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The Effect of Humic Acid Substances on the Thyroid Function and Structure in Lead Poisoning

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ABSTRACT

Lead (Pb) is a heavy metal, which adversely affects thyroid gland function and structure. Due to its high molecular weight and abundant functional groups, humic acid substances (HAS) can form chelates with heavy metals. The experiment was conducted to evaluate the prophylactic effect of HAS on thyroid hormone levels and histopathological lesions of laying hens exposed to lead (Pb) poisoning. After a week of adaptation, 192 Lohmann White laying hens (25 weeks of age) were fed one of four diets: a basal diet (BD) or the BD with HAS (0.15%), with Pb (0.3 g/kg), or with both. Experimental groups were replicated in 12 cages, with four hens each. Pb poisoning did not alter triiodothyronine (FT_3; 3.22 ± 0.20 ng/dL) or thyroxine (FT_4; 0.71 ± 0.08 ng/dL) concentrations, but caused a 167% increase in thyroid stimulating hormone (TSH) concentration. HAS supplementation returned the high TSH levels of hens exposed to Pb poisoning to normal values. Degenerative changes in the epithelial cells of the thyroid gland of the hens exposed to Pb poisoning were evidenced. Connective tissue cells in the interfollicular area and total amount of colloids with partially atrophic follicles were observed. These histopathological findings were less severe when HAS was added to the diet. In conclusion, HAS alleviates the effects of Pb poisoning on thyroid gland function and structure, possibly preventing its internalization by the tissue by forming chelates and exerting anti-inflammatory effects.

INTRODUCTION

The thyroid gland mediates various metabolic processes in the body by secreting mainly thyroxine (T_4) and triiodothyronine (T_3) hormones. These hormones are derived from amino acids, and result from the iodination of thyroglobulin tyrosine residues. They are responsible for regulating the global metabolic activity of the body. The secretion of thyroid hormones is controlled by the thyroid-stimulating hormone (TSH) that is released from the anterior pituitary gland. Increased activity of the thyroid gland results in hyperthyroidism, whereas decreased activity results in hypothyroidism (Szkudlinski *et al*., 2002). Environmental, physiological, and genetic factors play a role in the development of both hyperthyroidism and hypothyroidism (Burger, 2004). The exposure to heavy metals is of the most important environmental factor that adversely affects the function of the thyroid gland (Cullent *et al*., 1984).

Lead (Pb) is one of the main heavy metals and non-essential toxic elements, and pose a risk to public health due to expanded urbanization and increased industrialization (Smith, 1984; Roper, 1991; Tong *et al*., 2000). It is widespread in nature and has been used in many industrial applications throughout history, such as manufacturing of batteries, some paints, glass, construction materials, agrochemicals, cosmetics,

and fuel additives (Elwood *et al*., 1984). Once taken into the body through different routes, Pb accumulates in the organs and tissues. In the bloodstream, 85-90% of Pb is bound to erythrocytes, and the remaining 15-10% to plasma proteins (Lyn Patrick, 2006). Lead poisoning causes hematological, neurological, circulatory, and immunological pathologies, accompanied by biochemical changes, liver and kidney dysfunctions, and disrupt glucose metabolism (Al-Saleh, 1994; Lavicoli *et al*., 2003). Pb poisoning adversely affects endocrine glands, particularly the homeostasis of thyroid hormones, reproductive hormones and stress hormones (Zacharewski, 1998).

Chelating agents are used for the treatment of heavy metal poisoning. Humic acid substances (HAS) are water soluble, and include humic, fluvic, and ulmic acids derived from humus, breakdown product of organic substances in the soil (Islam *et al*., 2005). Previous studies have shown that humic acid regulates abnormal thyroid hormone secretion and act as immunomodulators (Laurberg *et al*., 2003). Due to their high molecular weight and functional groups (Schnitzer and Khan, 1972; Fan *et al*., 2004), it was hypothesized that supplementing diets with HAS reduces the adverse effect of heavy metals on the endocrine functions through forming chelates. This preliminary experiment was conducted to evaluate the prophylactic effect of supplementing HAS on hormonal and histopathological changes in the thyroid gland in laying hens exposed to Pb poisoning.

