

## **Effect of Ellagic Acid Extracted from Pomegranate (*Punica granatum* L.) on Thyroid and Parathyroid Gland of Adult Rats Exposed to Lead Acetate**

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### **Abstract:**

The present study was carried out to investigate the protective and treatment effect of pomegranate extract (ellagic acid) to treat the toxic effect of lead acetate. Forty adult male rats were randomly divided into four equal groups. First group kept as control, second group (T1) dosed lead acetate (10 mg/kg B.W.), third group (T2) dosed ellagic acid (100 mg/kg B.W.) plus lead acetate (10 mg/kg B.W.), while the fourth group dosed (200 mg/kg B.W.) of ellagic acid plus lead acetate (10 mg/kg B.W.).

Blood samples collected at (2, 4, 6) weeks for measuring the following parameters: serum T<sub>3</sub>, T<sub>4</sub>, parathyroid hormone and Ca<sup>+2</sup> levels.

At the end of the experiment (42 days) six animals of each group were scarified to examine the histological structure of thyroid and parathyroid gland.

Results revealed a decrease in thyroid hormones (T<sub>3</sub>, T<sub>4</sub>) in lead acetate group (T1) and protective dose of ellagic acid (T2) group at day 28 and 42 days of experiment compared with control group, while treatment dose of ellagic acid (T3) causes a significant increase of thyroid hormones at the two period above compared with lead acetate and protective dose of ellagic acid (T1 and T2) groups. On other hand exposure of rats to lead acetate revealed a decrease in parathyroid hormone and Ca<sup>+2</sup> levels compared with control group at 28 and 42 days of experiment for parathyroid hormone and at all period of treatment for Ca<sup>+2</sup>, also protective and treatment dose of ellagic acid caused elevation of parathyroid hormone at 28, 42 days of experiment compared with T1 group, while treatment dose of ellagic acid resulted in significant increment of Ca<sup>+2</sup> levels at second and last period of experiment.

It is concluded that the deleterious effect of lead acetate on thyroid and parathyroid gland of rats may be treated by ellagic acid especially in treatment dose which lead to a significant improvement of their functions activity.

## تأثير حامض الايلاجك المستخلص من الرمان (*Punica granatum L.*) في وظيفة الدرقية وجنيب الدرقية في الجرذان البالغة المعرضة لخلات الرصاص

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### الخلاصة:

أجريت الدراسة الحالية لمعرفة التأثير الوقائي والعلاجي لحامض الايلاجك المستخلص من الرمان لمعالجة التأثيرات الضارة لخلات الرصاص. تم استخدام (40) جرذاً بالغاً، قسمت عشوائياً الى اربعة مجاميع متساوية اعتبرت المجموعة الاولى مجموعة سيطرة، بينما اعطيت المجموعة الثانية (T1) خللات الرصاص (10 ملغم/كغم من وزن الجسم) يومياً، المجموعة الثالثة (T2) فقد اعطيت حامض الايلاجك (100ملغم/كغم من وزن الجسم) يومياً اضافة الى خللات الرصاص (10ملغم/كغم من وزن الجسم) اما المجموعة الرابعة (T3) فقد اعطيت 200ملغم/كغم من وزن الجسم حامض الايلاجك اضافة الى خللات الرصاص (10ملغم/كغم من وزن الجسم).

سحبت عينات الدم في الاسبوع (2، 4، 6) من فترة العلاج لغرض حساب مستوى هورمون الدرقية وهورمون جنيب الدرقية (T<sub>3</sub>, T<sub>4</sub>) وكذلك الكالسيوم في مصل الدم. وفي نهاية التجربة (42 يوم) تم قتل 6 حيوانات من كل مجموعة لدراسة الصفة النسجية-التركيبية للغدة الدرقية وجنيب الدرقية.

اشارت النتائج الى حصول انخفاض (T<sub>3</sub>, T<sub>4</sub>) معنوي في مستوى هورموني الدرقية المعرضة لخللات الرصاص (T1) والجرعة الوقائية لحامض الايلاجك (T2) في اليوم 28، 42 من فترة المعاملة مقارنةً بمجموعة السيطرة بينما تسبب الجرعة العلاجية لحامض الايلاجك بزيادة مستوى هورموني الدرقية خلال الفترتين اعلاه.

