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Assessment of the Protective Effects of Selenium and Zinc against Potassium Dichromate Induced Thyrotoxicity in Preimplanted *Wistar albinos Rats*

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Abstract

Hexavalent chromium is an environmental pollutant considered to be an endocrine-disrupting metal. Selenium and zinc are essential trace elements, known to play a crucial role in thyroid homeostasis. The purpose of the current work is to investigate the effects of potassium dichromate ($K_2Cr_2O_7$) administrated subcutaneously (s.c) on the 3rd day of pregnancy in preimplanted rats by using graded doses (10, 50, 100 mg/kg, s.c) or $K_2Cr_2O_7$ (10 mg/kg, s.c) in association with Selenium (0.3 mg/kg, s.c) and Zinc chloride (20 mg/kg, s.c). The hormonal profile, apoptosis induction and histological changes in thyroids were evaluated. Our main findings showed that $K_2Cr_2O_7$ promoted hypothyroidism with a significant decrease in plasma T3 and T4 levels ($P<0.001$), while plasma TSH level increased significantly ($P<0.001$), in addition, a hypertrophy of the thyroid was noted ($P<0.01$). Moreover, $K_2Cr_2O_7$ (10 mg/kg, s.c) induced apoptosis via the caspase 3 pathway ($P<0.001$) and altered the gland histoarchitecture. The co-treatment with Se or $ZnCl_2$ has ameliorated the hormonal status and restored partially the thyroid histoarchitecture. We concluded that the administration of Selenium and Zinc can prevent the hazardous effects of potassium dichromate on the hormonal and histological status of the thyroid gland in preimplanted *Wistar albino* rats.

Keywords: Potassium Dichromate, Preimplanted Rat, Selenium, Thyrotoxicity, Zinc

1. Introduction

The thyroid gland is vulnerable to numerous physiological changes or environmental chemicals, due to its complex histological structure and function, it's often the target of a wide range of Endocrine Disruptors (EDs) that can interfere at any level of the thyroid hypophysis hypothalamus axis, because of the structural similarity of some of them with thyroid hormones, they act via multiple mechanisms such as, binding to thyroid hormone receptor as agonists or antagonists, or interfering with their synthesis, release, transport, metabolism and actions on target tissues[1], by influencing their cellular uptake and gene expression[2], such as the industrial biocides[3], antithyroid drugs[4–7], fungicides[8], insecticide[9] and heavy metals[10–12]. One of the alleged thyroid-disrupting metals is hexavalent chromium (CrVI), it's widespread in the environment, naturally or anthropogenically by its improper disposal, resulting in its excessive usage in various industries including leather tanning, metallurgy and the production of paints and pigments[13,14]. The amounts of CrVI that have been dispersed through water, soil and air continue to increase in the last decades. Promoting its bioaccumulation in living beings by the route of contaminated food and water[15].

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CrVI induces cytotoxicity and mutagenicity by originating reactive oxygen species during its cellular reduction to trivalent chromium (CrIII)[16], which causes the inhibition of biomolecule synthesis and membrane transport[17].

CrVI and its compounds are classified as sensitizing agents and carcinogens[15,18]. Furthermore, they can exert hazardous effects on living beings, hematotoxic[19], hepatotoxic[20], nephrotoxic[21], reprotoxic[22] and genotoxic[23,24]. Since thyroid function can be affected as well by malnutrition[25] that can cause several deficiencies for instance iodine, zinc and selenium intake[26–29]. Thus, the availability of micronutrients and trace elements is the key to ensuring an appropriate thyroid hormone synthesis and activation[30,31].

Selenium is an essential trace element; it is incorporated in 50-70 selenoproteins encoded by 25 human genes[32]. The Se content in endocrine tissues is higher than in the other organs, it accumulates in the thyroid, adrenals testes and ovaries[33] and mainly in the pituitary gland, it deposits in somatotrophs, thyrotrophin, corticotrophs, and gonadotroph cells[34–36]. Selenoproteins play a definite role in the endocrine system; they are implicated in several physiological processes and biochemical pathways, for instance, spermatogenesis, thyroid hormone deiodination, the regulation of genomic transcription and protecting the cell from reactive oxygen species by peroxide degradation[37].

Zinc is found in all tissues, and it acts as a cofactor for more than 200 enzymatic systems[38]. It is a potent antioxidant, and it's required for many biological functions such as reproduction, fetal development, immunity, growth and DNA synthesis[39,40]. Zinc is known to play an integral role in the hormonogenesis of the thyroid-hypophysis-hypothalamus axis[41–43]. Thus, in the present work, we aimed to evaluate the deleterious effects of short-term exposure to different doses of potassium dichromate on thyroid hormonal and histological status in preimplanted *Wistar albinos* rats and the potential protective effect of selenium and zinc chloride against the thyrotoxicity induced by potassium dichromate.

2. Materials and Methods

2.1 Materials

Potassium dichromate ($K_2Cr_2O_7$, H_2O), selenium (Se) and zinc chloride ($ZnCl_2$) were purchased from Sigma Aldrich (Chemie Gmbh., Taufkirchen, Germany). All chemicals were dissolved in sterile saline (NaCl 0.9%) and the pH was adjusted when necessary to 7.5.

2.2 Animals

This study was carried out on healthy female *Wistar albinos* rats purchased from Pasteur Institute, Algiers, Algeria, weighing 180-250 g. All animals were maintained in polypropylene cages, under controlled conditions of light/dark (12/12 hours) and temperature (23±1˚C) and had free access to food and water. Female rats were caged with males overnight. A positive mating smear was taken as day zero of gestation. The standards established by the Institutional Animal Care and Use Committee at Batna university were followed.

Forty-two pregnant *Wistar albino* rats were allocated in seven groups of six each, they were housed separately from day zero of pregnancy.

All the treatments were performed on day 3 of pregnancy by subcutaneous route; each animal was anaesthetized with diethyl ether and weighed before each experiment.

- The control group was injected subcutaneously (sc) with a single dose of 0.3 mL/rat of NaCl 0.9%.
- The 2nd, 3rd and 4th groups were treated sc respectively by 10, 50 and 100 mg/kg, s.c. bw of $K_2Cr_2O_7$

The doses were chosen based on previous studies[19,44]. Based on our findings during the experiment, the lowest dose of $K_2Cr_2O_7$ (10mg/kg, s.c.bw) was chosen to be the one that affected the most the hormonal and the histopathological status of the thyroid glands of preimplanted rats. Thus, the remaining groups 5th, 6th and 7th were cotreated respectively by $K_2Cr_2O_7$ (10mg/kg, s.c. bw) and Se (0.3 mg/kg, s.c. bw)[19,45], or with $ZnCl_2$ (20mg/kg, s.c. bw)[46], or with the three of them simultaneously.