MATERIALS AND METHODS

Animals, diets and experimental design

A total of 192 Lohmann White laying hens of 25 weeks of age randomly were assigned to one of the four dietary treatments.

A week before introducing the dietary treatments, all birds were fed the same iso-nitrogenous and iso-energy basal diet formulated to meet their nutritional requirements (NRC, 1994). Birds were then fed a basal diet, or the basal diet with 0.15% HAS (50.6% humic acid, 9.4% fulvic acid) (Farmagülatör XP, Farmavet International, Istanbul, Turkey), 0.3 g Pb/kg (lead acetate trihydrate, Acros Organics, New Jersey, USA), or the same levels of Pb+HAS.

Each treatment (n=48 birds) was replicated in 12 cages (42 x 48 cm), housing four hens each. The experiment lasted 10 days. During the adaptation and experimental periods, hens were subjected to a light regimen of 17L:7D. Feed and fresh water were provided *ad libitum*.

Blood sampling and biochemistry

At the end of the experiment, blood samples were collected from axillary veins of one randomly selected hen per cage (n=12 per treatment) into additive-free blood tubes. Blood samples were centrifuged at 1,500 *g* for 15 min, and sera were placed in Eppendorf tubes. The sera (250 µl) were first digested with a mixture of 2 mL HNO_3 (30%; Merck-KGaA, Darmstadt, Germany) and 3 mL H_2O_2 (70%, Merck-KGaA) in a Microwave Digestion System (Berghoff, Eningen, Germany) for 25 min. The specimens were then subjected to analysis of elemental Pb using Inductively Coupled Plasma Emission Spectroscopy (Optima 2100 DV, ICP/OES, Perkin-Elmer, Shelton, CT). Moreover, the sera were analyzed for T3, T4, and TSH levels using a diagnostic automatic analyzer (Modular Analytics Evo, F. Hoffmann-La Roche Ltd., Berlin, Germany).

Histopathology

After blood sampling, birds were sacrificed by cervical dislocation for harvesting the thyroid glands. Tissue samples were fixed in 10% buffered formaldehyde. Fixed tissues were embedded in paraffin blocks. The sections (5 µ) were stained with hematoxylin eosin (HxE), and evaluated under a light microscope after performing Masson's Trichrome and Periodic Acid Shift staining methods.

Statistics

Data were analyzed by one-way analysis of variance using the PROC GLM procedure (SAS, 2002). Statistical differences among group means were determined by the LSD option and considered significant at $p \leq 0.05$. Data were presented as least square mean ± standard error of the mean (LSM±SEM).

RESULTS

Serum lead and thyroid hormone levels

Table 1 summarizes serum Pb and thyroid hormone level results. The addition of Pb in the basal diet caused a 3.73-fold increase in serum Pb level compared with the control group ($p<0.0001$). HAS supplementation decreased serum Pb level by 16.5% compared with the Pb-poisoned group ($p<0.05$).

Serum FT_4 level (0.71 ng/dL) and FT_3:FT_4 ratio (5.49) were similar across the experimental groups. Lead poisoning caused a 2.67-fold increase in TSH level ($p<0.05$), and was suppressed by HAS supplementation to the control level.

Table 1 – Effects of the dietary supplementation of humic acid substances (HAS) on serum lead and thyroid hormone levels of laying hens exposed to a 10-day lead (Pb) poisoning.

	Experimental Groups*				
Variable	Control	HAS (0.15%)	Pb (0.3 g/kg)	Pb+HAS	SEM
Pb, mg/L	0.062^{c}	0.068^{c}	0.231^{a}	0.193^{b}	0.04
FT_3, pg/mL	2.94^{b}	3.30^{ab}	3.22^{ab}	3.67^{a}	0.08
FT_4, ng/dL	0.715	0.682	0.719	0.726	0.20
FT_3:FT_4	4.34	5.80	4.79	7.04	0.89
TSH, µIU/L	0.003^{b}	0.005^{b}	0.008^{a}	0.004^{b}	0.001

*Data are the least square mean ± standard error of the mean (SEM) (n=12 cages per group, 4 hens per cage). Different superscripts within the same rows differ ($p<0.05$).