من جانب اخر فقد حصل انخفاض في مستوى هورمون جنيب الدرقية جراء تعرض الحيوانات لخللات الرصاص T1 مقارنة بمجموعة السيطرة بعد مرور 28، 42 يوم من فترة المعاملة، في حين حصل انخفاض بمستوى الكالسيوم في طيلة فترة التجربة.

لقد تسبب حامض الايلاجك بالجرعة العلاجية بحصول ارتفاع في مستوى هورمون الPTH ولجميع فترات التجربة بينما ارتفع مستوى الكالسيوم بعد مرور 28 و 42 يوم على فترة المعاملة مقارنة بمجموعة السيطرة.

نستنتج من الدراسة الحالية ان التأثيرات السلبية التي تحدثها خللات الرصاص في وظيفة الغد الدرقية وجنيب الدرقية يمكن التغلب عليها من خلال استخدام حامض الايلاجك وخاصة بالجرعة العلاجية التي تسببت بتحسن واضح في النشاط الوظيفي في هاتين الغدتين.

### Introduction:

Herbalism is a traditional or folk medicine practice based on the use of plant and plant extracts, many plants synthesize substance that are useful to the maintenance of health in human and other animals. These include aromatic substance most of which are phenols or their oxygen - substituted

derivatives such as tannis (1, 2).

The use of herbs as medicines has played an important role in nearly every culture on earth, including Asia, Africa, Europe and the Americas (3). There are several study used different type of plant for medicinal purposes like *nigella sativa* (4), parsley (5, 6)

*Pimpinella anisum* (7) and pomegranate (8).

The pomegranate is one of the puniceae families, is a native plant of northern Africa and the caucasus mountains is widely distributed throughout the southern united states and middle East (9, 10) pomegranate extract primarily composed of alkaloids and polyphenols, which composed from Anthorganidins, pelargonidin, Ellagotannins, Gallic acid and Ellagic acid(11). Ellagic acid has demonstrated a variety of beneficial function including anti-oxidant and antiviral activity (12), also has anticancer and anticarcinogenic activity (13).

Lead acetate as a heavy metal cannot be destroyed through biological degradation and have the ability to accumulate in the tissue of animals and human and may be produce degenerating changes like oxidative stress in the body (14, 15).

There is no or little information about the protective or treatment role of ellagic acid as antioxidant to depress harmful effects of lead toxicity, so that we attempt to demonstrate this role in this study.

### **Materials and Methods:**

1. Extraction: fruit was brought from gardens of Al-Mukdadia-Diala-Iraq, middle part which lies between seed and husk were collected and dried grinded with electrical grinds then put the powder of drical pulp in centrifugation, till it used (16). Taken 100gm of dry powder and heated with

50% ethyl alcohol to (60-70) for two hours by using soxhelt extractor then separated by centrifuge 5000vpm for 20 minutes. The supernatant solution was collected in sterile container, this process was returned three times then the solution was collected in sterile container (17).

After the above process done the ethyl-alcohol was removal by rotary evaporator for one hour then the final result of the extracted material was kept. The extract then measured by (HPLC) to know the quantity and quality of phenolic acid in the alcoholic extract (18).

2. Measurement and diagnosis of extracted Elegiac acid: The alcoholic extract of EA were measured by HPLC according to (19) method.

3. Preparation of lend acetate solution lead acetate purchased from Gonane office for medical devices-Iraq. Hi media (India) the water solution was prepared as 1ml of solution contains 1mg of lead acetate (1gm/L) and each animal of treated groups received 1ml/100gm from body weight (20,4).

4. Animals: Forty albino Wister male rats were used in this study, the age of these animals ranged between (10-12) weeks and weight was around (180-220) gm.

Rats were kept under suitable environment condition of (21-25 °C) in an air condition room and photoperiod of 12 hours daily and housed in plastic cage of diameters (50\*35\*15 cm), the food was given as Pellets of freshly prepared ration. The animals were kept

for at least 15 days for adaptation before beginning the experiment.

5. Study protocol: forty male rats divided randomly in to (4 groups) and handled as follows:

**Control Group:** Animals of this group were received 10ml/kg B.W. of ordinary tap water by oral dosage using gavages needle.

