Effects of Cyanide and Thiocyanate on Thyroxine in Rabbits

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Abstract:- **Effects of cyanide and thiocyanate on triiodothionine (T3) and the tetraiodothyonine (T4) of the thyroxine were determined on some rabbits selected from Keffi town in Nigeria. The rabbits were fed with food mixed with controlled amounts of cyanide and thiocyanate solutions over a period of six weeks. The effects of cyanide and thiocyanate on the thyroxine were determined spectrophotometrically on seven groups of the rabbit samples R1 (control) to R7. The results showed a significant (<0.05) depletion in T3 and T4 over the period of six weeks of the studies. For Rabbits R2 (T3: from 3.08ngml^{-1} to 3.00 ngm l^{-1}; T4: from 132.2 ngm l^{-1} to 132.0 ngm l^{-1}); R3 (T3: from 3.06 ngm l^{-1} to 3.06 ngml T4: from 132.00 ngm l^{-1} to 131.60 ngm l^{-1}); R4 (T3: no change; T4: from 132.40 ngml^{-1} to 129.50 ngm l^{-1}); R5 (T3: from 3.05 ngm l^{-1} to 3.00 ngm l^{-1}; T4: from 132.10 ngml^{-1} to 131.50 ngml^{-1}); R6 (T3: no change; T4: 132.00 ngml^{-1} to 130.00 ngml^{-1}) and R7(T3: from 3.08 ngml^{-1} to 2.90 ngml^{-1}; T4: from 133.00 ngml^{-1} to 129.50 ngm l^{-1}). Although the thyroid levels of the animal samples were affected by both the cyanide and the thiocyanate, no visible change in the size of the thyroid glands were observed within the period.**

Keywords: **Cassava, Cyanide, and Thyroxine.**

I. INTRODUCTION

Cassava *(Manihot esculenta Crantz)* is one of the world's most important root crop because its tubers are largely used for food, starch production and as livestock feed. It is used in many countries of Africa, Latin America and some Asian countries. Though it has its origin in South America, cassava has become a staple crop in the tropics having been widely grown and used. It has played vital roles in the diets of many African countries as a major source of low cost carbohydrate (Ravindran *et al)* 1992).

Cassava contains significant amount of iron, phosphorus, calcium, and is relatively rich in vitamin C (INN, 2000). In many parts of Western and Central Africa, cassava is being processed into a number of traditional staple foods. Dried tubers of cassava can be milled into flour. Maize may be added during the milling process to add protein to the flour. The flour can be used for baking breads. Typically, cassava flour may be used as partial substitute for wheat flour in making bread. Bread made wholly from cassava has been marketed in the U.S.A. to meet the needs of people with allergies to wheat flour. Garri, one of the staple food products from cassava, is obtained by fermentation and subsequent frying; a process similar to the production of injera (Ethiopia staple food) from teff. Garri can be fortified with legume like soybean to offer a nutritionally rich meal (Ravindran *et al.*, 1992).

However, the utilization of tubers of cassava for food is limited primarily due to the presence of the toxic substances cyanogenic glucoside, linamarin and lotaustralin. These are decomposed by linamarase, a naturally occurring enzyme in cassava, liberating hydrogen cyanide (HCN). Two important human diseases namely, tropical ataxic neuropathy and endemic goiter are closely associated with high cassava intake in some parts of Africa (Osuntokun, 1994). Cassava leaves contain more linamarin than do the plant's roots; somehow linamarin is transported from the leaves to the roots early in the plant's life. Turning off the linamarin-inducing genes in cassava leaves might reduce the levels of linamarin in the plant's roots. Linamarin protects cassava from being eaten by insects or animals. Plants with moderate linarnarin levels in their leaves and that contain nearly no linamarin in their roots are both protected from herbivores and contain far less of the cyanogen in their roots (Phillips, 1983).

There is a great concern about the levels of cyanide in many varieties of cassava food products. Cyanide is widely distributed in nature and is normal constituent of blood, usually at low concentration (<12 μmolL^{-1}) (Solomonson, 1981). Using improperly processed cassava can increase the cyanide content in the human body and eventually cause goiter, cretinism, paralysis and neurological disorders Delange, 1983). There is equally good evidence that some fibre depleted diets cause pathological effects (Umoh *et al.*, 1984). These effects are manifested not only in the gastro - intestinal tract but other anatomical structures and tropical ataxic neuropathy TAN) (Umoh *et al.*, 1984). The highest total cyanide reduction was obtained when the tubers were soaked in water, sun-dried and baked. The fibre content in garri and feeds generally has been regarded as being of no nutritive value, but its importance in recent times has been appreciated both clinically and in animal husbandry (Taye et al.,1999). The reliance on cassava as a food source and the resulting exposure to the goitrogenic effects of thiocyanate has been responsible for kwashikor and the endemic goiters seen in the Akoko area of southwestern Nigeria (Akindahunsi *et al*, 1998).