Thyroid Gland Histopathology

The thyroid tissues of the control hens did not show any histopathological changes (Figure 1). In the hens exposed to Pb poisoning, degenerative changes and necrotic areas were evident in the epithelial cells of the thyroid gland at various degrees of intensity (Figure 2A). Increases in connective tissue cell numbers in the interfollicular area and in the total amount of colloid with atrophic follicles were observed. Moreover, mononuclear cell infiltration, particularly lymphocytes in thyroid parenchyma and capsule and its surrounding fat tissue were evident (Figure 2B).

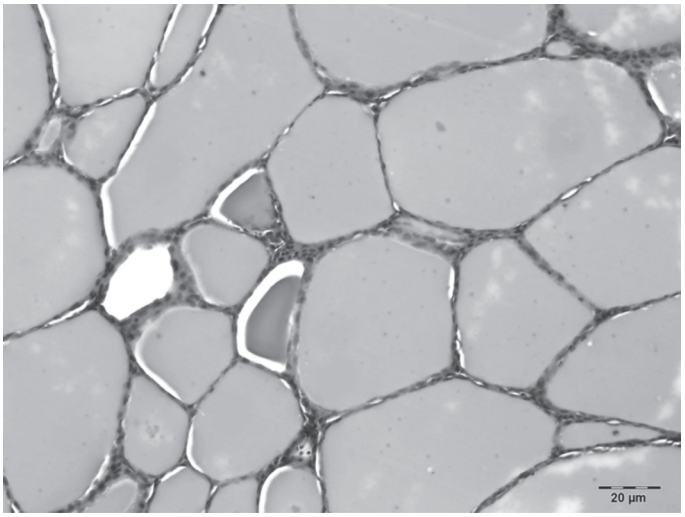
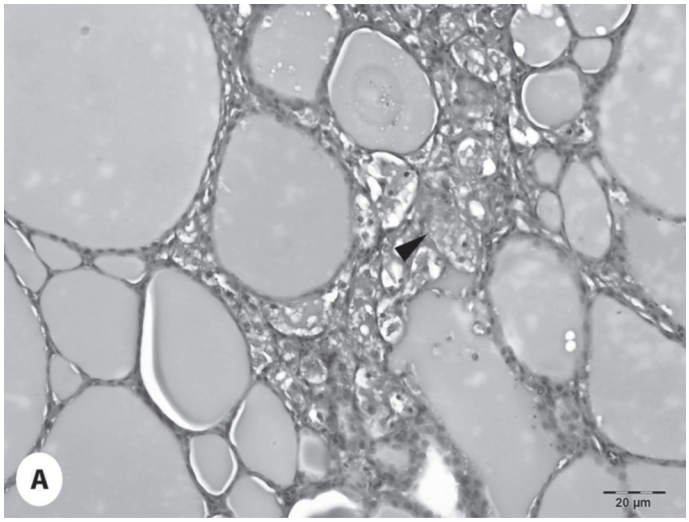


Figure 1 – The appearance of a normal thyroid gland (Control Group) (H&E).

Similar histopathological lesions were observed in the hens that were fed Pb+HAS, but at a lesser intensity. Follicular structure disappeared and colloid structure spreaded around to parenchymal and interfollicular areas in three cases (Figure 3A). In other cases, lesions were reduced and follicles presented normal histological appearance (Figure 3B).