Blood samples were collected into heparinized tubes from the jugular vein for hormonal quantification on days, 6 and 20 of gestation. After sacrifice on the 20th day, thyroid glands were quickly excised[47], rinsed in 0.9% NaCl at 4˚C and immersed in 10% neutral buffered formalin for the histological study.

2.3 Methods

2.3.1 Measurement of Body Weight Gain

The body weight of each rat was recorded by measuring the weight (g) on the 3rd, 6th and 20th day of pregnancy.

2.3.2 Biological Evaluation

The thyroid gland was removed from each rat, cleaned with a normal saline solution (0.9 %), dried with filtration paper and weighed. Absolute thyroid weight was recorded.

2.3.3 Hormone Quantification

On the 6th and the 20th day of pregnancy, blood was collected in heparinized tubes and centrifuged at 1500 × g for 15 min at 4°C. Plasma samples were drawn and kept at 20°C until hormone analysis. Total plasma T3, T4 and TSH concentrations were measured using Electrochemiluminescent Immunoassay (ECLIA) tests using Roche Cobas E411 analyzer and commercial kits from Eleccsys and cobas e analyzers. Roche Diagnostics, Germany), according to the manufacturer's instructions.

2.3.4 Histopathological Examination

Thyroid glands were fixed previously in 10% neutral buffered formalin and processed for histological procedures. Samples were transferred through baths of graded ethanol, cleaned in xylene and embedded with paraffin. A microtome is used to cut 5µm thick tissue sections. Finally, paraffin wax sections were stained with hematoxylin and eosin[48]. Photographs were taken using a light microscope AXIOSKOP from (ZEISS Germany) provided with a digital camera (VIVIDIA, DOM300).

2.3.5 Caspase-3 Activity

The caspases are cysteine proteases that play an essential role in apoptosis. The assay is based on the detection of the chromophore p- nitroaniline (pNA) after cleavage from the labelled substrate, the acetylated tetrapeptide (Ac- DEVD)[49]. The measure of caspase 3 activity was performed using a commercially available kit (Promega). According to the manufacturer's instructions, thyroid homogenates were incubated alone with the Ac–DEVD- pNA that was added to a 96-well microplate for four hours. In the presence of active caspase 3, cleavage and release of pNA from the substrate occur and a yellow colouration appears that is detected by a microplate reader (BiotekFLX800) at 405 nm, one unit of caspase is the amount of enzyme required to cleave one pmol of substrate hydrolyzed per hour at 30˚C.

2.3.6 Statistical Analysis

Data obtained from hormone levels and body weight were expressed as mean ± SEM, n = 6 of seven independent experiments, statistical comparisons were carried out by a two-way ANOVA test and followed by Dunnett's test. Absolute body weight was expressed as mean ± SEM, analyzed by a one-way ANOVA test and followed by Dunnett's test. Caspase- 3 activity values were analyzed by a one-way ANOVA test and followed by Tukey's test.

All statistical analysis was performed using GraphPad Prism 7. The differences were considered significant when $p<0.05$.

3. Results

3.1 Hormonal Profile

3.1.1 The Effect of the Graded Doses of $K_2Cr_2O_7$ on Plasma TSH, T3 And T4 Levels in Wistar Albino Preimplanted Rats

All three doses of $K_2Cr_2O_7$ (10, 50 and 100 mg/kg, s.c) induced a significant difference in T3, T4 and TSH levels during the exposure period when compared with the control group.

The obtained results showed that the T3 plasma levels were decreased at the 6th and 20th days of gestation for the three doses ($P<0.001$), the lowest dose of $K_2Cr_2O_7$ (10 mg/kg, s.c) decreased the T3 plasma level when compared to the control (7.067±0.187 vs. 3.683±0.280) and (5.560±0.0323 vs. 3.873±0.153) at the 6th and 20th days respectively (Figure

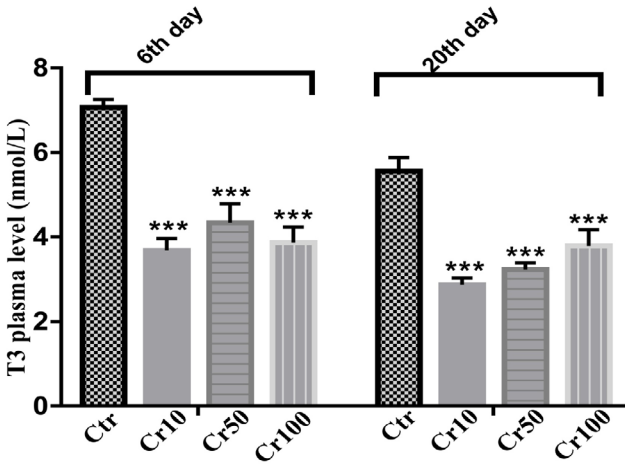


Figure 1. The mean level of serum Tri-Iodothyronine (T3) in pregnant *Wistar* rats exposed to $K_2Cr_2O_7$ at doses of 10, 50 and 100 mg/kg. Values are expressed as mean ± SEM (n=6).*$p < 0.05$, statistically significant compared to the control group.

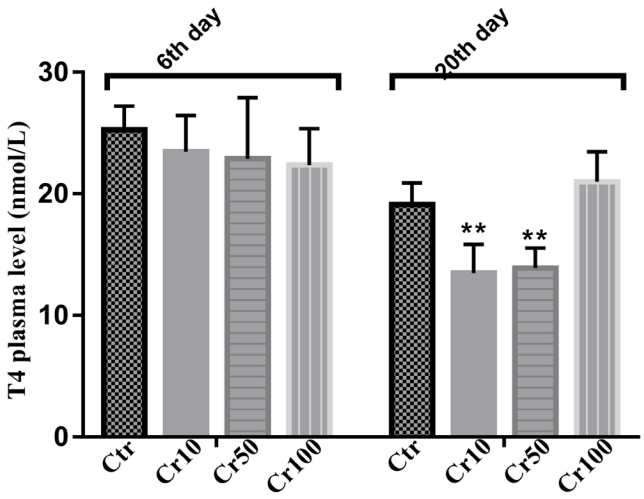


Figure 2. The mean level of serum thyroxin (T4) in pregnant *Wistar* rats exposed to $K_2Cr_2O_7$ at doses of 10, 50 and 100 mg/kg. Values are expressed as mean±SEM (n=6).*p < 0.05, statistically significant compared to the control group.

1). Similarly for the two other $K_2Cr_2O_7$ treated groups with 50 and 100 mg/kg, s.c, the plasma T3 concentration decreased significantly on the 6th day compared to the control (7.067±0.187 vs. 4.337±0.451) and (7.067±0.187 vs. 3.872± 0.366) respectively. On the 20th day, the reduction in the plasma T3 level remained statistically significant, (5.560±0.323 vs. 3.232±0.162) and (5.560±0.323 vs. 3.788±0.384) respectively (Figure 1).

