Chapter 8

URINARY THIOCYANATE AND IODINE LEVELS UNDER THE INFLUENCE OF THE SELECTED PLANT FOODS

Introduction

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). Another important source of thiocyanate is the enzymatic detoxification of cyanide by the enzyme thiosulfate sulphur transference or rhodnase. This enzyme is present in most mammalian tissues although their highest concentrations are found in liver, kidney, adrenals, thyroid and pancreas (Reinwein, 1961). Glucosinolates can be cleaved by the enzyme myrosinase. Any injury of the plant cell brings the substrate and the enzyme together creating isothiocyanate, nitriles, oxazolidinethione and thiocyanate (Conn, 1980). Some gastrointestinal microbes have a myrosinase like activity causing intestinal glucosinolates degradation. Isothiocyanate not only use the thiocyanate metabolic pathway but also form derivatives with thiourea like antithyroid effects (Gaitan, 1990). Furthermore detoxification of nitrile also leads to the formation of thiocyanate (Van Etten, 1969). Therefore, amount of thiocyanate in the urine is a good indicator of the presence of glucosinolates in the food (Gaitan, 1986). 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. Regular consumption of cyanogenic plant foods 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 Roy, 2001).

Iodine is an essential dietary element which is required for the synthesis of the thyroid hormones, thyroxine (T4) and triiodothyronine (T3). Iodine enters the body in the form of iodate or iodide in the water we drink; the iodate is converted to iodide in the stomach. The thyroid gland traps and concentrates iodide and uses it in the synthesis and storage of thyroid hormones. Iodide is rapidly absorbed from the gastrointestinal tract and distributed to
extracellular fluids. But the concentration of iodide in the extracellular fluid is usually low because of the rapid uptake by the thyroid gland and renal clearance. It is estimated that 75% of the iodide taken into the body each day enters the thyroid by active transport. About two-thirds of that is used in hormone synthesis, with the remaining amount released back into the extra cellular fluid. The thyroid gland contains the body’s largest pool of iodide, about 8 to 10 mg. Most of this iodide is associated with thyroglobulin, a thyroid hormone precursor and a source of hormone and iodinated tyrosine. 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 faces and in breast milk. However, over 90% usually comes out in the urine, presumably as iodine (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 (enlarged 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. However, in the environment where the precursors of thiocyanate (isothiocyanate etc.) have antithyroid or thyroid peroxidase and or (Na\(^{+}\)-K\(^{+}\))-ATPase inhibitory activities, adequate iodine supplementation would fail to prevent IDD (Chandra and Ray, 2001).

Therefore, urinary iodine and thiocyanate are the indicators to evaluate the consumption pattern of iodine and cyanogens 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 fed with plant foods as used in this study.

**Materials and methods**

**Maintenance of animals**

Described in the methodology section.

**Animal treatment**

Seventy two adult rats weighing 150±10 gm were allocated control and experimental groups. In the treatment schedule, rats were equally divided into nine groups each considered of eight
rats per group. One group was kept as respective controls and fed normal laboratory diet whereas experimental rats in each group received a normal laboratory diet with one-third of the diet replaced (Chandra et al., 2006) by either peanut seed coat or moringa leaves or spinach and or sugarcane juice obtained from a local market in Kolkata. The animals were maintained with above mentioned regimen dividing into two sets- first set treated for 30 day and the second set treated for 60 days respectively. Feed consumption, corrected for wasted feed, and body weight were measured every seven days. Animal sacrifice and all other experimental procedures were same as described earlier.

Analysis of urine
Described in the methodology section.

Results

![Urinary Iodine](image)

Figure 14. Selected plant-foods induced alteration in urinary iodine level of albino rats treated for 30 days and 60 days respectively. Each bar denotes mean ± SD; n = 8. One-way analysis of variance (ANOVA) test followed by Tukey’s post hoc test was done to determine differences across means of different groups. Mean values are significantly different by ANOVA at p < 0.05. a control 30 day versus treated 30 day group; b control 60 day versus treated 60 day group; c treated 30 day versus treated 60 day group.
Figure 15. Selected plant-foods induced alteration in urinary thiocyanate level in albino rats treated for 30 days and 60 days respectively. Each bar denotes mean ± SD; n = 8. One-way analysis of variance (ANOVA) test followed by Tukey’s post hoc test was done to determine differences across means of different groups. Mean values are significantly different by ANOVA at $p < 0.05$. a control 30 day versus treated 30 day group; b control 60 day versus treated 60 day group; c treated 30 day versus treated 60 day group.

Urinary iodine and thiocyanate levels in peanut seed coat, moringa leaves, spinach and sugarcane juice fed group of rats respectively were measured and compared with the control (figure 14 and 15). Wide variations in both the urinary iodine and thiocyanate levels were observed after feeding of studied plants.

Rats fed with peanut seed coat showed significant increase in urinary iodine ($p < 0.05$) compared to control. The increase was more in 60 days treated group rats when compared to 30 day treated group. Urinary thiocyanate level was also increased significantly in the peanut seed coat fed group ($p < 0.05$). Peanut seed coat fed group of rats treated for 60 day showed higher excretion pattern of urinary iodine and thiocyanate in comparison to that of 30 day treatment as well as control.

There was a significant ($p < 0.05$) increase in urinary iodine and thiocyanate in the moringa leaves fed rats in both the group treated for 30 day and 60 days respectively as
compared to control. The increase was more in 60 day treated group rats when compared to 30 days treated group.