**T1 Group:** Animals of this group were received 10mg/kg B.W. of lead acetate solution once daily.

**T2 Group:** Animals of this group were received (100mg/kg B.W.) of ellagic acid (8) and after 3 hour received (10mg/kg B.W.) of lead acetate solution daily (4, 20).

**T3 Group:** Animals of this group were received (10mg/kg B.W.) of lead acetate solution and after 3 hours received (200 mg/kg B.W.) of ellagic acid (8).

6. Blood collection: Fasting blood samples were collected at (7, 14, 21 days) of experiment via cardiac puncture after anesthetized animals by (ketamine 90mg/kg B.W. and xylazine 40mg/kg B.W.) blood samples were centrifuged at (3000 rpm) then serum samples stored in freezer at (-8 °C) till use.

7. Estimation of hormones and calcium: Serum T<sub>4</sub>, T<sub>4</sub> and parathyroid hormone were estimated by radioimmunoassay according to (21). Also a Ca<sup>+2</sup> level was estimation by eolorimatricolly using commercial chemical kits.

8. Histological study: Six animals from each group were sacrificed at

the end of the experiment, tissues of thyroid and parathyroid were taken to histological study (22).

### Statistical Analysis

Results are expressed as mean SE. statistical analysis of data was performed on the basis of two way analysis of variance (ANOVA) Group difference were determined using significant difference (LSD) test at P<0.05 (23).

### Results:

Table (1, 2) showed a prominent decrease of thyroxin (T<sub>4</sub>) and triiodothyronin (T<sub>3</sub>) levels in lead acetate group (T1) and protective dose of ellagic acid (T2) at 28 and 42 days of experiment compared with control group. While treatment dose of ellagic acid (T3) showed significant increase of these two hormones compared with two treated groups (T1 and T2).

On other hand there were no significant differences within all treated groups (T1, T2 and T3) at all periods.

In the current study (table 3) showed a significant decrease in parathyroid hormone levels in lead acetate group (T1) and protective dose of ellagic acid (T2) compared with control group at 28 and 42 days of experiment, while treatment dose of ellagic acid caused predominant elevation of parathyroid hormone at 28, 42 days of experiment compared with T1 group and there was no significant differences within group for all periods.

**Table 1: Effect of lead acetate and ellagic acid on serum thyroxine (T<sub>4</sub>) in adult rats (ng/ml)**

Groups Period	Control group	T1 group	T2 group	T3 group
14 days	4.06 ± 1.14 A a	3.88 ± 0.38 A a	3.98 ± 0.06 A a	4.01 ± 0.68 A a
28 days	4.20 ± 1.08 A a	3.01 ± 1.42 B a	3.80 ± 0.91 B a	4. ± 0.36 A a
42 days	4.16 ± 1.74 A a	3.14 ± 1.81 B a	3.12 ± 0.85 B a	4.15 ± 0.07 A a

L.S.D. = 1.02

- T1: Given lead acetate (10 mg/kg B.W.). T2: Given ellagic acid 100 mg/kg B.W. (protective dose) and lead acetate (10 mg/kg B.W.). T3: Given lead acetate (10 mg/kg B.W.) and ellagic acid 200 mg/kg B.W. (treatment dose).

- Values are presented as means ± SE (n= 10 rat/ group), Capital letter denote significant differences between groups (P<0.05), Small letter denote significant differences within groups (P<0.05).

**Table 2: Effect of lead acetate and ellagic acid on serum triiodothyronin (ng/ml) (T<sub>3</sub>) in adult rats.**

Groups Period	Control group	T1 group	T2 group	T3 group
14 days	0.70 ± 0.09 A a	0.60 ± 0.18 A a	0.62 ± 0.02 A a	0.70 ± 0.01 A a
28 days	0.80 ± 0.06 A a	0.60 ± 0.32 B a	0.50 ± 0.82 B b	0.74 ± 0.14 A a
42 days	0.81 ± 0.13 A a	0.62 ± 0.20 B a	0.68 ± 0.21 B a	0.88 ± 0.28 A b

L.S.D. = 0.12

- T1: Given lead acetate (10 mg/kg B.W.). T2: Given ellagic acid 100 mg/kg B.W. (protective dose) and lead acetate (10 mg/kg B.W.). T3: Given lead acetate (10 mg/kg B.W.) and ellagic acid 200 mg/kg B.W. (treatment dose).