Cassava varieties are often categorized as either sweet or bitter, signifying the absence or presence of toxic levels of the cyanogenic glucosides. The so-called sweet (actually not bitter) cultivars can produce as little as 20 milligrams of cyanide per kilogram of fresh roots, whereas bitter ones may produce more than 50 times as much (lg/kg). Cassavas grown during drought are especially high in these toxins. A dose of 40 mg of pure cassava cyanogenic glucoside is sufficient to kill a cow. It can also cause severe calcific pancreatitis in humans, leading to chronic pancreatitis Adeoti *et al*, 2009).

The background of this study forms the locus of this study which is designed to identify likely goiter problem associated with consumption of cassava processed foods. Although cassava is the third-most important food source in tropical countries including Nigeria, the roots and leaves of poorly processed cassava products could increase the cyanide in the body and affect the thyroxine T3 and T4. Using improperly processed cassava can increase the cyanide content in the human body and eventually cause goiter, cretinism, paralysis and neurological disorders (Delange, 1983). There is equally good evidence that some fibre depleted diets cause pathological effects (Umoh *et al.*, 1984). These effects are manifested not only in the gastro - intestinal tract but other anatomical structures and tropical ataxic neuropathy (TAN) (Umoh *etal.,* 1984). This project work will have been successful if it can identify that poorly treated cassava food can cause goiter due to its effect on the thyroxine enzyme production in the body, which also has other significant side effects such as loss of weight, poor eye sight, weakness of the body, difficulty in walking, deafness and decreased thyroid and function. Goiter has no direct cure except for surgical removal, especially a mature one. Some women, for the fear of surgery, have left the disgusting sight of goiter unattended to. This project will bring the awareness of these findings to the Nigerian populace, the Nigerian government, Ministry of Health, Ministry of Agriculture and other concerned agencies to take very seriously the processing of cassava to remove the cyanide. Cassava marketers will be aware of the danger of the rush to get unprocessed or partially processed cassava for end use consumption. Local people processing cassava will be aware of the danger of poorly processed cassava foods.

II. MATERIALS AND METHODS

2.1 Collection of Samples

The samples used in this project included fourteen rabbits obtained from Keffi market in Nasarawa State of Nigeria. The Rabbits were grouped into seven, the groups include group 1 (R1 and R2), group 2 (R3 and R4), group 3 (R5 and R5), group 4 (R7 and R8) group 5 (R9 and R10), group 6 (R11 and R12), group 7 (R13 and R14).

2.2 Chemicals, Reagents and Instruments

Reagents/Apparatus / Instruments

Elecsys 2010 (Thyroxine analyser)- situated in National Hospital, Abuja, and EDTA bottles (for collection of Rabbit blood samples), Potassium cyanide (KCN), Potassium thiocyanate (KSCN), these reagents were of analytical grade.

2.3 Determination of Thyroid Levels (T3 and T4) using Rabbit Specimens:

Fourteen healthy male rabbits were used (as pregnant female rabbits will interfere with results; females are at risk of goiter during pregnancy as in the third trimester of pregnancy, the body releases human chorionic gonadotrophin (HCG) that can result in an enlarged thyroid gland (www.buzzle.com). Rabbits group 1 (R1 & R2) served as the control (not fed with food mixed either with cyanide or thiocyanate solutions); Rabbits group 2 to 4 were placed on different concentrations of cyanide solutions (KCN) of 0.00l, 0.003 and 0.005 ppm respectively mixed with corn chaff (dusa). Groups 5 to 7 were placed on foods mixed with different concentration of thiocyanate solution (KSCN) (of 0.001 ppm, 0.003 ppm and 0.005 ppm respectively. The rabbit specimens were all in duplicates, and the average of the duplicates were taken as results for the Groups 1 to 7.

The weight of each rabbit was taken on the first day before feeding commenced. The rabbits were fed for six weeks, while the thyroid levels of each specimen were determined on the first day, third week and the sixth week of feeding the rabbits. $2cm^3$ of blood samples were extracted from each rabbit into the EDTA bottle (to prevent blood coagulation before test was carried out). T3 and T4 were determined for each of the blood samples using the ELECSYS 2010. The instrument operates on the theoretical principle shown below:

In the reaction, 8-anilino- 1-napthalene sulfonic acid (ANS) caused the release of endogenous T4 from the binding proteins. The released endogenous T4 competed for antibody (Ab) binding sites with T4 labeled with the enzyme glucose-6- phosphate dehydrogenase (G6PDH-T4 conjugate). G6PDH-T4 conjugate bound to antibody has lower activity than does unbound conjugate. As the binding of endogenous T4 increased, the amount of the unbound enzyme conjugate increases. The active enzyme reduced nicotinamide adenine dinucleotide (NADH-) to NADH.

$$T4 + T4\text{-}G6PDH + NAD + 2Ab \rightarrow Ab{:}\ T4 + Ab{:}T4\text{-}G6PDH + NADH + H^+ \quad \text{. . . . . ………..(equation) 2.1}$$

The rate of change of the absorbance at 340 nm is due to the conversion of NAD+ to NADH and is directly proportional to the amount of endogenous T4 in the sample (Murphy, 1964).

