

Effect of radish (*Raphanus sativus* Linn.) on thyroid status under conditions of varying iodine intake in rats

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Cruciferous plants viz. cabbage, cauliflower, turnip, radish, mustard etc. that contain goitrogenic/antithyroid substances, constitute a portion of regular human diet. The effect of chronic feeding of fresh and cooked radish, *R. sativus* under varying state of iodine intake on morphological and functional status of thyroid in albino rats was evaluated by thyroid gland morphology and histology, thyroid peroxidase activity, serum triiodothyronine, thyroxine and thyrotropin levels. The consumption pattern of iodine and goitrogens of cyanogenic origin was evaluated by measuring urinary iodine and thiocyanate levels respectively. After chronic radish feeding, increased weight of thyroid gland, decreased thyroid peroxidase activity, reduced thyroid hormone profiles and elevated level of thyrotropin were observed resembling a relative state of hypothyroidism in comparison to control even after supplementation of adequate iodine.

Keywords: Glucosinolates, Iodine, Radish (*Raphanus sativus*), Thyroid hormone, Thyroid peroxidase, Urinary iodine, Urinary thiocyanate.

Cruciferous plants viz. cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*Brassica oleracea* var. *botrytis*), mustard seeds (*Brassica juncea*) and leaves, brussels sprouts (*Brassica oleracea* var. *gemmifera*), radish (*Raphanus sativus*) etc, which constitute a major portion of human diet contain naturally occurring goitrogens/anti-thyroid substances or thiocyanate precursors (glucosinolates and cyanogenic glucosides)¹⁻⁴. Extreme differences in the goitrogen content of these plants belong to same family and same taxonomy owing to their genetic and ecological backgrounds have also been reported⁵. Besides, the goitrogenic/anti-thyroid potential of the plant foods not only depends on their relative concentrations of the goitrogenic constituents as found in fresh plants but also on their processing as foods^{4,6}; in certain instances, the inactive precursors are also converted to active constituents after consumption of the plant foods in the body^{1,7}. Querido *et al.*⁸ suggested that dietary supplies of cyanogenic glucosides and glucosinolates present in the plant food may be estimated from the daily urinary excretion and concentration of thiocyanate. Goitrogenic/anti-thyroid potential of plants containing thiocyanate or

thiocyanate precursors are enhanced in iodine-deficient conditions³ and this anti-thyroid effect may have been reversed by iodine supplementation⁹. As 90% of body's iodine is excreted through urine, the urinary iodine level reflects the iodine nutritional status or iodine intake¹⁰. Concentration of iodine and thiocyanate in urine is thus considered as an index of the dietary supplies of iodine and thiocyanate (cyanogenic foods) of the body⁵.

Consumption of excess cyanogenic plants in relation to iodine intake is considered as an etiological factor for the persistence of iodine deficiency disorders in certain regions of India¹¹⁻¹⁴. Moreover, in spite of salt iodization, residual goitre still persists in many regions of India¹²⁻¹⁴. In a recent study, the goitrogen content of Indian cyanogenic plants and their *in vitro* anti-thyroid potential with and without extra iodine has been reported¹⁵. However, the effect of chronic consumption of cyanogenic plants containing not only naturally occurring thiocyanates but also thiocyanate precursors viz. glucosinolates and cyanogenic glucosides on the thyroid status under varying iodine intake except bamboo shoot¹⁶ in Indian context has not been studied adequately.

Therefore, in the present study, the effect of chronic or prolonged consumption of radish, a widely used plant consumed both in fresh i.e. uncooked and cooked condition respectively, under varying status of

iodine nutrition (with and without iodine supplementation) on morphological and functional status of thyroid gland in albino rats has been evaluated by measuring urinary iodine (I) and thiocyanate (SCN) levels, thyroid gland morphology and histology, thyroid peroxidase (TPO) activity, serum triiodothyronine (T3), thyroxine (T4) and thyrotropin (TSH) levels.

Materials and Methods

Prior to conduct the proposed study due approval from the Institutional Animal Ethical Committee was obtained.

Collections of plant foods—Fresh radish were collected from the local markets for the estimation of goitrogenic constituents and also for feeding experiments.