- Values are presented as means ± SE (n= 10 rat/ group), Capital letter denote significant differences between groups (P<0.05), Small letter denote significant differences within groups (P<0.05).

**Table 3: Effect of lead acetate and ellagic acid on serum parathyroid hormone (ng/ml) in adult rats.**

Groups Period	Control group	T1 group	T2 group	T3 group
14 days	18.70 ± 0.24 A a	16.80 ± 0.07 A a	18.40 ± 0.38 A a	18.40 ± 0.10 A a
28 days	18.73 ± 0.93 A a	15.60 ± 0.13 BC a	16.40 ± 0.84 B a	17.82 ± 0.71 A a
42 days	17.70 ± 0.57 A a	15.31 ± 0.07 B a	15 ± 0.91 B a	17.80 ± 1.30 A a

L.S.D. = 2.20

- T1: Given lead acetate (10 mg/kg B.W.). T2: Given ellagic acid 100 mg/kg B.W. (protective dose) and lead acetate (10 mg/kg B.W.). T3: Given lead acetate (10 mg/kg B.W.) and ellagic acid 200 mg/kg B.W. (treatment dose).

- Values are presented as means ± SE (n= 10 rat/ group), Capital letter denote significant differences between groups (P<0.05), Small letter denote significant differences within groups (P<0.05).

**Table 4: Effect of lead acetate and ellagic acid on serum calcium in adult rats (mg/dl)**

Groups Period	Control group	T1 group	T2 group	T3 group
14 days	10.70 ± 0.52 A a	9.20 ± 0.99 B a	10.90 ± 1.70 A a	10.90 ± 3.20 A a
28 days	11 ± 0.30 A b	9.40 ± 1.29 B a	10.70 ± 0.30 A a	12.18 ± 0.90 B b
42 days	10.74 ± 0.18 A ab	9 ± 0.14 B a	10.20 ± 0.12 A a	11.60 ± 0.42 B b

L.S.D. = 1.12

- T1: Given lead acetate (10 mg/kg B.W.). T2: Given ellagic acid 100 mg/kg B.W. (protective dose) and lead acetate (10 mg/kg B.W.). T3: Given lead acetate (10 mg/kg B.W.) and ellagic acid 200 mg/kg B.W. (treatment dose).

- Values are presented as means ± SE (n= 10 rat/ group), Capital letter denote significant differences between groups (P<0.05), Small letter denote significant differences within groups (P<0.05).

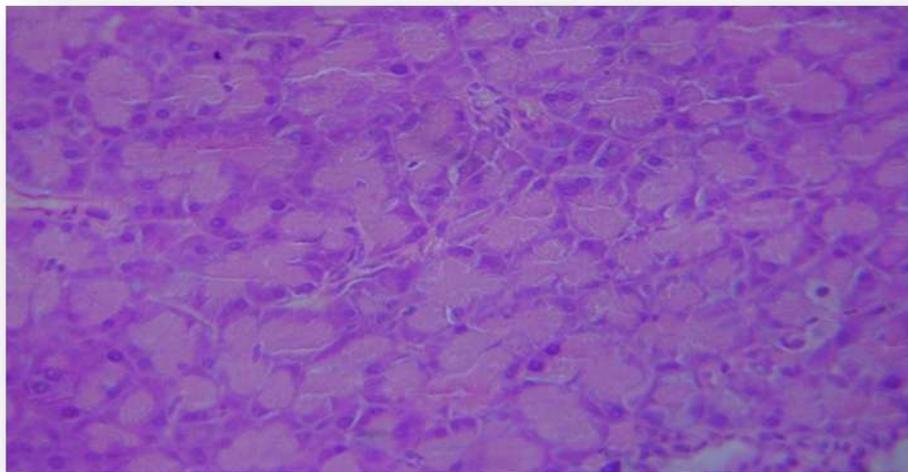


Figure 1: Histological section in thyroid gland of untreated rat (control group). Note: thyroid follicles with epithelial cells filled within colloid secretion (H&E, 40X).

