Histological Alterations in the Thyroid Follicular Cells Induced by Lead Acetate Toxicity in Adult Male Albino Rats

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ABSTRACT

Lead exposure can cause multiple systemic toxicities, particularly affecting the hematopoietic, nervous, and renal systems. Experimental studies have shown that lead has potent endocrine-disrupting activity. However, its effects on the thyroid structure and functions are not well elucidated and the published studies are controversial. The aim of this study was to evaluate the changes occur in thyroid tissue, optically and ultrastructurally, and hormonal changes induced by low-dose sub-chronic lead acetate toxicity. Twenty adult male albino rats were divided into two groups, each containing 10 animals; group I (normal control) received distilled water as placebo and group II (Lead acetate group) was treated with 100 mg/kg BW of lead acetate by oral gavage for 8 weeks. The results showed that lead acetate treatment caused biochemical changes that are consistent with hypothyroidism i.e. low T4 and T3 levels and a significant increase in TSH. Microscopic examination of thyroid sections in Lead-acetate treated group revealed that the majority of the thyroid follicles were irregular and enlarged, others were distended with vacuolated colloids, and some of them were small with no colloid, congested blood vessels, prominent mast cells, and exfoliated cells in the lumen of the follicles. In conclusion, lead acetate exposure resulted in subclinical hypothyroidism associated with evident morphological alterations in the thyroid tissue.

Key Words: *Follicular cells, Lead Acetate, Thyroid, Thyroid Stimulation Hormone, Thyroxine.*

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INTRODUCTION

Pesticides and heavy metals are considered the most common environmental pollutants because of the high utilization in agriculture and industries. Heavy metals such as cadmium (Cd) and lead (Pb) are common environmental pollutants [1-6]. They are intensively used in modern products such as leaded gasoline, mining wastes, smelting, electroplating, petroleum, and lead paint, as well as, batteries, pigments, and plastics [7, 8]. Therefore, humans are exposed to these pollutants during their lifetime through different routes such as skin, ingestion, and inhalation. People who are exposed to these elements, such as, lead (Pb) a toxic heavy metal, for long periods are considered at risk of toxicity [3, 9, 10]. Lead toxicity can be manifested in several systems such as, nervous, respiratory, immune, dermal, urinary and reproductive, in adults and during pregnancy [10, 11]. Organic and inorganic types of lead are known to cause toxicity, however, toxicity from inorganic type such as lead carbonate, lead chromate, lead monoxide, lead tetraoxide, and lead acetate is higher. Organic lead toxicity has been reported to the nervous system more than the inorganic type [9]. Other investigations have proved that a dysfunction endocrine system as a result of lead and cadmium toxicity [12-16]. The thyroid gland is vital gland in our body that regulates many body functions by secreting triiodothyronine (T3), thyroxine

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(T4), and calcitonin hormones. Thyroid hormones have a strong influence on metabolism, cardiovascular system, nervous system, lipid profile, energy consumption, and body weight. Abnormal function of the thyroid gland (hypothyroidism or hyperthyroidism) was manifested with a wide profile of symptoms such as respiratory distress syndrome, transient tachypnea, sepsis, apnea, and various problems for pregnant women and their fetuses [11, 12, 17, 18]. Additionally, it was also presented with weight gain and weight loss [13,19], dyslipidemia, mood alteration, cognitive deficit, cardiac dysfunction, osteoporosis, and fractures [20, 21].

Previous studies have found hypothyroidism in lead poisonings, but the histological characterizations in the thyroid tissue due to lead intoxication are not well documented and identified, yet. Due to the limited number of studies that have been previously conducted, which showed contradicting and inconclusive results [22-24]. Therefore, the present study was aimed to characterize the possible histological alterations in the thyroid follicular cells following experimental long-term lead poisoning with sub-toxic doses in male albino rats.

MATERIALS AND METHODS

Chemicals:

Lead acetate was purchased from Sigma, Aldrich (Jordan). Evaluation of Hormonal Levels TSH ELISA kits for rats were purchased from USCN Life Science Inc. (Wuhan, China), whereas total T3 and T4 ELISA kits for rats were prepared from MyBioSource Company (California, USA). The plasma samples were analyzed according to the relevant company's manuals.