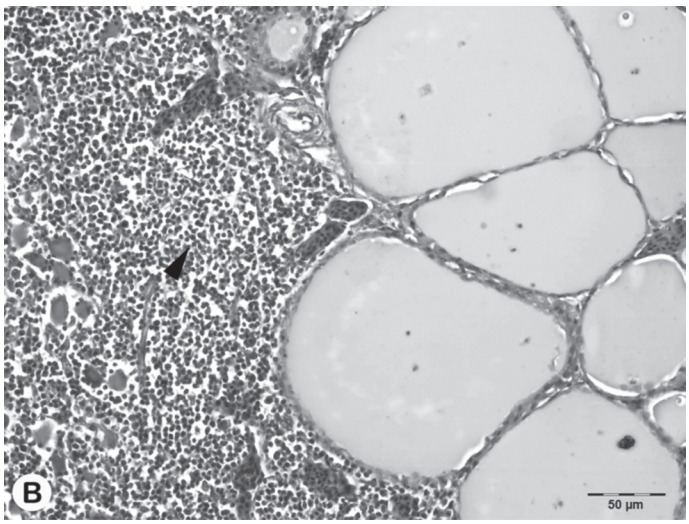


Figure 2 – Degenerative changes with debris and necrotic areas (arrowhead) (H&E) (Panel A) and lymphocyte infiltrations (arrowhead) (H&E) (Panel B) in the thyroid gland (Lead Group).

DISCUSSION

Lead is one of the most harmful heavy metals and can accumulate in soft (*i.e.*, liver, kidney) and hard (*e.g.*, bone) tissues (Lyn Patrick, 2006; Gillis *et al.*, 2012). Lead poisoning causes structural and functional changes in many organs, such as renal dysfunction, nervous system disorders, glucose metabolism abnormalities, liver dysfunctions, and hematological changes (Al-Saleh 1994; Lavicoli *et al.*, 2003). In particular, it may cause anemia by reducing hemoglobin concentration in the hematologic system (Hilliard *et al.*, 1973; Lynch *et al.*, 1976).

Several agrochemicals are applied in crop production, and many poultry feedstuffs may be contaminated by environmental pollution. Lead can accumulate in the body, which may adversely affect animal health and pose a food safety risk. The Pb level of 0.3 g/kg diet

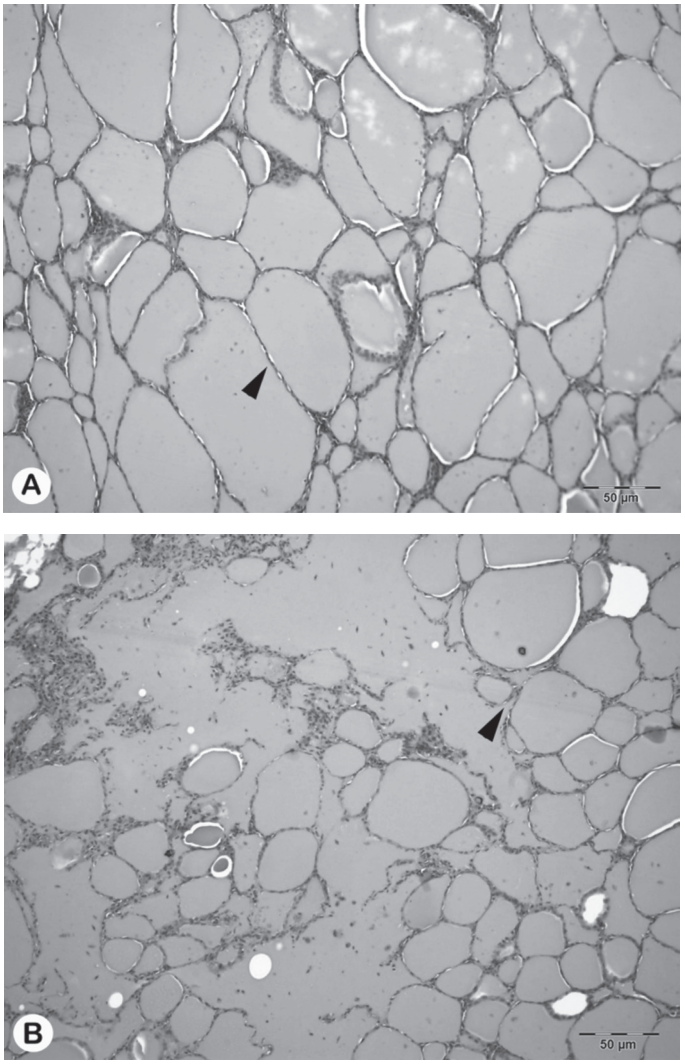


Figure 3 – Large colloid areas with lost follicular structure scattered in the parenchyma and interfollicular area (arrowhead) (H&E) (Panel A), and restoration of the thyroid gland to normal (arrowhead) (H&E) (Panel B) (Humic Acid Substances + Lead Group).