The principle for the determination of the T3 is similar. The fT3 test is a solid phase competitive enzyme immunoassay. Patient serum samples, standards, and T3- Enzyme Conjugate Working Reagent is added to wells coated with monoclonal T3 antibody. fT3 in the patient specimen and the T3 labeled conjugate compete for available binding sites on the antibody. After 60 minutes incubation at room temperature, the wells are washed with water to remove unbound T3 conjugate. A solution of H207/TMB was then added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of 3N HC1, and the absorbance is measured spectrophotometrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled fT3 in the sample. By reference to a series of ff3 standards assayed in the same way, the concentration of fT3 in the unknown sample is quantified (Alpco, 2011).

III. RESULTS AND DISCUSSION

Table 3.1 Thyroid Content of the Control Rabbit

Week	Food content	Test	R1 (1.68 ± 0.04 kg)
0	CN and SCN free	T3 ($ngml^{-1}$)	3 .05±0.03
		T4 ($ngml^{-1}$)	132.20±0.04
3	CN and SCN free	T3	3 .05±0.05
		T4	132.3 0±0 .04
6	CN and SCN free	T3	3.05±0.03
		T4	132.30±0.05

Table 3.2 Effect of Cyanide on the Thyroid Content of the Rabbits

Week	Food content	Test	R2(1 .60±0.04kg)	R3(1.55±0.05kg)	R4(1.10±0.07kg)
0	SCN	T3 ($ngml^{-1}$)	3.08±0.02	3.06±0.04	3.09±0.05
		T4(ngml-1)	132.20±0.05	132.00±0.05	132.40±0.03
3	CN	T3	3.05±0.04	3.04±0.03	3.09±0.04
		T4	132.10±0.02	131.80±0.05	129.50±0.05
6	CN	T3	3.00±0.04	3.02±0.02	
		T4	132.00±0.04	131.60±0.01	

Table 3.3 Effect of Thiocyanate on the Thyroid Content of the Rabbits

Week	Food content	Test	R5(:1.55±0.06kg)	R6(1.65±0.07kg)	R7(1 .80±0.04kg)
0	SCN	T3 ($ngml^{-1}$)	3.05±0.04	3.00±0.05	3.08±0.05
		T4(ngml-1)	132.10±0.05	132.00±0.03	133.00±0.03
3	SCN	T3	3.02+0.03	3.00±0.04	2.95±0.05
		T4	132.00±0.02	131.80*0.02	131.00±0.04
6	SCN	T3	3.00±0.04	3.00±0.01	2.90±0.02
		T4	131.50±0.03	130.00±0.05	129.50±0.05

Discussion

Both cyanide and thiocyanate have been reported to decreased thyroid gland activity in young rats, particularly in the restricted groups. Plasma thyroxine concentrations were maintained in the mature cyanide-treated rats, even though secretion rates were decreased. However, the mature thiocyanate-treated animals showed decreased plasma thyroxine concentrations, despite thyroid gland enlargement (USEPA, 1985). In this study, there was a decrease in the T3 and T4 levels for rabbits fed with cyanide and thiocynate treated meals. For the effect of cyanide on the rabbits, R2 had a decrease from 3.08 $ngml^{-1}$ to 3.00 $ngml^{-1}$ T3, 3.06 to 3.02 $ngml^{-1}$ (Table 3.3). The effect of SCN^- on the T3, indicated a decrease of 3.05 $ngml^{-1}$ to 3.00 $ngml^{-1}$ for R5, and 3.08 $ngml^{-1}$ to 2.90 $ngml^{-1}$ for R7 (see Table 3.3). For effects on the T4: R2 had a decrease from 132.20 $ngml^{-1}$ to 132.00 $ngml^{-1}$ R3 had a decrease from 132.00 $ngml^{-1}$ to 131.60 $ngml^{-1}$, R4 had a decrease of 132.40 $ngml^{-1}$ to 129.50 $ngml^{-1}$ (Table 3.3). The effect of SCN on the T3, indicated a decrease of 3.05 $ngml^{-1}$ to 3.00 $ngml^{-1}$ for R5, and 3.08 $ngml^{-1}$ to 2.90 $ngml^{-1}$ for R7 while R6 shows no significant difference.

IV. CONCLUSION AND RECOMMENDATION

4.1 Conclusion

In this study, there is a link between the depletion of thyroxine level of the body and the presence of cyanide residue in cassava processed foods consumed by the rabbits.

4.2 Recommendations

1. Research should be done on cassava processed food marketed in Nigeria in order to ascertain their cyanide levels.
2. Cassava processing should not be done in a hurry, or human lives will be at the expense of quick money-making.
3. SON/NAFDAC should establish standards for cassava processing, to ensure that no substandard cassava processed foods should be sold in the market.
4. Further research should be done to correlate the increasing cases of goiter in Nigeria with increasing consumption of cassava and cassava products.

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