Experimental animals and protocol:

Twenty mature male albino rats (Sprague Dawley strain), average weight 150-200g, were obtained from the animal facility of the Faculty of Medicine, Mutah University. They were randomly assigned to the control or treated group, housed in cages (10/cage) and maintained under standard conditions, namely, a 12:12-hour light:dark cycle, a temperature of 24±1 °C, and 50±10% relative humidity. All animals were fed a standard laboratory control diet and provided with tap water ad libitum. The experiments were conducted according to the ethical forms approved by the Faculty Ethics Committee. Body weight of the animals was recorded at the beginning and on alternate days. The rats were divided into two equal groups, each containing 10 animals. Group I (normal control) rats received distilled water as placebo. The test group of animals (Group II) received an oral gavage dose of lead acetate (Pb Ac) 100 mg/kg of Body Weight per day for 8 weeks. Lead acetate was prepared by dissolving 1g of lead acetate in 1L of distilled water based on the previous study [25]. At the end of the trial, blood samples were collected from all rats by cardiac puncture for the determination of serum levels of thyroid hormones; Total serum thyroid-stimulating hormone (TSH), Triiodothyronine (T3), and Thyroxin (T4) concentrations were quantitatively determined using enzyme immunoassay kits (ELISA) following the manufacturer instructions.

Histopathology:

Two specimens were excised from the dissected thyroid gland. The specimens were subjected to light and electron microscopic examination: The first specimen was immediately removed after scarification and immediately fixed in 10% formalin and processed to get 5 µm thick paraffin sections. These sections were stained with Haematoxylin & Eosin stain (H&E) and toluidine blue for routine histological examination. The second specimen was fixed in 3% phosphate-buffered glutaraldehyde at 4 ºC for 2 hours and further processed for examination by a transmission electron microscope.

Statistical Analysis:

The collected data for serum levels of hormones was statistically analyzed using SPSS software v.20. The values were expressed as mean±SE. The difference between groups was determined using the ANOVA test, $p < 0.05$ values were considered significant. (table 1)

Table 1: Serum T3, T4, and TSH levels after 8 weeks

	Group I	Group II	P- value
T3 Mean ± SD **Median**	3.0 ± 0.53 3.0	2.9 ± 0.55 3.1	0.77
T4 Mean ± SD **Median**	1.5 + 0.30 1.5	1.2 ± 0.34 1.2	0.78
TSH Mean ± SD **Median**	2.3 ± 0.9 1.9	4.0 ± 1.0 4.8	0.007*

*: Significant

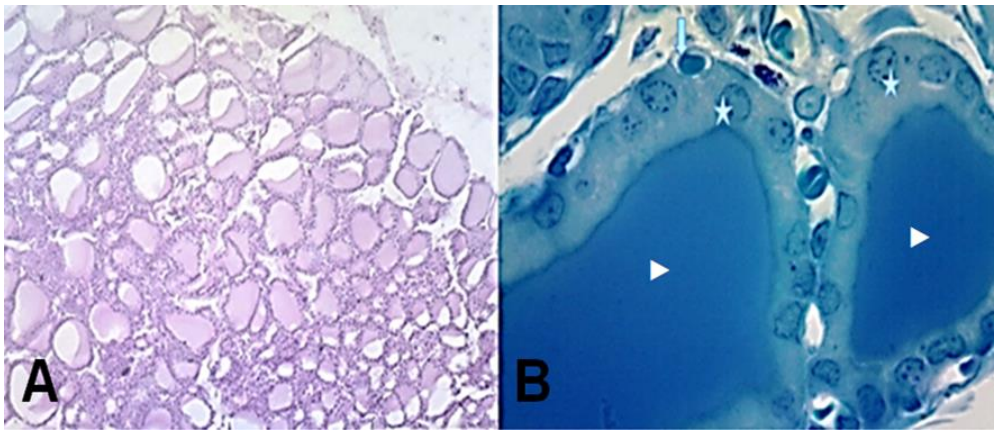

Figure 1: Photomicrograph of the thyroid gland of groups I (control group), showing normal structure of the thyroid gland. (A) Stained with H&E. X100. (B) Stained with toluidine blue. X1000. Showing thyroid follicles filled with colloid (arrowhead) lined by simple cuboidal epithelium with round nuclei (starred) and large pale parafollicular cells (arrowed). Small blood capillaries present between the follicles.