Preparation of plant foods—(i) Uncooked plant foods were prepared by coarsely chopping the radish in a cutter and then incubate in an oven at 30-40°C for 4 hour following the method of de Groot *et al.*⁴ (ii) Cooked plant foods were obtained by boiling in an equal weight of tap water for 15 min. The cooked material was not drained and immediately used for preparing diets following the method of de Groot *et al.*⁴.

The uncooked and cooked plant foods were then mixed with the other ingredients of the diet and also for the measurement of goitrogenic constituents.

Animals—Wistar rats (60) weighing 80 ± 5 g were allocated to control and five experimental groups of 10 each. Animals were caged in unheated well-ventilated stainless steel cages as per INSA guidelines and maintained in the laboratory on standardized normal diet (20% protein) containing (%) wheat (70), Bengal gram (20), fish meal powder (5), dry yeast powder (4), refined til oil (0.75), shark liver oil (0.25) and water *ad libitum*¹⁷.

Animal maintenance and treatment—Control rats were fed normal laboratory diet. Rats of radish fed group were fed normal laboratory diet replacing one-third portion of the diet with fresh/uncooked and cooked radish respectively. In potassium iodide (KI) supplemented group, rats were fed normal laboratory diet with extra iodide 12-14 $\mu\text{g}/\text{rat}/\text{day}$ ¹⁸. In radish-fed potassium iodide (KI) supplemented group, rats were fed both radish (1/3 portion of diet) and extra iodide 12-14 $\mu\text{g}/\text{rat}/\text{day}$ ¹⁸. The animals were maintained on the above-mentioned regimen for 90 days.

Feed consumption, corrected for feed wasted and body weight were measured every seven days. In the last week of the treatment each group of animals was kept in metabolic cages for 24 hr to collect the urine over xylene for the analysis of urinary iodine and thiocyanate. At the end of the experimental period, body weights of the rats were recorded and the animals were sacrificed following ether anaesthesia. After overnight fasting just before sacrifice, blood samples were collected from each rat from the portal vein under ether anaesthesia and the serum were separated for the assay of T4, T3 and TSH and stored at -20°C till analysis. Just after sacrifice, thyroid glands were weighed after removing connective tissues and preserved for assay of thyroid peroxidase activity (TPO) and also for histological studies.

Measurement of cyanogenic glucosides—Cyanogenic glucosides were measured following the method of Lambert *et al.*¹⁹. Edible parts of cooked plant foods (10 mg-1 g) were hydrolysed by the enzyme glucosidase (β -glucosidase obtained from Sigma Chemicals Co.) and the hydrocyanic acid thus liberated was trapped in sodium hydroxide. Cyanide content of trapped hydrocyanic acid was then determined quantitatively.

Measurement of glucosinolates—The enzyme thioglucosidase reacts with thioglucosides present in plant producing thiocyanate. Following this principle, thioglucosides were measured in cooked radish as per Gmelin and Virtanen²⁰. The thiocyanate thus produced was estimated by the method of Aldridge²¹ as modified by Michajlovskij and Langer²².

Measurement of thiocyanate—The cooked plant food was extracted with clean sand and water and refluxed subsequently. The extract containing thiocyanate was then allowed to react with benzidine hydrochloride and the intensity of colour thus formed was measured photometrically following the method of Aldridge²¹ as modified by Michajlovskij and Langer²².

Histological study of the thyroid gland—After completion of the experimental period, thyroid glands of rats in each group were dissected out and fixed in formol for subsequent histological examination and then embedded in paraffin bath after usual processing. Sections (5-6 μm thick) were stained with haematoxyline and eosin, micro photographed and compared.

Estimation of iodine in urine—Iodine in urine was measured by drying urine at 600°C in presence of

potassium carbonate and the iodine present in the ash was measured by ceric-arsenite system, as per Karmarkar *et al.*²³.

Estimation of thiocyanate in urine—Urinary thiocyanate (SCN) concentration was measured in the same urine samples using the method of Aldridge²¹ as modified by Michajlovskij and Langer²². Trichloroacetic acid was added to urine sample, mixed and centrifuged. To the supernatant, saturated bromine water was added and 4% arsenic trioxide (As₂O₃) was then added to oxidise all bromine present in the sample. After that, benzidine hydrochloride and pyridine mixture were added and the colour developed gradually. After 30 min, optical density was measured at the wavelength of 525 nm.