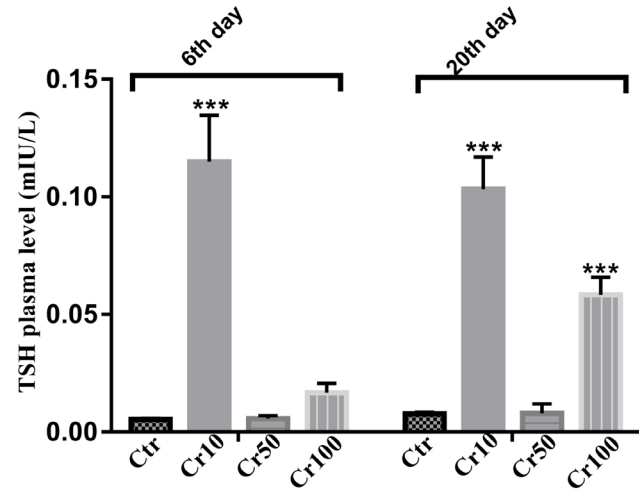


Figure 3. The mean level of serum TSH in pregnant *Wistar* rats exposed to $K_2Cr_2O_7$ at doses of 10, 50 and 100 mg/kg. Values are expressed as mean±SEM (n=6).*p < 0.05, statistically significant compared to the control group.

The plasma T4 levels were decreased significantly (p<0.01) only on the 20th day of pregnancy for both doses of $K_2Cr_2O_7$ (10 and 50 mg/kg, s.c), (19.115±0.720 vs. 13.478±0.967) and (19.115±0.720 vs. 13.885±0.0676) respectively. The highest dose of $K_2Cr_2O_7$ (100 mg/kg, s.c), had no effect (Figure 2).

Conversely to the decrease of thyroid hormones level, the level of the plasma TSH was increased significantly (P<0.001) at the 6th (0.005±0.0002 vs. 0.115±0.008) and 20th (0.008±0.0003 vs. 0.103±0.006) days of pregnancy with 10 mg/kg, s.c of $K_2Cr_2O_7$. This increase in plasma TSH level was maintained with the highest dose (0.008±0.0003 vs. 0.0058±0.003) on day 20 of gestation (P<0.001) (Figure 3).

3.1.2 The Potential Protective Effect of Se and $Zncl_2$ In $K_2Cr_2O_7$ Treated Wistar Albino Preimplanted Rats on Plasma T3, T4 and TSH Levels

The administration of $K_2Cr_2O_7$ (10 mg/kg s.c) in combination with Se (0.3mg/kg, s.c), induced on the 6th day of pregnancy a significant increase (P<0.01) in plasma T3 level compared to the $K_2Cr_2O_7$ treated group (3.683±0.280 vs. 4.657±0.129) (Figure 4).

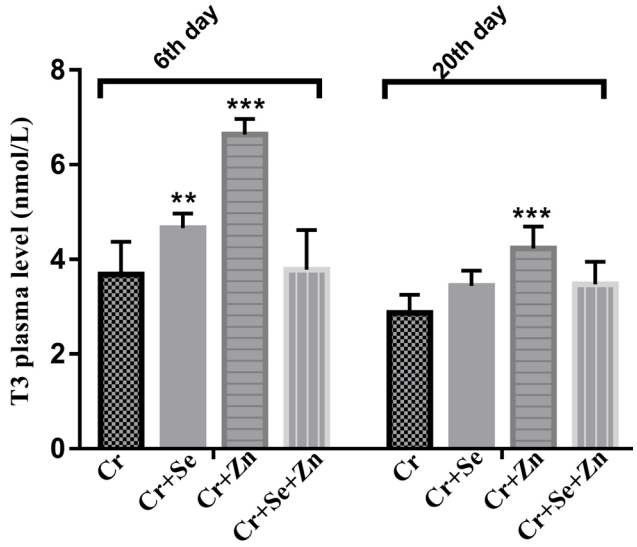


Figure 4. The effect of the administration of both Se (0.3 mg/kg) and $ZnCl_2$ (20 mg/kg) on the level of serum T3 in pregnant Wistar rats exposed to $K_2Cr_2O_7$ at a dose of 10 mg/kg. Values are expressed as mean±SEM (n=6).*p < 0.05, statistically significant compared to the $K_2Cr_2O_7$-treated group.

Likewise, a significant increase in the plasma T4 level was noted (P <0.01) on the 20th day of pregnancy (13.478±0.967 vs. 17.062±0.179) (Figure 5).

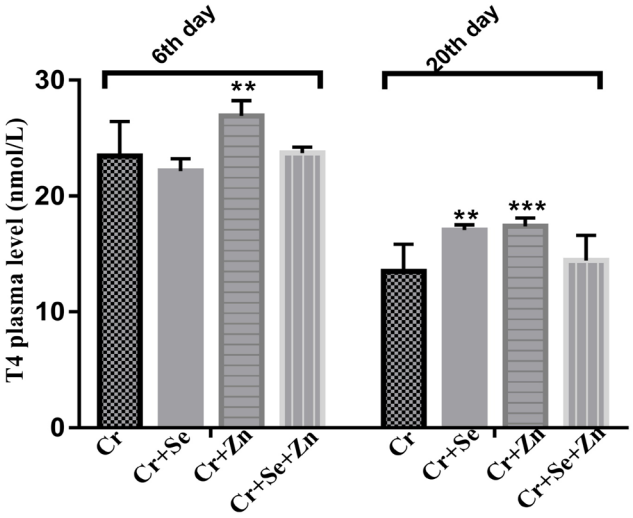


Figure 5. The effect of the administration of both Se (0.3 mg/kg) and $ZnCl_2$ (20 mg/kg) on the level of serum T4 in pregnant *Wistar* rats exposed to $K_2Cr_2O_7$ at a dose of 10 mg/kg. Values are expressed as mean±SEM (n=6). *p < 0.05, statistically significant compared to the $K_2Cr_2O_7$ treated group.

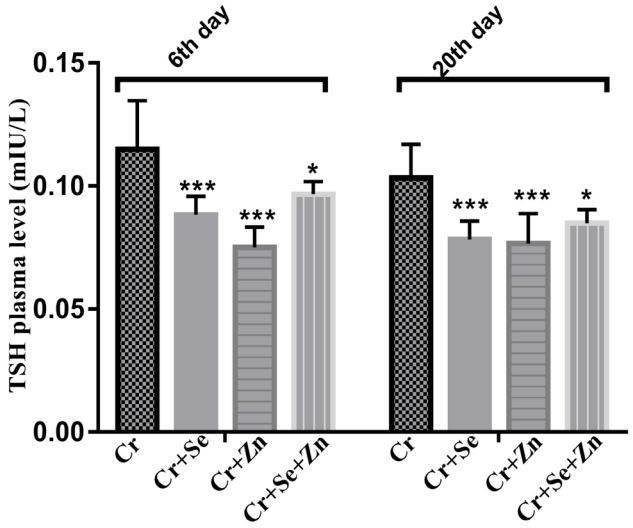


Figure 6. The effect of the administration of both Se (0.3 mg/kg) and $ZnCl_2$ (20 mg/kg) on the level of serum TSH in pregnant *Wistar* rats exposed to potassium dichromate at a dose of 10 mg/kg. Values are expressed as mean±SEM (n=6). *p < 0.05, statistically significant compared to the $K_2Cr_2O_7$ treated group.