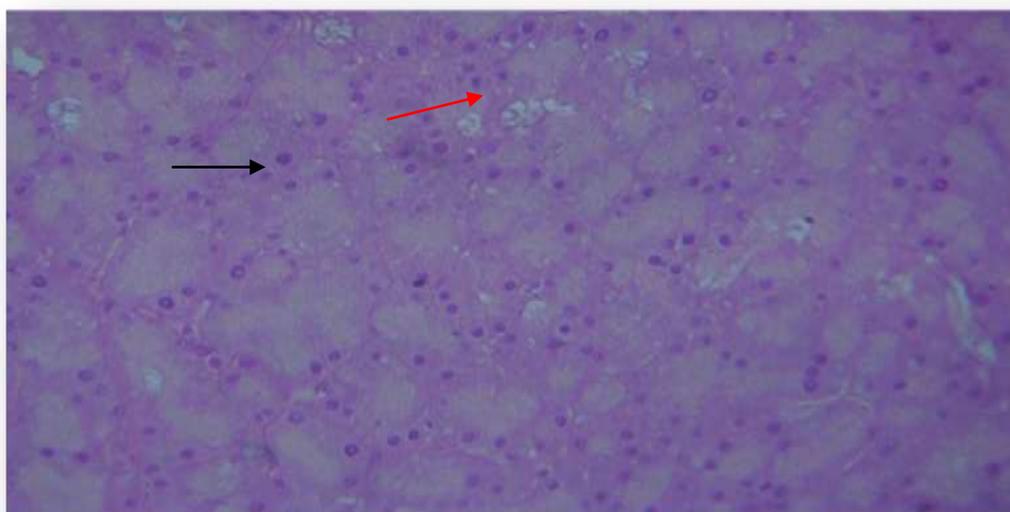


Figure 2: Histological section in thyroid gland of lead acetate rat (T1 group). Note: Hypoerplasia of follicular cells ( —————> ), vacuolation of cells with little colloid secretion ( —> ) (H&E, 40X).

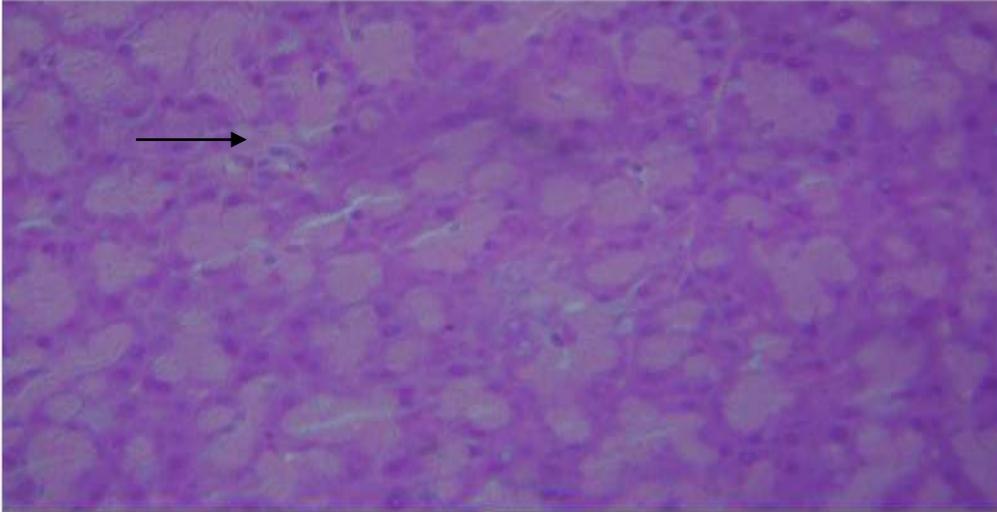


Figure 3: Histological section in thyroid gland of treatment dose of ellagic acid. Note: Few infiltrations of inflammatory cells (————→) and less hyperplasia (H&E, 40X).