Measurement of thyroid peroxidase activity—A 10% homogenate was prepared using thyroid tissues in phosphate buffer (pH 7.2, 100 mM) and sucrose solution (500 mM) at 4°C. Homogenisation was carried out in a Potter-Elvehjem glass homogeniser for 45-60 sec at 2000 rpm and about 15 strokes/min. The homogenate was centrifuged at 1000 g for 10 min and this low speed supernatant was further centrifuged at 10,000 g for 10 min at 4°C to get the mitochondrial fraction. The microsomal fraction containing most of the peroxidase activity was obtained by centrifuging the post mitochondrial supernatant at 1,05,000 g for one hour. After centrifugation, the precipitate was suspended in phosphate buffer. Thyroid peroxidase activity was measured following I₃⁻ formation from iodide using spectrophotometer (UV-1240 Shimadzu, Japan) at 353 nm as per Alexander²⁴. For performing the kinetic assay, in a 1 ml cuvette, 0.9 ml of sodium acetate buffer (pH 5.2, 50 mM), 10 µl KI (1.7 mM) and 20 µl microsomal fraction of thyroid tissue containing 0.03-0.04 mg protein were added and the reaction was started by the addition of 20 µl freshly prepared H₂O₂ (0.3 mM) according to laboratory standardization. The thyroid tissue protein level was determined by the method of Lowry *et al.*²⁵ using bovine serum albumin as standard. The results are expressed as ΔOD/min/mg protein.

Assay of total circulating thyroxine, triiodothyronine and thyrotropin—Total serum thyroxine and triiodothyronine were measured using ELISA Kits supplied by Lilac Chemicals according to manufacturer's instructions. Serum TSH level was measured by radio immuno assay using radioisotope ¹²⁵I.

Statistical analysis—The data were statistically analyzed and presented in the table as mean ± SD. Comparison among the groups was performed by ANOVA and the level of significance was expressed at *P*<0.05.

Results

Goitrogen content—Goitrogen content of cyanogenic origin namely cyanogenic glucosides, glucosinolates and thiocyanate were 1.28±0.4 mg/kg, 2.64±0.2 mg/kg and 13.28±0.9 mg/kg respectively, in the edible portion of fresh radish as observed in earlier study¹⁵. The concentration of cyanogenic glucosides, glucosinolates and thiocyanate in cooked radish were 0.005±0.002 mg/kg, 1.82±0.3 mg/kg and 13.12±1.04 mg/kg. Therefore, significant reduction in glucosinolates and cyanogenic glucosides contents was noted in cooked radish as compared to its uncooked/fresh counterparts, while thiocyanate content was unaltered.

Urinary iodine and thiocyanate levels, thyroid weight, thyroid peroxidase activity and serum levels of total T₃, T₄ and TSH in control, radish-fed (both fresh and cooked), KI-supplemented and radish fed KI-supplemented groups of rats treated for 90 days were measured and presented in Table 1.

Thyroid weight—Significant increase in thyroid weight was noted after feeding radish under different dietary regimens with varied goitrogen as compared to control (*P*<0.05). On the contrary, it was reduced significantly in KI-supplemented groups in comparison to control (*P*<0.05). The increase in thyroid weight was more in the rats fed fresh radish as compared to cooked radish fed rats. However, the differences between the KI-supplemented-uncooked and cooked radish-fed groups were not significant (Table 1).

Urinary iodine concentration—Urinary excretion of iodine was increased slightly in the fresh radish fed group (6.25%) and very slightly in cooked radish fed groups (1.68%) as compared to their respective control. Consistent with the observation the excretion of iodine was not increased in KI supplemented fresh (5.96%) and cooked (1.87%) radish fed group as compared to KI supplemented control.

Urinary thiocyanate concentration—Fresh and cooked radish fed rats had shown a significant increase in urinary thiocyanate concentration as compared to control and the increase was more pronounced in fresh radish-fed groups and differences

between the two radish-fed groups under different conditions were significant ($P < 0.05$). Extra KI supplementation increased the excretion of thiocyanate further in both the radish fed groups in comparison to KI-supplemented control (Table 1).