On the other hand, the cotreatment with Se has significantly (P<0.001) decreased the level of plasma TSH, its level changed significantly on day 6 (0.115±0.008 vs. 0.088±0.003) and 20 (0.103±0.006 vs. 0.078±0.003) (Figure 6).

In addition, $ZnCl_2$ (20 mg/kg, s.c) administered in combination with $K_2Cr_2O_7$ (10 mg/kg, s.c) has enhanced significantly (P<0.001) the hypothyroidism induced by the $K_2Cr_2O_7$ exposure, the plasma T3 level at the 6th (3.683±0.280 vs. 6.638±0.134) and at the 20th day (2.873±0.153 vs. 4.227±0.19) (Figure 4).

Furthermore, the plasma T4 level was also increased in a significant way on the 6th (P<0.01) and the 20th (P<0.001) day of pregnancy (23.468±1.211 vs. 26.907±0.544) and (13.478±0.967 vs. 17.375±0.302) (Figure 5). Additionally, a significant decrease (P<0.001) in plasma TSH level was observed on the 6th and 20th day of pregnancy (0.115±0.008 vs. 0.075±0.003) and (0.103±0.006 vs 0.077±0.005) respectively compared to $K_2Cr_2O_7$ (10 mg/kg, sc.) (Figure 6).

The simultaneous administration of Se and $ZnCl_2$ decreased the TSH plasma level on the 6th (0.115±0.008 vs. 0.097±0.002) and 20th day (0.103±0.006 vs. 0.085±0.002) in a significant way (P<0.05). On the other hand, the combination of Se and $ZnCl_2$ didn't improve significantly the plasma levels of T3 and T4 even though; the two elements play an essential role in thyroid hormone synthesis. Based on our results demonstrated in Figures 4 and 5, the concentration of T3 and T4 increased but the differences stayed insignificant when determined on the 6th and 20th day (Figure 6).

3.2 The Evaluation of Body Weight Gain and Thyroid Absolute Weight in $K_2Cr_2O_7$ Treated Groups (10, 50 and 100 mg/kg, s.c) and Cotreated Groups with Se and $ZnCl_2$

3.2.1 Body Weight Gain

All the rats that survived throughout the experimental period were weighed on the 3rd, 6th and 20th day of gestation. All three groups treated with the graded doses of $K_2Cr_2O_7$ (10, 50 and 100 mg/kg, s.c) when compared with the control, provoked a significant decrease in body weight (P<0.001) (Table 1).

The cotreatment with Se or with $ZnCl_2$ induced an elevation in body weight gain when compared with $K_2Cr_2O_7$ treated group (10mg/kg, s.c) on the 3rd, 6th and 20th day of the experimentation. In Se cotreated group, the body weight

Table 1. Effects of $K_2Cr_2O_7$ with 10, 50 and 100 mg/kg.sc on body weight and thyroid absolute weight in pregnant rats exposed to $K_2Cr_2O_7$ (10 mg/kg) at the 3rd, 6th and 20th gestational days

Groups	Days	Body weight (g)	Thyroid absolute weight (g)
Ctr (0.3 ml/rat)	3rd day	241.5±8.318	
	6th day	246.333±6.965	
	20th day	299.5±7.469	0.128±0.01
Cr (10 mg/kg, sc.)	3rd day	183.833±3.790***	
	6th day	190.333±2.512***	
	20th day	222.5±2.232***	0.169±0.007**
Cr (50 mg/kg, sc.)	3rd day	197.5±2.335***	
	6th day	201±3.357***	
	20th day	233.667±6.339***	0.154±0.004
Cr (100 mg/kg, sc.)	3rd day	186.5±10.155***	
	6th day	196.167±8.685***	
	20th day	226.667±12.617***	0.171±0.009**

Values are mean±SEM, n = 6. *P<0.05 considered significant, Dunnett test.

Table 2. Potential protective effects of Se and $ZnCl_2$ on body weight and thyroid absolute weight in pregnant rats exposed to $K_2Cr_2O_7$ (10 mg/kg) at the 3rd, 6th and 20th gestational days

Groups	Days	Body weight (g)	Thyroid absolute weight (g)
Cr (10mg/kg, sc.)	3rd day	183.833±3.790	
	6th day	190.333±2.512	
	20th day	222.5±2.232	0.169±0.007
Cr +Se (0.3mg/kg, sc.)	3rd day	219±11.027*	
	6th day	222±10.320*	
	20th day	270±10.564**	0.159±0.008
Cr +$ZnCl_2$ (20mg/kg, sc)	3rd day	222.5±12.902*	
	6th day	217±11.237	
	20th day	270.167±5.706***	0.178±0.003
Cr +Se (0.3mg/kg, sc.) +$ZnCl_2$ (20mg/kg, sc.)	3rd day	233.167±10.499**	
	6th day	241±9.363**	
	20th day	271.33±13.134**	0.149±0.005

Values are mean±SEM, n = 6. *P<0.05 considered significant, Dunnett test.

gain passed from 183.833±3.790 to 219±11.027 on the 3rd day, 190.333±2.512 to 222±10.320 on the 6th (P<0.05) and from 222.5±2.232 to 270±10.564 (P<0.01).

For the $ZnCl_2$ cotreated group, it changed from 183.833±3.790 to 222.5±12.902 on the 3rd (P<0.05) and from 183.833±3.790 to 270.167±5.706 on the 20th day (P<0.01). The concomitant administration of Se and $ZnCl_2$ showed a significant increase in body weight gain in pregnant rats (Table 2).

3.2.2 Thyroid Absolute Weight

Our results clearly showed a significant increase in absolute thyroid weight in pregnant rats treated with 10 and 100 mg/kg, s.c of $K_2Cr_2O_7$ when compared to the control (P<0.01) (Table 1 above).