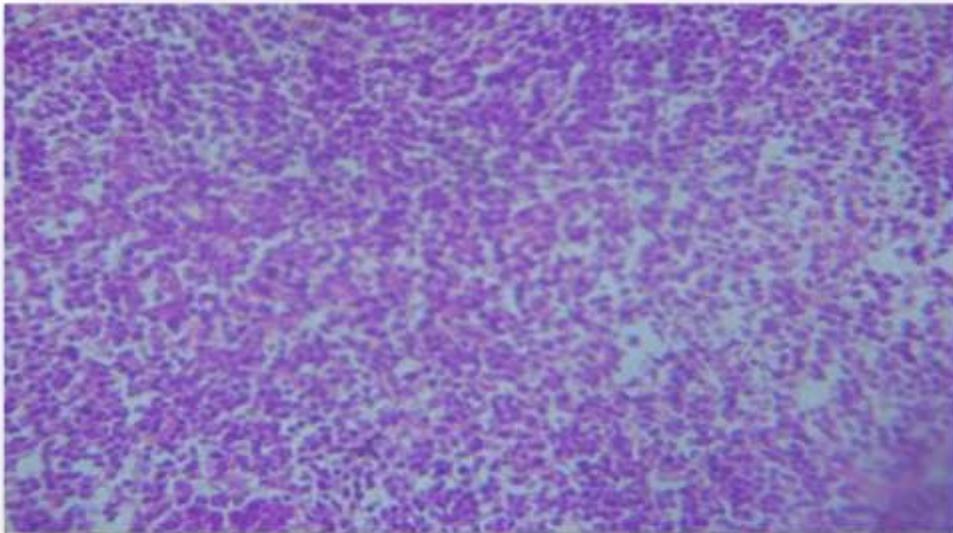


Figure 4: Histological section in parathyroid gland of untreated rat (control group). Note: normal structure of chief and oxyphil cells (dark and light cells) (H&E, 40X).

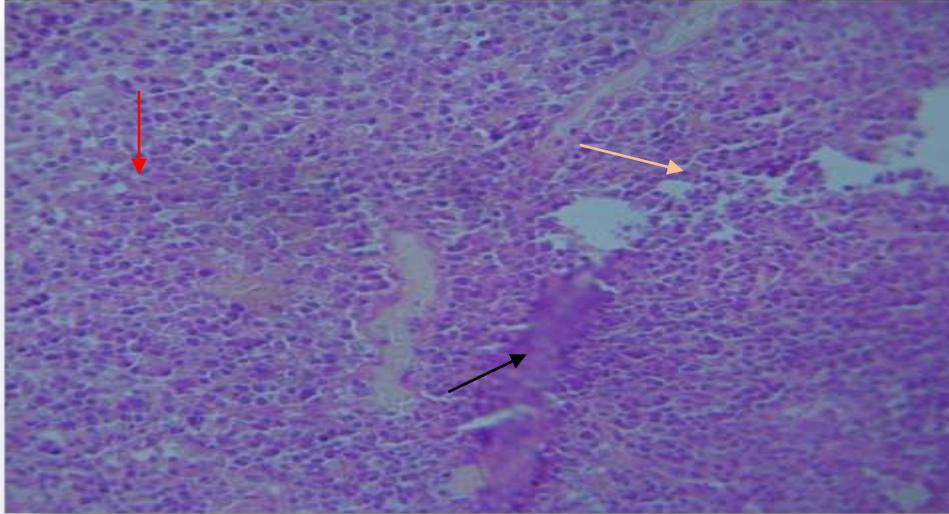


Figure 5: Histological section in parathyroid gland of lead acetate treated rat (T1 group). Note: Infiltration of inflammatory cells (—→), increase of dark cells (—→) with severe congestion (—→) (H&E, 40X).

