

Cyanide-induced hyperthyroidism in male Wistar rats

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ABSTRACT

Background: Cyanide is one of the major environmental pollutants termed thyroid disruptor. Regardless of its origin, it is a primary toxic agent. This study was designed to understand the impact of prolonged low dose cyanide exposure on the structure and function of the thyroid gland. **Materials and Methods:** Twelve F1 male Wistar rats were used for this study. They were divided into two groups of six animals each. The first group served as the control group and received 0.25M sucrose while the second group being the treated group received 2 mg/kg body weight (BW) potassium hexacyanoferrate III solution. The treatment duration was 56 days following which the animals were sacrificed by cervical dislocation. Blood samples were drawn to determine serum FT₃, FT₄ and thyroid stimulating hormone (TSH) levels. The thyroid gland was also excised and processed for light microscopic studies. **Result:** An increase in serum FT₃ and FT₄ with decrease serum TSH was obtained in the treated group. Application of one-way analysis of variance (ANOVA) statistical analysis showed that there were highly significant differences ($P < 0.05$) in the activities of FT₃, FT₄ and TSH when compared with those of the control group. Light microscopic examination of thyroid gland from the treated group revealed marked epithelial hyperplasia with cellular degeneration and scanty cytoplasm while the control group revealed normal thyroid architecture. **Conclusion:** Results obtained revealed that hyperthyroidism was induced by cyanide.

Key words: Cyanide, hyperthyroidism, thyroid gland, thyroid hormones

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INTRODUCTION

Thyroid gland is one of the largest endocrine glands in the body with the primary function of secreting thyroid hormones.¹ Thyroid hormones are needed for the functioning of most of the body tissues where they perform important roles in growth, development, differentiation and metabolism, and are of greater effects on oxygen (O₂) consumption and metabolic rate.^{2,3} Thyroid hormones are made up of triiodothyronine (T₃) and thyroxine (T₄). These hormones are synthesised and secreted by follicular cells in vertebrate animals, regulated by thyroid stimulating hormone (TSH), which is secreted by the pituitary gland.⁴ Serum levels of T₃, T₄ and TSH are 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 and/or secretion as well as disorders in their extra-thyroidal peripheral metabolism.⁵

Cyanide is one of the major environmental pollutants termed thyroid disruptor.⁶ Regardless of its origin, it is a primary toxic agent.⁷ Large proportion of the population is exposed to very low level of cyanide in the general environment. Environmental factor of cyanide has been associated with much intoxication in humans and animals resulting from exposure to environmental pollution, chemical war and industrial sources, which is a form of occupational hazard.⁸ Cyanides are components of electroplating, printing and photography solutions, fertilizers, fumigant mixtures, metal polishes and rodenticides.^{7,9} It also may be released into the environment during the course of industrial usage, from smoke, cigarettes smoke or vehicle exhaust. Such smoke may contain the incomplete combustion products of nitrogen-containing organic polymers which react to form cyanide.^{10,11}

This present study adopted chemical induction of cellular alterations through cyanide intoxication. This was aimed at understanding the impact of low dose cyanide exposure on the structure and function of follicular cells of the thyroid gland.

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MATERIALS AND METHODS

Potassium hexacyanoferrate III salt ($K_3Fe(CN)_6$; Mol Wt = 329.25) and sucrose salt (β -D-fructofuranosyl- α -D-glycopyranoside; $C_{12}H_{22}O_{11}$; Mol. Wt = 342.30) used for this study was procured from Sigma, Aldrich Germany. Five grams of the $K_3Fe(CN)_6$ salt was dissolved in 1000 ml of 0.25 M sucrose in order to obtain a final working solution of concentration 5 mg/ml of potassium hexacyanoferrate in 0.25 M sucrose solution.

Twelve (12) first filial (F1) generations in bred adult male Wistar rats (*Rattus norvegicus*) with an average weight of 250 g were procured from the animal facility of College of Health Sciences, Osun State University, Osogbo, Osun State. The animals were kept under standard laboratory condition of good lighting, moderate temperature and adequate ventilation in a hygienic environment. They were on standard feed with water *ad libitum*. The animals were placed under standard laboratory protocols as stipulated by the Institutional Animal Care and Use Committee¹² and as adopted by the animal research ethical committee of the College of Health Sciences of Osun State University.

