyanate in those plant foods which degraded in the animal body yielding thiocyanate, goitrin, isothiocyanate that mainly affected the activity of the enzyme acting at different levels.

**Total serum T₃ and T₄ under the influence of plant foods**

Serum total T₃ level was decreased significantly in cooked cabbage, cauliflower, mustard and radish fed group of animals. Among all the plants maximum reduction was found in cauliflower fed group followed by cabbage, radish and mustard fed groups. Further reduction in serum T₃ level was observed under the influence of each of those plant foods in fresh or uncooked condition; maximum reduction was with cauliflower followed by cabbage, mustard and radish.

In consistent with T₃ level, serum total T₄ level was also reduced in fresh or uncooked cabbage, cauliflower, mustard and radish fed rats as compared to control. Maximum reduction was found in cabbage fed rats followed by cauliflower, radish and mustard fed rats in uncooked condition. No significant change in T₄ level was found in between the different uncooked and cooked plant fed groups.

Reduced TPO activity might be responsible for decrease in thyroid hormone levels because the enzyme regulates the synthesis of thyroid hormones (Pommier et al. 1973; Taurog 1970).

It has been discussed earlier that the presence of cyanogenic glucosides, glucosinolates and thiocyanate in the foodstuff and their degradation products like thiocyanate, isothiocyanate, and goitrin are responsible for the inhibition of thyroid peroxidase activity (Greer et al. 1966; Virion 1980; Kohler 1989; Gaitan 1990; Orgiazzi and Millot 1994; Schone et al. 2001). The main product of transformation of cyanide is thiocyanate, which competes with iodide in the Na⁺/I⁻ symporter in the thyroid gland, consequently inhibiting the synthesis and clearance of thyroid hormones (Dohan et al. 2000). Glucosinolates load through rapeseed meal decreased serum concentration of thyroid hormones and resulted in goitre formation (Schone et al. 1997). Salts of thiocyanic acids in crucifers also interfere with the uptake of iodide by the thyroid gland (Gmelin and Virtanen 1960). 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 shown to raise the rate constant for exit of iodide but only slightly reduce the rate of entry (Wollman 1962; Scranton et al. 1969; Van Middlesworth 1985). Greer et al. (1966) reported that thiocyanate has inhibitory effect mainly on the uptake of iodine in the thyroid. 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 the thyroid gland and inhibits the normal metabolism of iodine (Green 1978). Capen (1992) reported that thiocyanate causes inhibition of iodide trapping mechanism. Laurberg et al. (2002) also found thiocyanate inhibits iodide transport, a similar observation with Maloof and Soodak (1959). Knudsen et al. (2002) showed that thiocyanate acts as a competitive inhibitor of iodide uptake. Bourdoux et al. (1978) found that when thiocyanate intake is high, an adaptation is obtained only at the expense of a drop of plasma T4 level with a very high increase of plasma TSH level. Lakshmy and Rao (1997) reported a partial suppression of thyroid 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. Rao and Lakshmy (1995) showed that the addition of thiocyanate to food deprived of potassium iodide brought down significantly circulating levels of T4 in rats. A study with crushed cabbage hydrolysate containing glucosinolates caused marked decrease in serum T3 and T4 concentrations analogous with methimazole, a well-known anti-thyroid drug (Heary et al. 1992). Lakshmy et al. (1995) also showed that supplementation of one-third dry cabbage with normal diet significantly reduced plasma T4 level. Spiegel et al. (1993) reported that serum thyroxine concentrations were reduced significantly in pigs fed rapeseed press cake meal containing glucosinolates but serum T3 concentration remained unaltered. de Groot et al. (1991) reported diet supplemented with Brussels sprouts reduced plasma T4 level significantly. Hershman et al. (1985) showed that feeding of cassava reduced the levels of serum T3 and T4. Tripathy et al. (2001) reported reduced plasma T3 and T4 level in calves fed with mustard meal. Vermorel et al. (1987) also reported that when rape seed diets were given as powder or mash causes a 30% and 50% decrease in the plasma T4 level. Plasma T4 concentration was reported to reduce after rapeseed meal supplementation (Papas et al. 1979). 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 (Mukherjee et al. 1979).

In addition to thiocyanate, thiooxazolidone, another break down or intermediate product of glucosinolates also inhibits the synthesis of thyroid hormones (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 shoot, rich in cyanogenic glucosides when supplemented to albino rats causes reduction in thyroid hormone profiles (Chandra et al. 2004c).

On the contrary, Dahlberg et al. (1984) found no significant changes in serum T3, T4 and TSH levels during the treatment with thiocyanate through milk. Spiegel et al. (1993) also reported no significant changes in serum T3 level in rats fed rapeseed press cake meal containing high glucosinolates. Administration of thiocyanate to rats caused a significant increase of serum free T4 fraction coincided with the significant decrease of TSH level as shown by Michalovskij et al. (1978).

Most of the earlier workers used fresh / uncooked foods to evaluate the thyroid functional status but the studies on the effects of cooked foods on thyroid functional status are less. Inclusion of cooked Brussels sprouts (150 gm daily for 4 weeks) into a normal diet of 10 volunteer subjects had no effect on thyroid function as determined by measurement of thyrotrophic hormone, thyroxine and triiodothyronine even though the sprouts contained high concentrations of glucosinolates and for the reported antithyroid activity of 5-vinlyoxazolidine-2-thione. It is suggested that this lack of activity of cooked Brussels sprouts is due to inactivation during cooking of myrosinase, the specific glucosinolates-degrading enzyme by McMillan et al. (1986). In a study Slominski and Campbell (1989) observed that during cooking the concentration of glucosinolates in the foods are decreased up to 10% and the reduction is due to the formation of thiocyanate and other break down products of glucosinolates. Thus no significant change is found in thyroid hormones levels after the feeding of uncooked and cooked foods containing glucosinolates mostly. de Groot et al. (1991) reported more pronounced decrease in serum T4 level after feeding cooked brussel sprouts compared to control and uncooked.

Decreased circulating levels of serum T3 and T4 in cabbage, cauliflower, mustard and radish fed animals as observed in the present study were for the interference of
glucosinolates, cyanogenic glucosides and thiocyanate or their degradation products like goitrin, isothiocyanate, nitrile, thiooxazolidone and thiocyanate etc. formed by the enzymatic degradation in the body that inhibited thyroid hormone synthesis by interfering thyroid peroxidase activity at different levels in addition to their inhibition of iodide uptake in thyroid gland or stimulating iodide efflux or suppressing the normal metabolism of iodine.

The inhibition in TPO activity as observed in the study in uncooked and cooked cabbage, cauliflower, mustard and radish fed groups was for the presence of cyanogenic constituents in the studied plants. The inhibitory effect was more marked in fresh cyanogenic plant fed rats because of higher concentration of goitrogenic constituents as compared to their cooked counterparts. Inhibited TPO activity associated with the decreased concentration of iodide / iodine in the thyroid gland may be the probable reason for the reduced synthesis of thyroid hormones as reflected by circulating thyroid hormone levels in the selected plant fed rats.