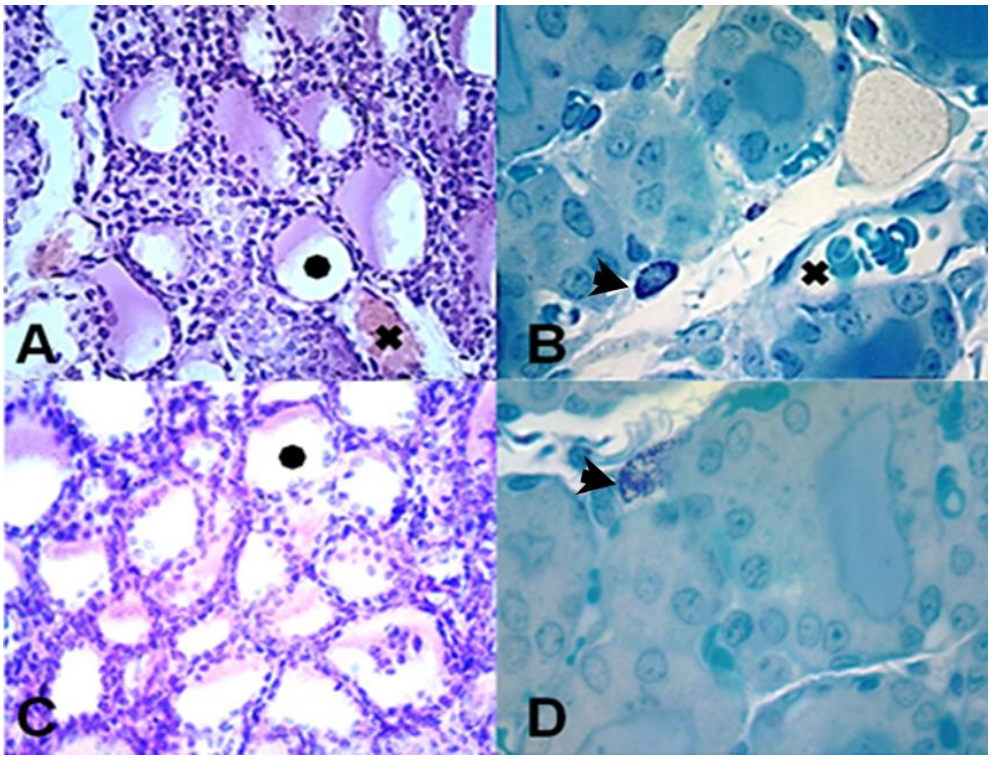

Figure 2: Photomicrograph of thyroid gland of group II (lead acetate treated group), showing abnormal structure of thyroid gland. A and C stained with H&E. X100. B and D stained with toluidine blue. X1000. Images showing that most of the thyroid follicles were irregular and enlarged, while other follicles were swollen with vacuolated colloids (●). Basophilic mast cells were seen between the follicles (arrowhead) and congested blood vessels (✖) with exfoliated cells in the lumen were also seen between follicles.