Absolute thyroid gland weight remained statistically insignificant when compared to the 10 mg/kg, s.c group in the cotreated groups with only Se, $ZnCl_2$ or both of them (Table 2 above)

3.3 The Evaluation of the Potential Protective Effect of Se and $ZnCl_2$ on the Caspase 3 Activity

Caspase 3 is one of the executioner caspases; it participates in the proteolysis process that leads to apoptosis. Results showed that caspase 3 activity was elevated in thyroids of $K_2Cr_2O_7$ (10mg/kg, s.c) group compared to the control (0.319±0.007 vs. 0.714±0.058) (P<0.001). Moreover, our data revealed that the co-treatment with Se (0.714±0.058 vs. 0.482±0.035) (P<0.05), $ZnCl_2$ (0.714±0.058 vs. 0.454±0.0016) or simultaneously with them both (0.714±0.058 vs. 0.473±0.072) has ameliorated the apoptotic rate by decreasing the caspase 3 activity compared to $K_2Cr_2O_7$ treated group (P<0.01) (Figure 7).

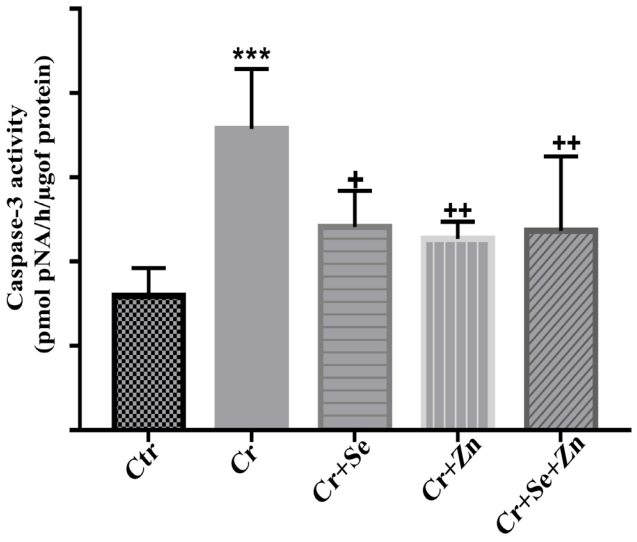


Figure 7. The effect of the administration of $K_2Cr_2O_7$ (10 mg/kg) on the caspase-3 activity in the thyroid of pregnant *Wistar* rats and the potential protective effect of Se and $ZnCl_2$ against apoptosis induction. Values are expressed as mean ± SEM (n=6).
* $p < 0.05$.

3.4 The Histopathological Study

To evaluate the histological changes caused by the different doses of $K_2Cr_2O_7$ and the potential protective effect of Se and $ZnCl_2$ in restoring the thyroid gland histoarchitecture, we used HE staining. The microscopic analysis of the thyroid slices from the control group demonstrated normal thyroid parenchyma composed mainly of follicles of variable sizes with the predominance of the larger ones (macro follicles), filled with eosinophilic colloid composed mostly of thyroglobulin and surrounded by a monolayer of flattened epithelium lining composed of thyrocytes. The interfollicular space was thin and contained connective tissue with capillaries beds and clustered parafollicular cells. The epithelial cell height was normal (Figure 8 (A1, A2)).

In $K_2Cr_2O_7$ treated groups with the increased doses (10, 50 and 100 mg/kg, s.c), the thyroid parenchyma appeared less well organized in the three treated groups, it was characterized by the predominance of the micro follicles over the larger-sized ones, the desquamation of the epithelial lining and disruption of the follicular walls led to the collapse of many follicles, their colloid matter was filled by exfoliated cells, especially in 10 mg/kg, s.c of $K_2Cr_2O_7$ treated group (Figure 8 (B1, B2)). The epithelial cells became hyperplasic and vacuolated, the colloid was diluted and reduced remarkably and the presence of the resorption vacuoles was evident at the periphery of follicles. The connective tissue was disintegrated, the follicular area was reduced and the interfollicular space was massively enlarged (Figure 8 (B1, B2, C1, C2, D1, D2)).

In Se $K_2Cr_2O_7$ treated group, the histopathological findings showed an improvement in restoring the normal architectural pattern of the thyroid gland, the follicles were rather large and filled with rich colloid, they were lined with a flat epithelium and the interfollicular space wasn't apparent. However, there are some follicles where the lumen is filled with squamous cells and micro follicles with slightly vacuolated epithelial cells (Figure 9 (E1, E2)).

In $ZnCl_2$, $K_2Cr_2O_7$ treated group, the microscopic analysis showed that apart from the presence of some vacuoles at the edges of the colloidal matter, the administration of $ZnCl_2$ has almost restored the thyroid histoarchitecture of preimplanted *Wistar* rats exposed to $K_2Cr_2O_7$ (Figure 9 (F1, F2)).

In the Se, $ZnCl_2$, $K_2Cr_2O_7$ treated group, the thyroid slices showed the presence of micro follicles, their epithelial cells height was increased and the colloid was exceedingly vacuolated. The collapsed follicles merged with neighbouring ones due to the desquamation of the epithelial lining. Whereas the administration of the $ZnCl_2$ or the Se alone in the thyroid of pregnant rats exposed to $K_2Cr_2O_7$ had a protective effect, the administration of both of them didn't prevent the deleterious effects of chromium on thyroid architecture based on our findings (Figure 9 (G1, G2)).