Histology of thyroid gland—The morphological status of thyroid gland in fresh and cooked radish-fed rats with and without iodide supplementation was also studied histologically (Fig. 1). In radish-fed rats, thyroid follicles were lined by high cuboidal cells with distinct nucleus showing hypertrophy and hyperplasia filled with less homogeneous colloid; some follicles were invaded by epithelial cells. Increase in the number of comparatively small follicles was one of the characteristic features in radish-fed rats. On the contrary, in control rats thyroid follicles were almost equal in size, lined by low cuboidal cells filled with colloid. Alteration was found in the thyroid structure between the fresh and cooked radish-fed rats. Variation in the number and size of follicular cells and colloid content were observed in KI-supplemented and non-supplemented radish-fed groups of rats. In addition, colloid stained more with eosin in radish-fed rats as compared to control and KI-supplemented group of rats.

Thyroid peroxidase activity—Highest TPO activity was noted in KI-supplemented group of rats. Inhibition in TPO activity was found in fresh radish fed rats (58.7%) and cooked radish-fed (29.9%) respectively and inhibition was more profound in the fresh radish-fed group. In KI-supplemented fresh and cooked radish-fed groups, the inhibition was 54.8% and 28.2% as compared to KI-supplemented control indicating that TPO activity was not corrected even after iodine supplementation (Table 1).

Serum T3, T4 and TSH level—Total circulating serum T3, T4 and TSH levels were assayed in the experimental groups of rats maintained under different conditions and the obtained results were compared with their respective control. Serum T3 and T4 levels were reduced significantly, in both radish-fed groups. Extra KI-supplementation in the radish-fed groups showed very slight improvement in serum T3 and T4 levels. In consistent with the serum thyroid hormone profiles, serum TSH level showed a proportionate rise in radish fed groups. Iodide supplementation to radish-fed rats showed a slight decrease in TSH level as compared to their respective non-supplemented rats (Table 1).

Table 1—Uncooked/fresh and cooked radish induced alterations on urinary thiocyanate and iodine and thyroid weight and functional status under varying iodine intake in albino rats for 90 days

[Values are mean \pm SD of 10 observations; figures in parenthesis are % change as compared to control group]

Groups	Thyroid weight (mg/100g body weight)	Urinary iodine (μ g/dl)	Urinary thiocyanate (mg/dl)	TPO activity (Δ OD/min/mg protein)	Serum T4 (μ g/dl)	Serum T3 (ng/dl)	Serum TSH (ng/dl)
Control	8.89 \pm 0.34	416.29 \pm 4.74	1.2 \pm 0.13	5.45 \pm 0.28	4.32 \pm 0.16	141.5 \pm 1.2	0.59 \pm 0.13
Fresh radish	11.17 \pm 0.51* (25.6%)	442.75 \pm 4.08* (6.25%)	6.25 \pm 0.19* (420.8%)	2.25 \pm 0.19* (58.7%)	3.61 \pm 0.11* (16.4%)	117.05 \pm 2.27* (17.3%)	2.28 \pm 0.14* (286.4%)
Cooked radish	10.29 \pm 0.52* (15.7%)	423.22 \pm 5.45 (1.68%)	5.67 \pm 0.18* (372.5%)	3.82 \pm 0.14* (29.9%)	3.67 \pm 0.18* (15.0%)	123.67 \pm 2.06* (12.6%)	1.83 \pm 0.1* (210.2%)
KI supplemented	7.84 \pm 0.24	2396.24 \pm 3.89	1.46 \pm 0.19	6.24 \pm 0.52	4.82 \pm 0.13	152.04 \pm 3.02	0.31 \pm 0.11
Fresh radish fed KI supplemented	10.08 \pm 0.36** (28.6%)	2539.02 \pm 5.38** (5.96%)	8.82 \pm 0.14** (504.1%)	2.82 \pm 0.14** (54.8%)	3.72 \pm 0.22** (22.8%)	120.55 \pm 2.31** (20.7%)	1.26 \pm 0.16** (306.5%)
Cooked radish fed KI supplemented	9.69 \pm 0.62** (23.6%)	2441.68 \pm 6.03** (1.87%)	7.48 \pm 0.27** (412.3%)	4.48 \pm 0.27** (28.2%)	3.9 \pm 0.11** (19.1%)	129.48 \pm 1.58** (14.8%)	0.92 \pm 0.12** (196.8%)

P values:

Values bearing superscripts are significantly different by ANOVA at < 0.05 .