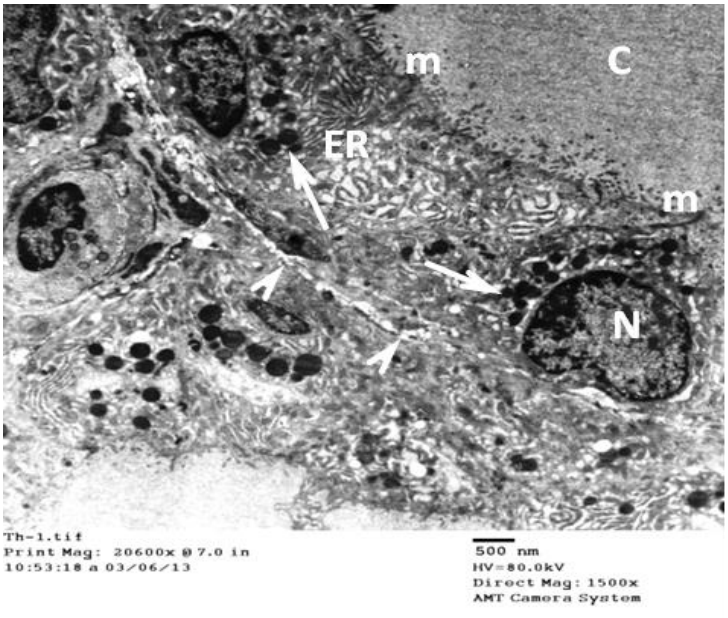

Figure 3: Electromicrograph of the thyroid gland of the control group (group I), showing part of a thyroid follicle lined by simple cuboidal epithelium and filled with a homogenous colloid (C). Cuboidal cells had euchromatic nuclei (N), parallel cisternae of rough endoplasmic reticulum (ER), and pleomorphic electron-dense lysosomes (arrowed). Cells' apical surface is folded into numerous microvilli (m) while a basal lamina (arrowheads) separates basal surface from blood capillaries present in the adjacent parafollicular space.