Animals were randomly and equally divided into two groups. Those in group I received 0.25M sucrose while those in group II were treated with 2 mg/kg body weight (BW) of potassium hexacyanoferrate III solution for the duration of 56 days.

The animals were force fed orally using oral canula. The animals were held with a glove with the left hand (such that the neck region will be held by the fingers to still the neck) while being fed with the canula. Treatment was carried out at 07.00 am every day before the animals were fed.

Twenty-four hours after the last administration, the animals were sacrificed by cervical dislocation. Blood samples were collected by jugular venepuncture into plain universal bottle under aseptic conditions. The thyroid gland was excised following midline-abdominal incision to the neck region. The specimens for routine histological investigations were fixed in formol saline and processed for paraffin wax embedding. Serial sections of 6 μ m thickness were stained with Hematoxylin and Eosin¹³ and Periodic Acid Schiff (PAS).¹⁴

Blood samples were immunoenzymometrically analysed to determine serum FT3, FT4 and TSH levels by the methods of Wild,¹⁵ Lee *et al.*,¹⁶ and Fisher,¹⁷ respectively.

Values were reported as mean $\pm$ SEM (standard error of the mean). Significance was determined statistically by application of one-way analysis of variance (ANOVA) using statistical software SPSS version 17 at 95% confidence interval. Differences between means were considered statistically significant at $P < 0.05$.

RESULTS

The control group (Group I): light microscopic examination of sections from the thyroid gland of rats in the control group administered with 0.25M sucrose was composed of follicles lined with a single layer of cuboidal follicular cells [Figure 1a and b]. Their colloid is vacuolated with scalloped boundaries with lumen containing stained secretion.

The experimental group (Group II): light microscopic examination of sections from thyroid gland obtained from the treated group revealed markedly distended follicle has ruptured and coalesced to form a large cyst with marked epithelial hyperplasia and cellular degeneration [Figure 2a]. The coalesced follicle observed in Figure 2b is not as enlarged as that in Figure 2a. Follicles in Figure 2b are generally markedly reduced when compared with that of the control with some having almost obliterated lumen (O). The nuclei of the thyroid epithelial cells were observed to have ballooning degeneration and some bearing prominent nucleoli [Figure 2a and b]. Some vacuolated colloids were also observed (V) [Figure 2a and b].

A highly significant increase in the levels of serum FT3 and FT4 with a very highly significant decrease in serum TSH was obtained in the treated group when compared with the control group.

Statistics and data analysis

Result obtained revealed that cyanide also significantly affected the activity of FT3, FT4 and TSH when compared with the control group [Table 1].

DISCUSSION

Upshots on the structure and function of the thyroid have drawn much attention because thyroid hormones act on cells of almost all tissues consequently involved in several physiological processes. This study was performed to evaluate the influence of cyanide on the structure and function of the thyroid follicular cells. The structure of the follicular cells was evaluated by light microscopic examination while its function was assessed by measuring the serum levels of FT3, FT4 and TSH.

Two histological techniques were looked into to explain likely structural changes linked to cyanide response in the thyroid follicular cells. Harri's hematoxylin and eosin technique demonstrated the general tissue structure while the PAS technique demonstrated the integrity of the basement membrane and the colloid of the follicles. In this study, these two techniques demonstrated structural changes in the thyroid follicles of Wistar rats treated with potassium hexacyanoferrate to mimic hyperthyroidism condition.

