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Acute Low Dose Oral Cyanide Induced Thyrotoxic State by Oxidative Stress in Rats

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ABSTRACT

This study assessed the cyanide induced thyrotoxic state by oxidative stress on the thyroid gland. Twelve (12) first filial (F1) generation in bred adult male Wistar rats were randomly and equally divided into two groups. Group I received 0.25M sucrose while Group II received 20mg/KgBW of potassium hexacyanoferrate III solution both for the duration of 14 days. Animals were sacrificed by chloroform ether anesthesia, blood samples collected to determine the serum *Free Tri-iodothyronine* (FT3), *Free Tetra-iodothyronine (*FT4) and Thyroid Stimulating Hormone (TSH) concentration and thyroid gland excised and processed for light microscopic examination and histomorphometry while the activities of Malondialdehyde (MDA) and Superoxide dismutase (SOD) were assayed from the thyroid tissue homogenates. Light microscopic examination of the thyroid gland from the treated group showed enlarged hyperplastic thyroid follicle lined by degenerated columnar follicular cells while the control group showed normal thyroid follicle architecture. Maximum diameters of the follicles from the slides of thyroid tissues of both groups of animals were measured and data acquired were analyzed using Image J software and student's T-test. The result obtained showed a highly significant difference in the diameters of follicles of the two groups of animals on comparism. Increased activities of MDA and SOD with increased serum FT3 and FT4 and decreased serum TSH concentrations were observed in the treated group. Results obtained from MDA, SOD, FT3, FT4 and TSH activities were highly significant ($P<0.05$) on application of one-way ANOVA statistical analysis when compared with those of their control. The study showed that acute cyanide exposure effectively induced thyrotoxic state by oxidative stress.

Keywords: Cyanide, thyroid hormones, thyrotoxic state, oxidative stress.

INTRODUCTION

Cyanide is a naturally occurring and highly ubiquitous chemical listed among the potent toxic and rapid-acting poisons. Potential human sources of cyanide are numerous[1, 2]. Cyanide ecological feature has been linked with countless intoxication sequence in humans and animals ensuing from chemical war, homicide, suicide, environmental pollution, occupational factors, ingestion of foods and use in some drugs such as nitroprusside and laetrile[3].

Cyanide has long been implicated with the ability to induce oxidative stress. This is due to the hyperthyroid state it confers on the thyroid gland resulting in increased mitochondrial oxygen consumption demands[4]. But then, mitochondria are one of the preferred targets of thyroid hormones. This thyroid circulating hormones are implicated in the physiologic changes of the mitochondrial respiration process[5]. In thyroid hormone synthesis, the mitochondrial system provides a constant source for the production of hydrogen peroxide which is very essential for the oxidation of thyroidal iodide in the presence of thyroid peroxidise[6, 7]. Coupling reaction between the inactive iodotyrosines; Monoiodotyrosine (MIT) and Diiodotyrosine (DIT), are as well catalysed by thyroid peroxidase through a mechanism involving an extreme reactive free radical mediated reaction[8].

Epidemiological records and experimental analyses established that hyperthyroidism is connected with a general increase in tissue oxidative stress[7, 9]. Tissue damage in hyperthyroidism is as a result of increase free radical and changes in the antioxidant defense system[10]. An increase in the antioxidant capacity would reduce the damage produced by free radicals[6].

This study was aimed at assessing the impact of oxidative stress on the thyroid gland following chemical induction of cellular changes through cyanide intoxication. For this reason, the morphology of cells in the thyroid follicles was examined and the enzymatic activities of superoxide dismutase (SOD) and the level of malondiadehyde (MDA) in the thyroid gland

homogenates from rats were measured.

MATERIALS AND METHODS

Ethical Approval

Research proposal for this research work was submitted to the Health Research Ethics Committee (HREC) of the College of Health Sciences of Osun State University, which considered the work and gave an ethical approval (Code Number 2013/08/014/A) for carrying it out.

Experimental Animals

Twelve (12) in bred first filial (F1) generation adult male Wistar rats (*Rattus novergicus*) with an average weight of 250 gm were procured from the animal facility of College of Health Sciences, Osun State University, Osogbo, Osun State. The animals were kept under standard laboratory conditions of good lighting, moderate temperature, and adequate ventilation in a hygienic environment. They were on standard feed with water *ad libitum and* were placed under standard laboratory protocols as stipulated by the Institutional Animal Care and Use Committee[11]and as adopted by the Health Research Ethics Committee (HREC) of the College of Health Sciences of Osun State University.

Animals were randomly divided into two groups of six animals per group, with those animals in group I being treated with 0.25M sucrose while those in group II were treated with 20mg/KgBW of potassium hexacyanoferrate III solution. Administrations were for the duration of 14 days.