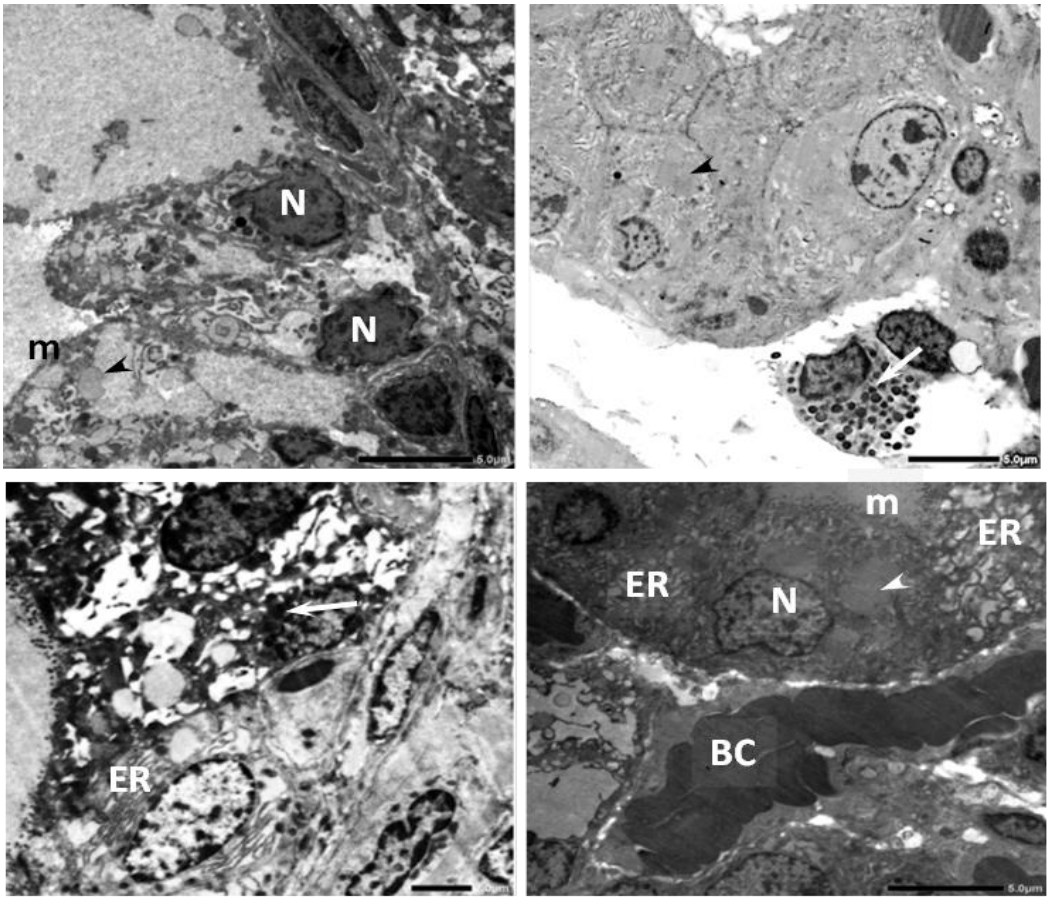

Figure 4: Electromicrograph of the thyroid gland of lead acetate treated group (group II), showing follicular cells' appear with irregular heterochromatic nuclei (N) and a cytoplasm containing extensively dilated cisternae of rough endoplasmic reticulum (ER) filled with flocculent material, membrane-bound vacuoles (arrowheads), and polymorphic lysosomes (arrowed). The luminal surface has few blunt microvilli (m) and congested blood capillaries (BC) were observed in the interfollicular space.

RESULTS

General and biochemical findings

Animals in the control group (group I), gained some weight after 8 weeks, however, group II (lead acetate treated group) showed weight loss. Statistically, this weight loss in group II was significant when compared with the weight of the control group (group I), indicating that lead acetate strongly affected the body weight of the animals. The change in body weight for both groups (weight gain in group I and weight loss in group II) was significant after eight weeks (T-test, $p < 0.05$).

Biochemical results showed changes in the serum levels of thyroid hormones in animals of both groups**.** TSH levels were significantly increased in the lead acetate group (group II) compared to the control group (group I) (T-test, $p = 0.007$). On the other hand, lead acetate treatment caused an insignificant increase in T4 levels (T-test, $p = 0.78$). Similarly, T3 serum levels showed an insignificant decrease in the lead acetate treated group (group II) in comparison to the control group (T-test, $p = 0.77$).

Histological findings

Light microscopic evaluation of H&E and toluidine blue-stained parts of group I (control group) showed normal histology of the thyroid gland, which includes normal-sized thyroid follicles, filled with homogenous colloid and lined by simple cuboidal cells with rounded nuclei. Large pale parafollicular cells and small blood capillaries were seen between the follicles (Figure 1). Lead acetate treated group (group II) showed abnormal histology of the thyroid sections which includes; irregular, enlarged and swollen follicles with vacuolated colloids. Few follicles were small with no colloid. Parafollicular space contains prominent mast cells, congested blood vessels and exfoliated cells in the lumen (Figure 2). Transmission electron microscope examination of ultra-thin sections of the control group (group I) showed part of the thyroid follicle lined with cuboidal follicular cells and filled with homogenous colloid. The microstructure of follicular cells showed euchromatic nuclei, parallel cisternae of rough endoplasmic reticulum, and pleomorphic electron-dense lysosomes. Abundant microvilli were seen on the luminal surface of the follicular cells while a basal lamina separates from the blood capillaries present in the parafollicular space (Figure 3). Lead acetate treated group (group II) showed the abnormal microstructure of the thyroid glands which included, follicular cells with irregular and heterochromatic nuclei, polymorphic lysosomes, and extensively dilated cisternae of rough endoplasmic reticulum filled with flocculent material. Luminal surfaces of follicular cells had short few blunt microvilli. Parafollicular space showed congested blood capillaries (Figure 4).

DISCUSSION

Our results revealed that systemic administration of lead acetate caused a significant increase in TSH serum levels while the changes in T3 and T4 levels were insignificant. Additionally, rats exposed to lead toxicity exhibit a significant weight loss with abnormal histological changes in the thyroid follicles cells and colloids. Abnormal function of the thyroid gland as a result of lead toxicity has been demonstrated in animal and human studies, which support our results. Lead acetate toxicity in experimental animal studies showed mixed effects on the thyroid gland. For example, eight weeks of daily administration of 5 mg/kg lead acetate orally in sheep significantly reduces serum levels of all thyroid-related hormones i.e. TSH, T4, and T3 [26]. In a preclinical trial, rats showed a significant decrease in T3 and T4 after six weeks of lead administration, while TSH levels peak after three weeks before suddenly diminished [27]. This study also showed that follicles were swollen with shed epithelial. After 14 days of lead administration, a significant decrease was observed in T3 and T4, while TSH was significantly increased in rats [28]. Furthermore, abnormal thyroid function was observed in rats exposed to lead systemically [29].