When compared to control group *

When compared to KI supplemented group **

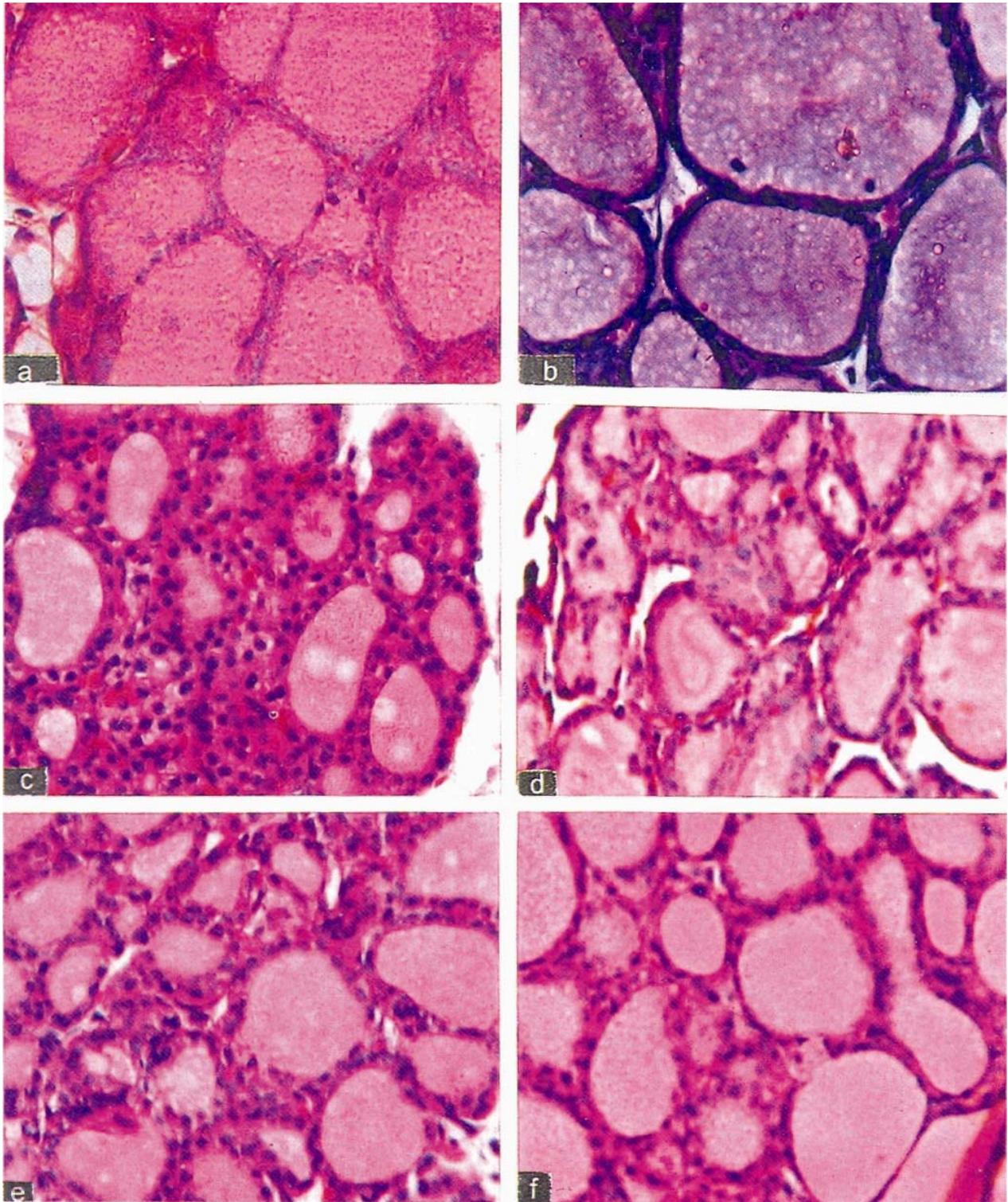


Fig. 1—Photomicrographs of thyroid gland in (a) control, (b) KI supplemented, (c) fresh radish, (d) fresh radish fed KI supplemented, (e) cooked radish, and (f) cooked radish fed KI supplemented after 90 days treatment (HE, 20 \times).