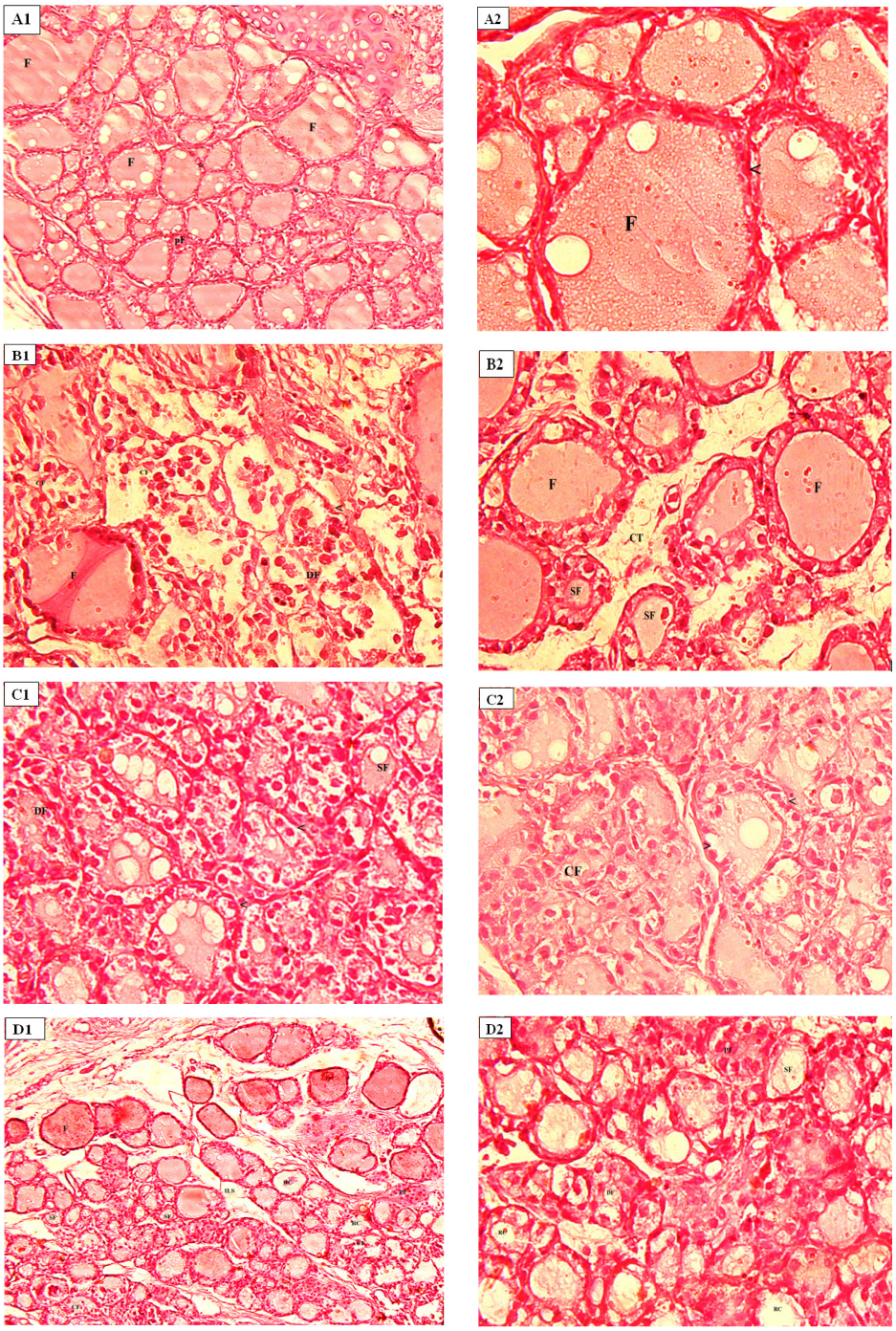


Figure 8. Photomicrographs of thyroid gland sections of the control group (A1×100, A2×400), $K_2Cr_2O_7$ (10, 50 and 100 mg/kg) respectively (B1, B2, C1, C2, D2 ×400, D2×100) stained with Haematoxylin-Eosin showing the thyroid parenchyma composed of macro Follicles (F), Microfollicles with swollen epithelial cells (SF), Parafollicular cells (pF), connective tissue (asterisk), Reduced Colloidal matter (RC), squamous hyperplasic epithelial cells (arrowhead), wide Interfollicular Space (IFS), the dissolution of the Connective Tissue (CT), Degenerated Follicles filled with squamous cells (DF), collapsed and scattered follicles with exfoliated epithelium (CF), follicles filled with exfoliated epithelial cells (arrow).

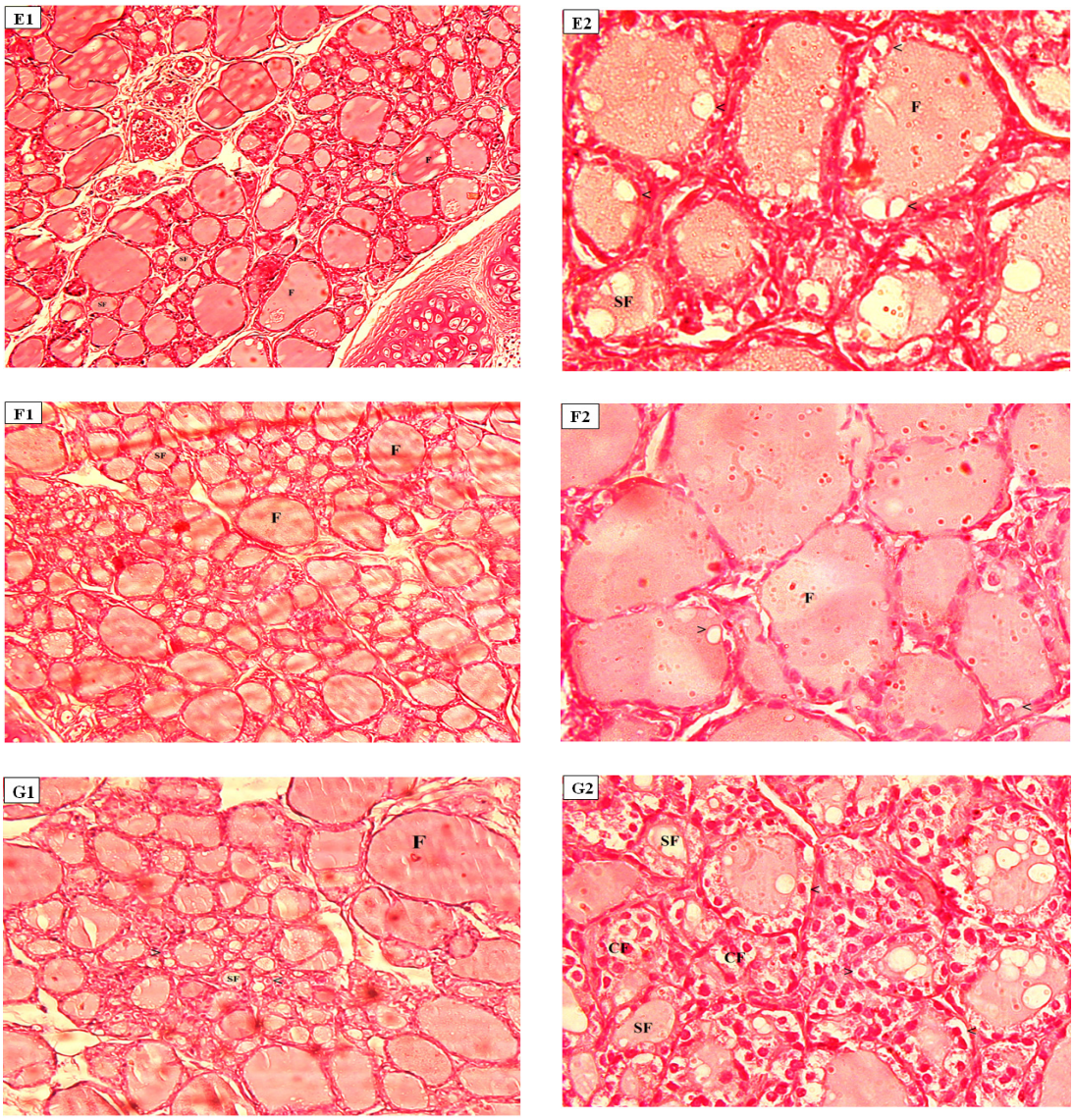


Figure 9. Photomicrographs of thyroid gland sections of the Se+ $K_2Cr_2O_7$ (10 mg/kg) treated group (E1×100, E2×400), $ZnCl_2$+ $K_2Cr_2O_7$ (10 mg/kg) treated group (F1×100, F2×400), Se+ $ZnCl_2$+ $K_2Cr_2O_7$ (10 mg/kg) treated group (G1×100, G2×400), stained with H&E, showing the presence follicles of variable sizes (F) with peripheral resorption vacuoles in their colloid (arrowhead) and epithelial cells (arrowhead), micro follicles (SF), follicles filled with exfoliated epithelial cells (arrow), Collapsed Follicles (CF) and large Interfollicular Space (IFS).