In human, several previous studies suggested that abnormal levels of lead may affect thyroid function [30]. For example, among occupationally exposed workers, elevated blood lead was associated with a significant release of TSH [31], while another study on workers exposed to lead revealed no statistically significant difference in thyroid hormones' levels [32]. In a national survey in the USA, the results showed no association between lead toxicity and thyroid hormones [23]. Conversely, another study based on the survey data showed a correlation of lead exposure with the malfunction of thyroid with the possible influence of gender [22]. In these cross-sectional studies, the etiology and the effect could not be conclusive, because there were confounding factors e.g. age of participants, and variable durations of cumulative occupational exposures, which in total might give false outcomes. Alternatively, a recent systemic review did not support a causal relationship between lead exposure and thyroid dysfunction in males [33].

In the present study, a low-dose administration of lead acetate was shown to cause a mild decrease in the serum level of T_3 and T_4, which mimics the environmental exposure effect without apparent systemic toxicity. Although the alteration in the concentration of thyroid hormone was mild, the fact that these very low doses of lead acetate cause some levels of hormonal changes should not be ignored since they can reflect subclinical metabolic changes in the system [34]. Although T_3 is a

weak indicator of overt or subclinical hypothyroidism, a low decrease in serum level of T_3, that was observed in group II, subchronically administered with lead acetate, is probably due to the low level of T_4, not decrease in its synthesis, since T_4 has to be converted to T_3 for the biological efficiency of the hormone [35]. Moreover, the relatively low level of T_3 is probably due to the deficiency in the synthesis of 5′-deiodinase, which is an enzyme that converts T_4 to the more metabolically active T_3. Lead acetate inhibits the activity of type-I iodothyronine 5′-monodeiodinase (5′-D). The low enzyme activity has been attributed to pathological changes in the organs responsible for its synthesis due to lead acetate toxicity [36].

The present study revealed evident structural and morphological changes in the thyroid glands at both optical and ultrastructural levels. To our knowledge, it is the first time to demonstrate lead acetate induced changes at the ultrastructural level. A low decrease in T_4 level in lead acetate treated group, could be due to the damage of the thyroid follicles, which was probably caused by oxidative stress induction via lead acetate [37]. Lead acetate on its own causes hypothyroidism either by oxidative stress induction or by inhibiting iodine uptake [8, 10, 38, 39]. The significant increase in the TSH concentration in group II is probably due to the attempt of the body to increase the synthesis and elaboration of T_4 by stimulating the thyroid gland in order to compensate the significant deficit in the system. The low T_4 concentration in the (lead acetate) group might have stimulated the hypothalamic neurons to secrete thyrotropin-releasing hormone (TRH), leading to increased stimulation of TSH synthesis [15, 22, 40-42]. The observations made in this study indicate that lead acetate damages the structure and function of the thyroid gland, leading to interference in the synthesis and/or secretion of T4 as a result of the damage of thyroid follicular cells, decrease transformation rate of the T4 to T3 in peripheral tissue, and interference with pituitary gland or hypothalamus gland. The severity of the disturbances increases with the time of exposure. We obtained important data on lead acetate interference in the thyroid gland, yet this problem requires further studies since mechanisms of this action are still poorly recognized.

CONCLUSION

In the present study, we demonstrated that eight weeks of lead acetate exposure resulted in subclinical hypothyroidism i.e. a mild decrease in T4 and T3 levels and significantly high TSH serum level associated with evident damage to the thyroid tissue at both optical and ultrastructural levels. Since thyroid hormones were not evidently affected at this stage, these abnormalities are possibly resulted by lead toxicity through oxidative damage and inflammation of thyroid tissue.

Conflict of Interest

The authors declare no conflict of interest.

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