Treatment solution and mode of administration

A final working solution of 5 mg/ml of potassium hexacyanoferrate III, $K_3Fe(CN)_6$ in 0.25 M sucrose solution was obtained by dissolving 5 gm of the CN salt in 1000 ml of 0.25M standard isotonic sucrose solution (β-D-Fructofuranosyl-α-D-Glycopyranoside; $C_{12}H_{22}O_{11}$), and was administered to the animals using an oral cannula with a ball point at the tip. The animals were held with a gloved left hand with the neck region stabilized by fingers while being fed with the cannula. Treatment was done pre-prandial at 07.00 hour each day for the duration of the research work.

Methods

The animals were anesthetized with chloroform ether twenty four hours after the last administration. Blood samples were collected by jugular venepucture into plain sample bottles under aseptic conditions. The thyroid glands were excised following midline-abdominal incision that was made up to the neck region. The specimens for routine histological investigations were fixed in formol saline and processed for paraffin wax embedding. Serial sections of 3µm thickness were stained with Haematoxylin and Eosin (H&E).

Specimens for biochemical assay were preserved separately in 0.25M sucrose and homogenized with Polter-Elvhjem homogenizer. The homogenates were centrifuged at 5000rpm for 10 minutes. The supernatants were immediately stored in the freezer (-20°C) and assayed spectrophotometrically for the activities of SOD and MDA within 48 hours by the methods of Marklundand Marklund[12] and Pasha and Sadasivadu[13] respectively. Blood samples were immunoenzymetrically analyzed to determine serum FT3, FT4 and TSH levels by the methods of *Wild, Lee et al[14] and Fisher[15] respectively.*

Statistical analysis

The values obtained were described as mean ± SEM (standard error of the mean). Values were considered statistically significant at $P<0.05$ by application of T-test and one-way analysis of variance (ANOVA) using statistical software SPSS version 17.

RESULTS

Histological observations

Micrographs from thyroid tissue of animals in group 1 showed the normal architecture of thyroid follicles lined by simple cuboidal epithelium and vacuolated colloid with scalloped boundaries, while micrographs from thyroid tissue of animals in group 2 showed enlarged thyroid follicle lined by degenerated columnar follicular cells with loss of cell boundary. The colloidal material is also vacuolated with scalloped edges in micrographs of tissues form group 2 animals. Maximum diameters of the follicles from the slides of thyroid tissues of both groups of animals were measured and data acquired were analyzed using Image J software and student's T-test.

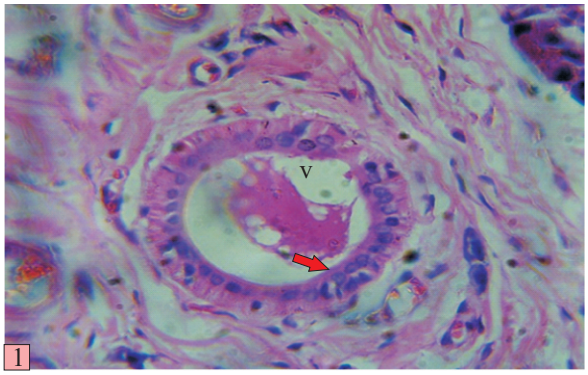

Figure 1: Photomicrograph of section revealing the thyroid follicle of rats from the control group (group 1) showing: normal thyroid follicle lined by simple cuboidal epithelium (red-arrow). Their colloid is vacuolated with scalloped boundaries and lumen containing stained secretion (V). (H&E X400).

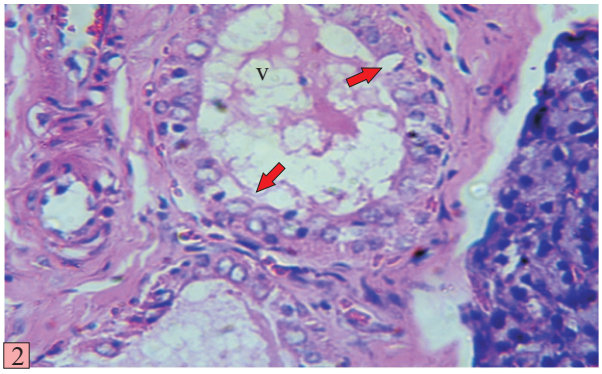

Figure 2: Photomicrograph of section revealing the thyroid follicle of rats from the cyanide-treated group (group 2) showing: enlarged thyroid follicle lined by degenerated thyroid follicular cells and cytoplasmic vacuolation (red-arrows). Each thyroid epithelial cell is large and columnar. The colloidal material is vacuolated with scalloped edges (V). (H&E X400).

The result obtained showed a highly significant difference in the diameters of follicles of the two groups of animals on comparism (Table 1).

Table 1: Results of thyroid follicle histomorphometry in control and cyanide-treated groups

Groups	N	Mean ± S.E.M (μm)
Control	6	112.41±0.48
Treatment	6	164.59±0.64*

Mean ± S.E.M = Mean values ± Standard error of mean. N = diameter of six follicles

Analysis was done using T-test. Cyanide toxicity: significance from control group, *P<0.05. Cyanide significantly affected the activity of diameter of the follicles (P=0.00).