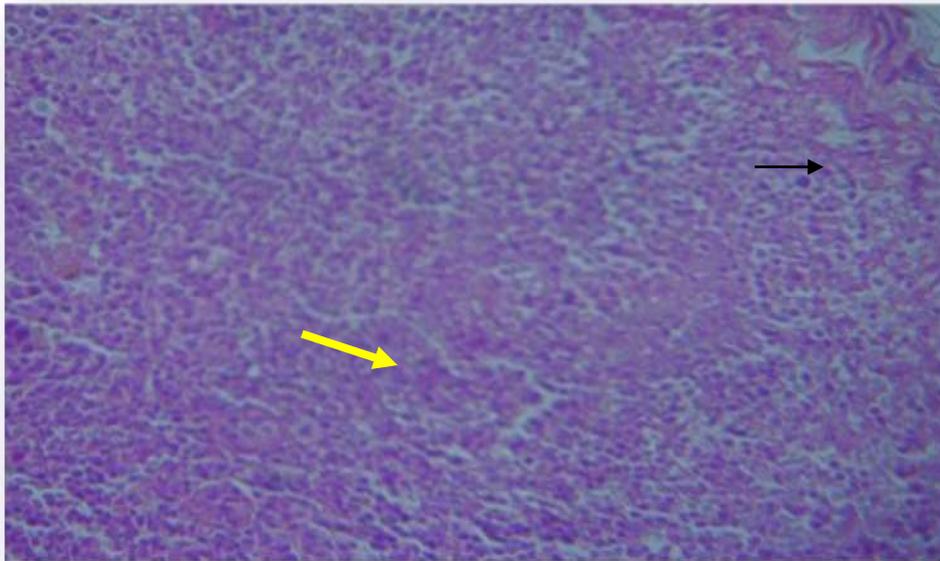


Figure 6: Histological section in parathyroid gland of treatment dose of ellagic acid. Note: Increase of light cells (—→) with few inflammatory cells (—→) (H&E, 40X).

**Discussion:**

The decrement of thyroid hormones ( $T_3$ ,  $T_4$ ) levels in animals exposed to lead may be due to interfere of lead acetate with the hypothalamic peptides thyroid releasing hormone (TRH) (24) or with thyrotropin stimulating hormone (TSH) (25), furthermore, the properties of lead as oxidative factor which release free radicals may be another cause of decrement of thyroid hormones (26) especially  $T_3$ , thus lead induced cytotoxicity and enhanced lipid peroxidase levels so that the activities of the antioxidant enzymes was depressed and this is accompanied by an increased cellular oxidative stress and reducing equivalents such as glutathione (GSH) and finally essential thiol (-SH) groups depleted and causing disruption of 5-D enzyme configuration leading to inhibition of deiodination of  $T_4$  to  $T_3$  (27).

The increment of thyroid hormones ( $T_3$ ,  $T_4$ ) in treatment dose (T3) may be due to antioxidant activity of ellagic acid which decrease or prevent the adverse effects of lead acetate on thyroid gland directly or hypothalamic- pituitary- thyroid axis (28) and the high dosage of ellagic acid in treatment group (T3) may be explain the significant effects of ellagic acid on  $T_3$  and  $T_4$  hormones compared with protective dose.

This study also reported significant hypocalcaemia in lead acetate group (T1) compared with control group at all periods of experiment (table 4). The protective dose of ellagic acid caused no alteration of  $Ca^{+2}$  levels

compared with control group at all periods, while treatment dose result in decrement of  $Ca^{+2}$  levels at second and end period compared with control and T2 groups.

The decrement of parathyroid hormone may be due to depressive effect of lead acetate on parathyroid gland function (29) and this effect lead to decrease of parathyroid hormone synthesis and secretion, hypocalcaemia may be have occurred as a result of hypoproteinemia or due to renal impairment and depressive effect of lead on parathyroid gland function (30, 31). Furthermore, hypocalcaemia may occur as a result of competition absorption between lead acetate and  $Ca^{+2}$  at the level of intestinal epithelium (32).

The elevation of parathyroid hormone in ellagic (T2, T3) may be due to enhancement of parathyroid gland activity by antioxidant effect of ellagic acid (26), also the increment of  $Ca^{+2}$  levels in treatment dose of ellagic acid (T3) during second and last period may be due to increase of parathyroid hormone (31) or due to increase of thyroxine hormone at these two periods which stimulate the active form of vitamin- $D_3$ , vitamin- $D_3$  increase the  $Ca^{+2}$  from small intestine by stimulation of  $Ca^{+2}$  binding protein (CBP) or by increasing their active diffusion across brush border (30, 31), also suggest that elevation of  $Ca^{+2}$  levels in blood is due to stimulation of PTH which stimulate renal 1, $\alpha$ -hydroxylase system (31) to increase the active form of vitamin- $D_3$  (32).

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