Light microscopic examination of sections from the thyroid glands of rats in the treated group revealed marked distortion

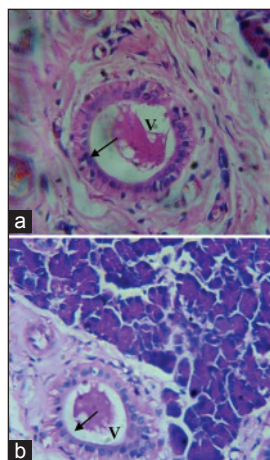


Figure 1: Photomicrographs of sections in the thyroid gland of rats from the control group (group I) showing normal thyroid follicle lined with simple cuboidal epithelium (arrows). Their colloid is vacuolated with scalloped boundaries and lumen containing stained secretion (V). 1a (H and E $\times 400$), 1b (PAS $\times 400$)

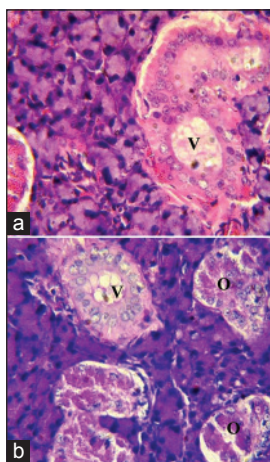


Figure 2: Photomicrographs of sections in the thyroid gland of rats from the experimental group (group II) showing markedly distended thyroid follicle that has ruptured and coalesced to form a large cyst in Figure 2a. The coalesced follicle observed in Figure 2b is not as enlarged as that in Figure 2a. Follicles in Figure 2b are generally markedly reduced when compared with that of the control with some having almost obliterated lumen (O). The nuclei of the thyroid epithelial cells were observed to have ballooning degeneration [Figure 2a and b]. Their colloids are vacuolated (V). 2a (H and E $\times 400$), 2b (PAS $\times 400$)

Table 1: Serum hormone levels in control and experimental groups

Treatment dose	FT ₃ (Pg/ml)	FT ₄ (Pg/ml)	TSH (μ IU/ml)
0.25M Sucrose	1.03 $\pm$ 0.29	9.38 $\pm$ 0.61	0.22 $\pm$ 0.07
2 mg/kg BW	2.78 $\pm$ 0.21*	17.22 $\pm$ 4.39*	0.01 $\pm$ 0.03*

Mean $\pm$ S.E.M – Mean values $\pm$ Standard error of mean; Analysis was done using one way ANOVA; Cyanide toxicity – Significance from control group; * $P < 0.05$. Cyanide significantly affected the activity of FT₃ ($P = 0.00$); FT₄ ($P = 0.00$) and TSH ($P = 0.00$)

of follicular structure. It was specifically observed that the follicle is markedly distended and has coalesced to form a large cyst with epithelial hyperplasia [Figure 2a]. Ballooning

degeneration of the thyrocytes was also observed [Figure 2b]. While the control group showed normal histologic features of thyroid gland [Figure 1a and b].

These finding showed features seen in thyrotoxic hyperplasia, a thyrotoxicosis or hyperthyroidism condition^{18,19} in the cyanide treated group. Although there is dearth information on cyanide-induced microstructure of the thyroid gland, the histological findings of thyroid gland from this study showed that hyperthyroidism was effectively induced by cyanide.

In the blood, both T₄ and T₃ are bound to proteins: thyroxine-binding globulin, transthyretin, and albumin. Only a small fraction of the hormones, 0.025% of T₄ and 0.35% of T₃, are free and unbound.²⁰ It is also important to note that the laboratory measurement of total T₃ and total T₄ measures mainly protein-bound hormone concentrations, results may as well be affected by conditions that affect protein binding. Because of this interference with total thyroid hormone levels, free hormone concentrations are preferable in diagnosis.²¹ This informs why serum-free T₃, T₄ and pituitary TSH were evaluated in this study to correlate the function of the follicular cells. Elevated concentration of free T₃ and T₄ was observed with a depressed concentration of TSH when compared with that obtained from the control group [Table 1]. On application of statistical analysis, this finding showed significant difference while comparing with the control group. This result perfectly describes thyrotoxicosis or a hyperthyroidism condition, which supported our histological findings.

Disruptions of thyroid function by endogenous or exogenous factors may produce various subclinical effects²² or direct clinical manifestations.²³ A clear example is the clinical hyperthyroidism also known as thyrotoxicosis, which can be activated by different disorder with a resultant upshot of excess thyroid hormones.²⁴

In conclusion, our results suggest that low dose cyanide effectively induced hyperthyroidism condition, which could eventually lead to goitre formation and or thyroid cancer with time. This result also suggest that cyanide not only induce hypothyroidism, but has also been documented in most findings.²⁵⁻²⁸

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