Discussion

The carbohydrate, protein, fat, minerals, fibre and moisture content of fresh radish are 3.4, 0.7, 0.1, 0.6, 0.8 and 94.4 respectively, when the values are expressed in g/100 g edible portion of radish²⁶. Fresh radish contains the goitrogenic/anti-thyroid substances of cyanogenic origin and it shows *in vitro* TPO inhibiting activity having inhibition constant (IC₅₀) 51.88±1.2 µg and PTU equivalence 1.73 (ref. 15). Consumption of cyanogenic food is considered as an etiological factor for the persistence of residual goitre during post iodization phase^{14,27}.

The concentration of glucosinolates and cyanogenic glucosides were markedly reduced after cooking, however, thiocyanate concentration remained unaltered as compared to those in fresh radish. Alteration in the concentration of goitrogenic principles in cyanogenic plants during their processing as food including crushing, heating, boiling, soaking etc. had already been reported^{1,4}. These goitrogenic principles viz. glucosinolates and cyanogenic glucosides are converted to thiocyanate and isothiocyanate like active goitrogenic/anti-thyroid agents after ingestion or during their processing as foods by widespread myrosinase, glucosidases and sulphur transferase enzymes present in the animal body or in the plant itself, through a series of intermediate products^{7,28,3}. The loss of cyanogenic glucosides content in the cooked radish as observed in the present study may be due to hydrolysis of cyanogenic glucosides into volatile hydrogen cyanide, a breakdown product of cyanogenic glucosides²⁹. Glucosinolates are also susceptible to thermal degradation³⁰ and their degradation causes the production of thiocyanate, isothiocyanate, nitriles etc.³, which are responsible for the decrease in glucosinolate content after cooking; while unaltered thiocyanate content in the cooked radish may be due to the conversion of glucosinolates and cyanogenic glucosides to thiocyanate to a certain extent on heating.

The amount of thiocyanate present in the urine is a good indicator of the presence of cyanogenic goitrogens in the foods³¹. Therefore, the increase in urinary thiocyanate level in radish-fed rats was for the conversion of cyanogenic constituents present in the plant to thiocyanate indicating that glucosinolates and cyanogenic glucosides after ingestion are metabolized to thiocyanate mostly in the animal body that ultimately excreted through urine.

Urinary iodine level reflects the iodine nutritional status of the body or the iodine intake through diet. Iodine excretion through urine was increased markedly after supplementation of iodide showing a better state of iodine nutriture in the iodine-supplemented groups. Feeding of plant foods containing glucosinolates and cyanogenic glucosides (thiocyanate precursors) enhanced the excretion of iodine in both KI-non-supplemented and KI-supplemented groups of rats. Thiocyanate and thiocyanate like compounds arising from glucosinolates, cyanogenic glucosides or thiocyanate interfere with iodine metabolism in thyroid gland by reducing iodide uptake, stimulating iodide efflux or replacing iodide by thiocyanate resulting in an increase in the excretory level of iodine^{32,33}. Moreover, thiocyanate or thiocyanate like compounds mainly inhibit the iodide concentrating mechanism of the thyroid gland by inhibiting unidirectional clearance of iodide from the gland^{34,3}. The differences in urinary level of iodine and thiocyanate between fresh and cooked radish fed groups may be due to the reduction in the concentration of thiocyanate precursors during cooking. The present results suggest that the iodine-retaining capacity of the thyroid/body is dependent on the consumption pattern of the cyanogenic plant foods.