4. Discussion

The current study highlighted firstly the disruptive effects of potassium dichromate on thyroid function in preimplanted *Wistar albinos* rats under subcutaneous exposure. Secondly, we tested the potential preventive effect of both selenium and zinc chloride in retrieving thyroid homeostasis.

The results of the hormonal profile TSH, T3 and T4 revealed that the subcutaneous exposure to $K_2Cr_2O_7$ provoked peripheral hypothyroidism as indicated by a significant increase in plasma TSH and a significant decrease in plasma T3 and T4 levels, even though during gestation the thyroid hormonal status witness hyperthyroidism to accommodate the presence of the fetal and placental tissues. Multiple related studies have shown that $K_2Cr_2O_7$ caused

hypothyroidism by disturbing the thyroid hormones and TSH levels in *Wistar albinos* male rats[50,51]. Other similar studies were conducted to investigate the contribution of the maternal thyroid during pregnancy, they reported that tributyltin has significantly reduced the T3 and T4 levels in dams[52]. Likewise, male pups from mothers treated with tributyltin showed reduced serum T4 levels[52,53], others also stated that cadmium caused a decrease in the T4 level and a non-significant increase in serum TSH concentration[54]. This might be explained, according to previous studies, by the fact that hexavalent chromium overt its toxicity by generating reactive oxygen species through its intracellular reduction, the ROS lead to oxidative deterioration of macromolecules and DNA damage by inducing DNA strand breaks, chromium DNA adducts and alteration of gene expression and activation of apoptotic cell death[23,55,56]. Indeed, thyrocytes are supposed to be highly sensitive to disruptions in the Endoplasmic Reticulum (ER) homeostasis due to their secretory function. Therefore, the expression of genes involved in thyroid hormone synthesis and their transcriptional regulators are down-regulated in thyrocytes during ER stress-causing hormone synthesis depletion[57].

Additionally, another study has demonstrated the toxic effect of $K_2Cr_2O_7$ on the anterior pituitary gland. Thus, affecting the hypothalamic-pituitary axis and the proper functioning of the endocrine system[58]. The reduction of the T4 plasma level observed in this study can be attributed to the inhibition of thyroid synthesis of this hormone. Thyroid hormonogenesis can be altered in iodine deficiency, the decrease in the intrathyroidal iodine pool by impairing its uptake by the thyroid gland was reported in female Wistar rats after the treatment with the methimazole[4]. It was indicated in several studies that methimazole exhibits hypothyroidism by either inhibiting the sodium iodide symporter[59]. Or by depleting the enzymatic activity of Thyroid Peroxidase (TPO) in catalyzing the incorporation of iodide into tyrosine residues on thyroglobulin[60]. In addition to that, Song *et al*., found that several chemicals have disturbed the human TPO activity in vitro[61]. Moreover, a significant number of chemicals including Polychlorinated Biphenyls (PCB) and bisphenol tend to bind with thyroid hormone-binding proteins with a high affinity[62,63]. Furthermore, both TPO and Dual Oxidase (DUOX) are inactivated by the increased amounts of H_2O_2 generated via oxidative stress, causing a conformational change and leading to a less active form of the enzymes[64]. As well, cadmium, another thyroid-disrupting heavy metal, has altered gene expression related to the hypothalamic pituitary thyroid axis by upregulating TSH and thyroglobulin mRNA and downregulating the expression of both thyroid hormone receptors ThRβ and ThRα in fish[65]. Besides, many authors suggested that cadmium influences the production and /or the secretion of T4 by the induction of oxidative phosphorylation disorders in the mitochondria of thyrocytes[66–68]. Hence, the declined plasma T3 levels demonstrated by our results indicate that $K_2Cr_2O_7$ influences negatively the enzymes implicated in the extrathyroidal metabolism of thyroid hormones such as the 5' monodeiodinases (5'DI) which catalyzes the conversion of T4 to the active T3. This can be confirmed by previous investigations on rats and mice treated with cadmium[69,70]. Consequently, the inhibition of (5'DI) is associated with cadmium-induced lipid peroxidation which affects the cell membrane integrity including the enzyme's binding sites[69]. Although their beneficial anti-inflammatory and antioxidant activity; several studies have shown that flavonoids inhibit the T4 5'deiodinase activity in vivo[71], thyroid type 1 iodothyronine deiodinase activity[72] and by the displacement of T4 from its receptor[73].

In our study, we demonstrated that the lower dose of $K_2Cr_2O_7$ has induced significant toxic effects on the thyroid in preimplanted rats than the higher doses; these results agreed with the fact that the endocrine disruptors may exert more potent effects at low doses[74].

In contrast, the cotreatment with selenium and zinc chloride has enhanced remarkably the thyroid hormonal status of preimplanted rats exposed to K_2Cr_2O7 (10mg/kg, s.c). Indeed, the co-administration of selenium alone, zinc chloride alone, or simultaneously with both of them in $K_2Cr_2O_7$ treated rats have revealed more or fewer values near to that of the control group. The plasma TSH level decreased, and the T3 and T4 levels increased back again significantly. This improvement can be explained by the fact that selenium is a key trace element that takes a major part in maintaining thyroid homeostasis. Since our results suggested that the subcutaneous administration of $K_2Cr_2O_7$ decreased the T3 level, the cotreatment with Se enhanced T3 plasma concentration through an upregulation in Deiodinase I (DIOI) activity and an increase in T4 conversion[75]. However, both animal models and human studies confirmed that low selenium intake causes altered DIOI activity; this latter implicates Se as a nucleophilic atom in the enzyme active site[27,76,77]. Therefore, the low Se status has been related to thyroid depletion in humans[78], rats[79] and mice[80]. Thus, alterations in the quantity of deiodinase mRNA in rat liver caused DIOI activity impairment and thyroid dysfunction[76].