was selected in the present experiment in order not to cause mortality, but to cause toxicity within 10 days (Vangris and Mare, 1974). Accordingly, mortality was not recorded, but the elevation of plasma Pb level was evident (Table 1). The changes in plasma Pb levels were associated with changes with the plasma levels of thyroid gland hormones (Table 1).

Lead adversely affects the production, secretion, and biological activities of thyroid and stress hormones and of hormone-related metabolism (Zacharewski, 1998). In the present study, Pb poisoning partially affected the thyroid hormones (Table 1), which may be related to the duration and level of Pb ingestion. Ibrahim *et al*. (2012) showed that lead acetate ingestion did not cause any significant reduction of plasma T_4 and T_3 levels. Similarly, in a study with 58 male gas station attendants and automobile mechanics with occupational exposures to high Pb levels, mean T_3 and T_4 levels did not differ (Singh *et al*., 2000). On the other hand, Robins *et al*. (1983) reported reduction of serum T_4 and FT_4 levels in humans exposed to excessive Pb levels. Dundar *et al*. (2006) evaluated the long-term and low-level Pb exposure among young individuals, and reported a negative correlation between blood lead and FT_4 levels, but no changes in TSH and FT_3 levels. In another study, high FT_3 level and low TSH level in the group of patients poisoned with lead compared with the control group were reported (Yılmaz *et al*., 2012). When Pb concentration exceeds 52 µg/dL, it triggers TSH secretion from the pituitary gland (Singh *et al*., 2000). In agreement with the present study, TSH level increased among workers exposed to lead (Gustatson *et al*., 1989; Lopez *et al*., 2000).

Lead can cause degenerative changes, satellitosis, neuronal vacuolation, neuronophagia, inflammatory changes and degenerative disorders in the nervous system, inflammatory cell infiltration and cytoplasmic vacuolation in the liver and kidneys (Taib *et al*., 2004; Ozsoy *et al*., 2011; Shalan *et al*., 2005). Degenerative changes, inflammatory cell infiltration, and interstitial connective tissue proliferation in the thyroid gland, indicating hypothyroidism, were observed in the present study (Figure 2).

Many agents, including vitamin C and L-carnitine, are applied for Pb poisoning treatment (Shaban El-Neweshy and Said El-Sayed, 2011; Flora *et al*., 2003). HAS are reported to alleviate abnormal thyroid hormone secretion and to act as immunomodulators (Laurberg *et al*., 2003). Nonetheless, HAS have multifunctional characteristics, such as to be able bind to metal ions to form chelates (Schnitzer and Khan, 1972). In the present study, dietary HAS supplementation partially alleviated feed intake and egg production reductions by 17.7% and 23.8%, respectively (data not shown) and reduced serum Pb level by 16.4% (Table 1) compared with the hens exposed to Pb poisoning and not supplemented. Moreover, it reduced serum TSH to normal levels (Table 1), which could be explained but its protective effects on the parenchymal cells of the thyroid gland. In addition to forming chelates with heavy metals, HAS has anti-inflammatory effects. The thyroid tissue showed similar, but less severe lesions when hens were supplemented with HAS (Figure 3).

In conclusion, in hens experimentally poisoned with Pb, FT_3 and FT_4 levels did not change, but TSH level increased by 167%. The increase in serum Pb and TSH levels of hens exposed to Pb poisoning was partially and completely normalized, respectively, by dietary HAS supplementation, and were accompanied by the

recovery of the degenerative changes observed in the thyroid gland. These results suggest that HAS may bind to heavy metals and play a role in the recovery of thyroid gland structure and function.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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