Biochemical observations

The toxic cyanide effect significantly affects the activities of SOD and MDA (Table 2) as well as the activities of FT3, FT4 and TSH (Table 3).

Table 2: Results of oxidative stress markers in control and cyanide-treated groups

Groups	SOD (unit/ml)	MDA (nmol/dl)
Control	29.83±1.66	0.0100±0.0013
Treatment	39.50±1.33*	0.0137±0.0013*

Mean ± S.E.M = Mean values ± 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 SOD (P=0.00) and MDA (P=0.00).

Table 3: Serum hormone levels in control and cyanide-treated groups

Groups	FT3 (Pg/ml)	FT4 (Pg/ml)	TSH (μIU/ml)
Control	1.03±0.29	9.38±0.61	0.22±0.07
Treatment	4.90±0.39*	12.92±0.31*	0.15±0.07*

Mean ± S.E.M = Mean values ± 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).

DISCUSSION

Using H & E meant for the demonstration of the cellular architecture, the histological observations of the thyroid follicles from the cyanide-treated group showed enlarged thyroid follicle lined by degenerated thyroid follicular cells and cytoplasmic vacuolation. The thyroid epithelial cells increase in size and edges of the colloid are scalloped, indicating active removal of stored colloid for processing into thyroxine (Figure 1). While the control group showed normal thyroid follicle architecture (Figure 2). These microscopic features from the cyanide-induced thyroid follicles showed the characteristic features of thyrotoxic hyperplasia, a thyrotoxicosis or hyperthyroidism condition[16]. To substantiate this finding, thyroid follicle histomorphometry was carried out. Results obtained were subjected to statistical analysis which showed data of maximum diameter obtained were highly significant (Table 1). Amazingly, there is paucity of histological findings addressing cyanide-induced hyperthyroidism. Our histological findings established cyanide can induce hyperthyroidism.

The levels of serum thyroid hormones were also measured in this study (Table 3). Increased concentration of free T3 and T4 with decreased concentration of TSH as seen in hyperthyroidism was observed in the treated group. This finding showed significant difference when compared with the control group on application of statistical analysis. The result obtained from hormonal assay further supported our histological findings. Despite previous research findings on serum thyroid hormones established hypothyroidism characterized by supressed FT3 and FT4 elevated TSH in both human and animal studies [17, 18, 19, 20, 21], thyrotoxicosis also known as hyperthyroidism condition is very rare. It is suggestive of Graves' disease confirm by suppressed TSH, elevated free T4 and/or T3 [22]. This observation again showed that cyanide effectively induced hyperthyroidism supporting the findings of Adeniyi *et al*[4].

In animal models, free radical-mediated lipid peroxidation plays a fundamental task in hyperthyroidism Pathophysiology and the consequent tissue injury. Lipid peroxidation is a self-catalytic mechanism involving the oxidative destruction of cellular membranes by free radicals[23]. Inhibition of enzymes, impaired functions of the mitochondria and Golgi apparatus and decrease membrane fluidity and function are different forms of harmful effects related with lipid peroxidation. Malondiadehyde (MDA) is an end-product of lipid peroxidation which is often assessed as an indicator of these processes[24]. It was reported that hypermetabolic condition in hyperthyroidism was associated with an increase in free radical formation and lipid peroxidation levels[25, 26].

This study showed increase in the value of MDA assayed from the homogenate obtained from the cyanide-treated group with significant difference when compared with the control group. This affirms the findings of Iangalenko *et al*[27] that lipid peroxidation was increased in hyperthyroid patients and Asayama and Kato[28], where lipid peroxidation was increased in liver, heart and some skeletal muscles of rats in experimental hyperthyroidism and our histological and serum hormone findings.

Superoxide dismutase (SOD) is among the antioxidant enzymes that convert free radical to less toxic compound, thereby, counteracting the possible laid up free radicals effects in a mechanism known as the antioxidant defense system[29, 30]. SOD is an essential intracellular oxygen radical-scavenging enzyme. From literatures, increase in metabolic rate and the increase in oxygen consumption are associated with hyperthyroidism thereby enhancing microsomal capacity and free radical formation[6, 7, 9]. On the contrary, increased free radical formation enhances intracellular scavenging enzymes, like SOD, in experimentally induced hyperthyroidism[31]. Regardless of the way in which hyperthyroidism influences antioxidant defense capacity, organism naturally protect itself against the effects of oxidative stress by increasing SOD activity as a protection mechanism. This explains the increase observed in the result of SOD obtained in the cyanide-treated group when compared with the control (Table 2) which was significant on analysis with statistical application.

In conclusion, our results suggested that acute low cyanide exposure effectively induced thyrotoxic state by causing oxidative stress in male rats.

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