Thyroid gland weights in radish-fed rats were increased significantly. Glucosinolates and their breakdown products cause morphological alterations in thyroid gland^{35,36}. Cyanogenic glucosides also affect thyroid gland weight largely³⁷. Goitrogenic effects of glucosinolates and cyanogenic glucosides are enhanced during iodine deficient state³⁸. Moreover, thiocyanate itself has pronounced effect on the thyroid enlargement^{39,40}. The increase in thyroid weight was more in fresh radish fed rats because of higher concentration of cyanogenic constituents. Extra iodide supplementation in radish fed groups reduced the gain in thyroid weight to an extent because iodine supplementation can counteract the effect of thiocyanate when present in low concentration³³, but the effects of higher concentration of thiocyanate, isothiocyanate, nitrile etc., breakdown products of glucosinolates cannot be antagonized by iodide administration³.

Increased thyroid weight accompanied by marked alteration in thyroid gland histological status was observed in radish-fed groups suggesting hypertrophy and hyperplasia of epithelial cells along with

increased number of small follicles with less colloid. This finding is the prototype of TSH induced diffuse goitre⁴¹. Alterations in the structures of thyroid gland of rats fed with and without KI supplementation were also observed. In addition, more eosin-stained colloid in the thyroid gland of radish fed rats as compared to control and KI-supplemented rats indicates the development of an iodine deficient state in the gland as observed by Sharpless⁴². Gaitan *et al.*⁴³ have also reported that colloid of iodine deficient thyroid gland takes more eosin than control and iodine sufficient thyroid. The present results reveal that in spite of adequate iodine intake (as reflected by urinary iodine level) or proper iodine nutrition, the thyroid gland gets little or less iodine (as observed by relatively eosinophilic colloid) due to the interference of cyanogenic constituents present in radish with the iodine uptake mechanism of thyroid gland.

Thiocyanate, a monovalent anion having the molecular size corresponding to that of iodine, inhibits the incorporation of iodide into thyroglobulin by competing with iodide at the thyroid peroxidase level³³. It inhibits the iodide oxidation i.e. conversion of I⁻ leads to I₂ by inhibiting TPO activity⁴⁴. Moreover, thiocyanate also increases the formation of an essentially insoluble iodinated thyroglobulin within the thyroid in iodine-depleted condition⁴⁵. At high concentration thiocyanate inhibits the incorporation of iodide into thyroglobulin³³. Besides, glucosinolates undergo a rearrangement to form isothiocyanate derivatives which react spontaneously with amino groups forming thiourea like strong anti-thyroid derivatives that interfere with organification of iodine and formation of thyroid hormone by inhibiting TPO activity and their action is not antagonised by iodide administration³. Glucosinolates also change the action of TPO because they are iodine antagonist thereby prevent iodination of thyroglobulin⁴⁶. Orgiazzi and Millot⁴⁷ have found that TPO is the target of thiocyanate there by blocks iodination of tyrosine residues and coupling of iodotyrosines into iodothyronines in thyroid gland. Thus inhibition in TPO activity as observed in both the radish-fed groups was for the presence of cyanogenic constituents in the studied plant. The inhibitory effect was more marked in fresh radish-fed rats because of its higher concentration of goitrogenic constituents. Extra KI-supplementation in radish-fed groups had shown as such almost no improvement in TPO activity because of the presence of excess thiocyanate.

Diet rich in thiocyanate or glucosinolates but deprived of iodide causes decreased circulating levels of T3 and T4^{40,46}. Inhibited TPO activity associated with decreased concentration of iodide 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 radish fed rats. Low circulating level of T4 and T3 stimulates the secretion of TSH from the pituitary by feedback mechanism and if this condition is continued for longer duration enlargement of thyroid gland occurs or goitre develops⁴⁸. Prolonged radish feeding had increased the serum TSH level because of the reduction in thyroid hormones profiles in circulation. Moreover, histological observations further showed TSH induced diffuse goitre. Therefore iodine supplementation in the radish-fed groups of rats showed almost no improvement in thyroid hormonal profiles.

The overall results showed that the prolonged consumption of radish may develop a relative state of morphological and as well as biochemical hypothyroidism even in the presence of iodine and this can explain a part for the persistence of goitre in the country during post salt iodization period.

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