SUMMARY

Cyanogenic foods viz. cabbage, cauliflower, mustard seeds and radish most often constitute a part of the human diet in India. These vegetables are eaten raw as well as in the processed forms. These plant foods have been considered as goitrogenic principles for the persistence of goitre and associated IDD in many regions of India during post salt iodization phase.

In the present study systematic quantification of goitrogen content in those fresh and cooked vegetables were made along with their in vitro thyroid peroxidase inhibitory activity and relative anti-thyroid potency by IC\textsubscript{50} (Inhibition Constant\textsubscript{50}) and PTU (6-n-Propyl-2- thiouracil) equivalence. The influence of those selected plant foods on the alteration of morphological and functional status of thyroid gland in albino rats were evaluated by substituting one third portion of the normal diet with these plants for a period of 60 days and by measuring urinary iodine (I) and thiocyanate (SCN) excretion pattern, thyroid gland morphology and histology, thyroid peroxidase (TPO) activity, serum total triiodothyronine (T\textsubscript{3}) and thyroxine (T\textsubscript{4}) levels. In addition, the consumption pattern of cyanogenic plant foods in relation to iodine as urinary iodine / thiocyanate ratio was also ascertained.

Cyanogenic glucosides, glucosinolates and thiocyanate, the three main dietary goitrogens of cyanogenic origin were found present in the edible parts of each of those fresh / uncooked and cooked plants. Cyanogenic glucosides content was found highest in cauliflower in fresh condition while in cooked condition it was highest in mustard seed; glucosinolates content was highest in cauliflower in both the uncooked and cooked conditions while thiocyanate content was highest in mustard seeds in fresh / uncooked as well as in cooked conditions.

Cyanogenic glucosides and glucosinolates contents were reduced markedly while thiocyanate content remained slightly elevated or almost same in the cooked plants in comparison to their uncooked / fresh counterparts. The loss of cyanogenic glucosides content in the cooked plants might be due to partial hydrolysis of cyanogenic glucosides into volatile hydrogen cyanide, a break
down product of cyanogenic glucosides. Glucosinolates were also susceptible to thermal degradation and their partial degradation had produced thiocyanate as end product, probably through certain intermediates viz. isothiocyanate, goitrin, nitriles etc. and this was responsible for the decreased glucosinolates content in cooked plants. Unaltered or slightly elevated thiocyanate content in the cooked plants might be due to partial conversion of glucosinolates and cyanogenic glucosides to thiocyanate on heating.

Varying degrees of inhibition in thyroid peroxidase activity were shown by the aqueous extracts of each plant in both the fresh and cooked conditions in in vitro experiment. Cooked extracts had shown more anti-TPO potency than their uncooked counterparts. The thyroid peroxidase inhibition was highest with mustard seeds among all the studied plants in both uncooked and cooked conditions. The presence of heat stable glucosides and more intact glucosinolates in cooked plants than their fresh or uncooked counterparts have caused greater inhibition of in vitro thyroid peroxidase activity.

The relative anti-TPO potency of the fresh and cooked plant foods was determined by IC₅₀ and PTU equivalence. The relative anti-TPO potency was highest with uncooked mustard seeds followed by cauliflower, radish and cabbage while cooked cauliflower showed highest IC₅₀ and PTU equivalence in cooked condition followed by cooked cabbage, mustard and radish. Anti-TPO activity of the plant extracts as observed in the present study was not consistent with goitrogenic contents present in the plants because of differences in moisture content of the plants or for the differences in conversion of inactive precursors to active constituents during mastication, boiling / cooking etc. from plant to plant.

Urinary thiocyanate level was enhanced markedly in the animals fed dietary goitrogen containing cyanogenic constituents viz. cyanogenic glucosides, glucosinolates and thiocyanate suggesting that the goitrogen precursors present in the studied plants were metabolised after ingestion and liberated thiocyanate mostly in the animal body that was excreted mainly through urine. Feeding of plant foods containing cyanogenic constituents had enhanced the excretion of iodine suggesting that dietary goitrogens of cyanogenic origin are metabolised to thiocyanate and thiocyanate like compounds that interfere with iodine
metabolism in the thyroid probably by reducing iodide uptake, stimulating iodide efflux or replacing iodine by thiocyanate resulting an increase in excretion of iodine through urine. Urinary iodine and thiocyanate levels were higher in fresh plant fed groups because of increased concentrations of cyanogenic constituents in the fresh plants compared to their cooked counterparts. This study suggests that the iodine retaining capacity of the thyroid / body may be dependent on the consumption pattern of the cyanogenic plant foods. The dietary supplies of iodine and thiocyanate are generally determined from the urinary iodine / thiocyanate ratio (I / SCN in μg/mg in dl). Urinary iodine and thiocyanate ratio was found higher in the group of rats fed with cooked cyanogenic plants in comparison to their fresh counterparts.

Thyroid gland weight in cyanogenic plant fed rats were increased significantly because glucosinolates and their breakdown products, cyanogenic glucosides and their derivatives thiocyanate and thiocyanate itself present in the plant foods had altered thyroid gland morphology. Among the cyanogenic plant fed groups the gain in thyroid weight was more in uncooked / fresh plant fed rats as compared to cooked plant fed groups of rats.

Marked alteration in thyroid gland histology was observed in the groups fed the selected plant foods – because the follicles were reduced in size, lined by cuboidal or columnar epithelial cells containing relatively deep eosin stained colloid resembling a relative state of morphological hypothyroidism with hypertrophy and hyperplasia of follicular epithelium. In addition, there was an increase in the number of small follicles without any colloid. In contrast, in control groups the follicles were lined with low cuboidal cells filled with colloid. The follicles were almost equal in size. The histological alteration of thyroid gland was almost of similar nature under the influence of selected cyanogenic plant foods but variations were in severity. Deep stained eosinophilic colloid in the centre of follicles indicated that the concentration of iodine in the follicles was reduced under the influence of goitrogenic constituents. All these histological alterations were the prototype of goitrogen induced TSH stimulated diffuse goitre / thyroid gland enlargement.
This study further reveals 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 as evidenced by relatively deep eosinophilic colloid in comparison to control group due to the interference of cyanogenic constituents on iodine concentrating mechanism of thyroid gland.

Prolonged consumption of selected plant foods had decreased in vivo thyroid peroxidase activity and serum total T₃ and T₄ levels developing a relative state of biochemical hypothyroidism because of the presence of cyanogenic constituents. Inhibition in in vivo TPO activity was probably due to the interference of thiocyanate and thiocyanate like other break down products of glucosinolates with iodine at the substrate site of thyroid peroxidase. Decreased circulating levels of T₃ and T₄ had reflected the reduced synthesis of these hormones in the thyroid gland because of the inhibition of TPO activity associated with less availability of iodide / iodine in the gland for the interference of thiocyanate or thiocyanate like metabolites arising from cyanogenic plants. The inhibitory effect was more pronounced in fresh cyanogenic plant fed rats because of higher concentration of goitrogenic constituents than their cooked counterparts.

Decreased TPO activity and the reduced concentration of iodide / iodine in the thyroid gland had prevented thyroid hormone synthesis as evidenced by reduced circulating level of thyroid hormones in spite of proper iodine nutritional status resembling a relative state of morphological as well as biochemical hypothyroidism in both the fresh and cooked cyanogenic plant fed rats. The goitrogenic / anti-thyroid potency of the studied Indian cyanogenic plant foods was reduced to an extent during cooking but failed to prevent it completely.