Further to this, when Se was supplemented to the diet of MMI-treated rats, the T4 plasma level was ameliorated partially, while the T3 level was returned to normal[4]. This concurs with the fact that Se participates actively in the antioxidant system. It's incorporated in many Se-dependent antioxidant enzymes such as thioredoxin reductase and glutathione peroxidases that protect thyrocytes from oxidative damage[81]. When ROS are produced at high levels, cellular components are damaged. Indeed, glutathione peroxidase is associated with peroxisomal catalase in H_2O_2 degradation, protecting thyroid cells from ROS attack[82]. The study conducted by Hala, Ibrahim and Hemmat suggested that selenium nanoparticles may be considered exogenous scavengers of ROS formed in the thyroid after CrVI exposure[83].

Analogously, $ZnCl_2$ cotreatment in $K_2Cr_2O_7$ preimplanted rats has demonstrated that $ZnCl_2$ has improved the plasma TSH, T3 and T4 levels significantly. This could be explained by the indispensability of zinc, not only for the activity of enzymes that convert T4 to its active form through its antioxidant properties but also for the synthesis of TSH in the anterior hypophysis and that of TRH in the hypothalamus[41,43,84]. As well, it's required for the T3 receptor to adopt its biologically active confirmation, that's why some of the effects of zinc depletion are due to the loss of Zn from the nuclear receptor which provokes the alteration of the T3 function[28], in addition, zinc therapy has improved the thyroid function in Down patients by influencing the pituitary–thyroid axis[85].

It is well known that thyroid hormones promote growth. Results from the body weight indicated a significant decrease on the 6th and 20th day of gestation since the treatment with chromium-induced hypothyroidism. On the other hand, thyroid weight has increased significantly compared with the control group, this change was correlated with the hypertrophy of the gland and the epithelial cells when examined histopathologically, several thyroid-disrupting chemicals have caused an increase in thyroid absolute weight[4,86,87]. Se cotreatment has reversed the thyroid absolute weight and body weight gain after the $K_2Cr_2O_7$ exposure, these findings are in agreement with the results of Ben Amara *et al*., (2009) who found that Se supplementation promoted a partial recovery in body and thyroid gland weights and plasma TSH levels of both mothers and pups[4]. It may be the case that selenium concentration was inversely associated with goitre and thyroid volume[77].

On the other hand, the results of the histological examination of thyroids of rats treated with 10, 50 and 100 mg/kg, s.c showed that $K_2Cr_2O_7$ has altered strongly the histoarchitecture, of the gland compared with the control group, the thyroid parenchyma appeared highly disorganized, with unevenness in the diameter and form of the follicles in both, the central or the peripheral regions of the gland.

The presence of the micro follicles was preponderant than the larger ones, these changes were common in the glands treated with the three doses of $K_2Cr_2O_7$. These findings were from other studies[12]. In addition, the desquamation of the epithelial lining of the follicles was noticed, which led to the disruption of the follicular walls and provoked their collapse. The colloid that serves as a pool for thyroid hormones seemed to be diluted and reduced considerably, vacuolated and filled by exfoliated cells. These alterations were more pronounced in the 10 mg/kg, s.c $K_2Cr_2O_7$ treated group and coincided with the results reported by ElBakry and Tawfik[50]. Owing to the degeneration and the collapse of follicles and the reduction of the follicular area, the interfollicular space has been enlarged massively. This widening has been seen in the thyroid of male, non-pregnant rats and mice, respectively treated with potassium dichromate[83], aluminum[88] and cadmium[54]. Furthermore, another study was conducted to investigate the toxic effects of chromium on the thyroid, disclosing the same histological alterations cited above, the presence of irregularity, less well-ordered and collapsed follicles were observed[51]. The apoptotic cells that have been exfoliated and discarded in the colloid. Afterwards, they have being removed by macrophages[89]. As regard thyrocytes, they appeared hyperplasic and highly vacuolated and filled with resorption droplets, their height seemed to be increased in particular in the 10 mg/kg, s.c $K_2Cr_2O_7$ treated group, as reported by Hala *et al*., and Khalaf *et al*[83,90]. Thyrocyte hyperplasia may be related to oxidative stress, DNA damage and modulation of the apoptotic regulatory gene p53 caused by hexavalent chromium toxicity[91]. It's more likely also that the enlargement of thyroid cells results from the increased circulating plasma TSH level to compensate for the peripheral hypothyroidism induced by $K_2Cr_2O_7$ exposure as revealed by our study, it leads to thyrocytes hypertrophy and hyperplasia[92]. The measurement of the caspase3 activity in thyroids revealed that the administration of 10 mg/kg, s.c of $K_2Cr_2O_7$ has increased significantly the caspase 3 activity which confirms the apoptotic properties of CrVI compounds. These results are from a recent study carried out by Elbakry and Tawfik (2014), by detecting apoptosis in thyroids exposed to $K_2Cr_2O_7$, by evaluating the Bcl2 proteins that were shown to be downregulated leading

to programmed cell death[50]. The detection of apoptosis in the thyroid of pregnant rats has been explained by several studies[56]. They declared that the chromium toxicity mechanism is based on inducing apoptosis, raising the mitochondrial cytochrome C and upregulated proapoptotic molecules[55]. Other researchers clarified that Cr (VI) tend to bioaccumulate in the hypothalamus and pituitary gland engendering apoptosis through the caspase 3 pathway[93]. Thus exerting a hazardous impact on the endocrine function of the hypothalamic-pituitary axis.

Conversely, the histological improvement in the thyroid can be explained by Zn and Se antioxidant properties, Se is implicated also in glutathione peroxidase[94], which protects thyrocytes from cellular oxidative stress by regulating cellular redox and transcription[37]. Se regulates the concentration of H_2O_2 in the follicular lumen. In the case of Se deficiency,

the apoptotic response to H_2O_2 increases[95]. Similarly, Zn enhances the antioxidant defences of the organism[96], it actively inhibits the propagation reactions of free radicals by firstly, promoting the synthesis of metallothionein[97] and secondly, by enhancing the dismutation reaction catalyzed by the superoxide dismutase[98]. Indeed, Zn can prevent the oxidation of the cell membrane components by occupying the cellular binding sites of prooxidants[96]. Thirunavukkarasu and Sakthisekaran (2001) reported that Se has antiperoxidative effects and preventive capacity against cancer[99]. Additionally, our results are those from other studies that showed the preventive impact of selenium in thyroid morphological alterations[4,83].

5. Conclusion

In conclusion, the present study revealed that the subcutaneous exposure to $K_2Cr_2O_7$ in preimplanted rats altered the plasma TSH, T3 and T4 levels. Besides that, our findings indicated that $K_2Cr_2O_7$ has impaired thyroid histoarchitecture and absolute weight and decreased body weight gain, it induced apoptosis via the activating the caspase 3 pathway. These toxic effects exerted by $K_2Cr_2O_7$ have been counterbalanced by selenium and zinc chloride cotreatment. Our results suggested that selenium and zinc have thyro-protective properties against the deleterious effects of $K_2Cr_2O_7$ in preimplanted *Wistar albinos* rats.

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