In consistent with those plant foods significant increase in urinary iodine and thiocyanate were observed in fresh spinach fed groups ($p<0.05$) when compared to control; the values were more in 60 day treated group fed with fresh spinach than 30 days treated group ($p<0.05$).

Urinary iodine and thiocyanate were also increased significantly ($p<0.05$) after sugarcane juice administration for 30 and 60 days respectively when compared with the control groups, with the 60-day treatment causing a more increase excretion of iodine and thiocyanate through urine.

Among all the selected plant foods, moringa leaves treated group had shown maximum excretion of iodine and thiocyanate in urine followed by peanut seed coat, sugarcane juice and spinach treated groups for 30-day and 60-days respectively.

**Discussion**

Peanut seed coat, moringa leaves, spinach and sugarcane juice respectively as those foods contain substantial portion of goitrogen / anti-thyroid substances as mentioned earlier. Thyroid physiology was evaluated in the present study. The selected plant foods were mixed with standard diet by replacing one third portion of the diet (Chandra et al., 2006) and fed to rats and this dietary regimen was maintained for 30 days and 60 days respectively and the excretion of iodine and thiocyanate in urine of the experimental animals were determined and compared with the control values.

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

Urinary iodine level was increased in peanut seed coat, moringa leaves, spinach and sugarcane juice supplemented groups; it was maximum in moringa leaves fed group followed by peanut seed coat, sugarcane juice and spinach fed group. The urinary iodine level was found higher than control. All the studied plants contain cyanogenic glycosides, glucosinolates, thiocyanate and polyphenols in different proportion.

Beyssen *et al.*, (1999) showed that drugs that cause hypothyroidism also increase excretion of urinary iodine when dietary iodine was restricted. Schone *et al.*, (2001) also reported that spot urine and faeces samples from sows fed rapeseed 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

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intestinal iodine excretion. Tewe et al., (1984) observed that dietary cyanide supplementation increased urinary iodine excretion. A study of Lakshmy et al., (1995) showed increased excretion of iodine in rats following supplementation of cabbage in diets. Moreover thiocyanate or thiocyanate like compounds primarily inhibits iodide concentrating mechanism of thyroid gland an also increased 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., 2013).

Therefore, the increased urinary excretion of iodine after feeding peanut seed coat, moringa leaves, spinach and sugarcane juice was for the glucosinolates, cyanogenic glucosides or other goitrogenic / antithyroid constituents present in the selected 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 investigations are consistent with the earlier observations. This study confirms earlier findings that the iodine-retaining capacity of the thyroid gland/body depends on the concentration of thiocyanate as well as the amount of iodine ingested (Chandra et al., 2009).

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 cyanogen containing plant foods. This study also suggests that the iodine retaining capacity of the thyroid / body is dependent to an extent on the consumption pattern of the cyanogen containing plant foods.

*Urinary thiocyanate level under the influence of plant foods*

Urinary thiocyanate level was markedly increased in peanut seed coat, moringa leaves, spinach and sugarcane juice fed group of rats. The highest thiocyanate level was observed in the moringa leaves fed group followed by peanut seed coat, sugarcane juice and spinach fed rats.

The studied plant foods contain cyanogenic glucosides, glucosinolates, thiocyanate and polyphenols in significant proportion as discussed earlier. Therefore, the increase in urinary thiocyanate level after the feeding of those plant foods was for the metabolism / conversion of the cyanogenic glucosides, glucosinolates into thiocyanate or the thiocyanate itself present in those plants.
After the ingestion, cyanogenic glucosides are readily converted into the active goitrogenic agent thiocyanate by glucosidases and sulphur transferase enzymes present in plant and animal tissues (Van Etten, 1969). Studied plant foods contain glucosinolates which are degraded by enzyme myrosinase to yield thiocyanate, nitrile and goitrin (Van Etten, 1973). In the animal organism thiocyanate is also formed endogenously from cyanides, nitriles and sulphur containing compounds and thus both the thiocyanate brought exogenously into the organism and thiocyanate autolysed plant foods 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 replacing one third supplementation of cabbage. The high levels of serum and urinary thiocyanate represent a biochemical indicator of the consumption of a goitrogenic foodstuff rich in cyanogenic constituents by that population. 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 mannar in blood plasma and urine after feeding Brussels sprouts. Similarly the dose response was more pronounced in urine than plasma. The glucosinolates derivatives converted to thiocyanate in the blood followed by its appearance in urine (Michajlovskij and Langer, 1959). Langer, (1961) also reported after 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$^{14}$ – labelled cyanide was carried out in rats exposed to a regular intake of cyanide 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$^{14}$CN$^-$ was given by orally 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. Chandra et al., (2013) also reported bamboo shoot (rich in cyanogenic glucosides) feeding in albino rats causes a marked increase in urinary thiocyanate level.

Therefore the increase in urinary thiocyanate level as observed in this study was for presence of glucosinolates, cyanogenic glucosides and thiocyanate in the studied plant foods. The urinary thiocyanate level was almost consistent with the consumption of the different plant foods containing those goitrogenic principles. Highest urinary thiocyanate level was found in moringa leaves treated group followed by peanut seed coat, sugarcane juice and spinach groups of rats according to their presence of cyanogenic constituents. 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 and excreted through urine along with excess iodine.