Original Research

Toxicology International, Vol 26(1&2), 54–63, 2019, DOI: 10.18311/ti/2019/v26i1&2/24438

ISSN (Print): 0971-6580
ISSN (Online): 0976-5131

Potential Protective Effects of Selenium and Zinc against Thyroid Toxicity Induced by Nickel in Preimplanted *Wistar* Albino Rats

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Abstract

Nickel is a heavy metal that may affect the function of the thyroid. The present study was undertaken to assess the protective effects of selenium and zinc against nickel-induced thyrotoxicity in preimplanted *Wistar* albino rats. Nickel is administered alone (100 mg/kg body weight) subcutaneously or in combination with selenium (0.3 mg/Kg body weight) either with zinc (20 mg/Kg body weight) or combined with both selenium and zinc. All groups of rats were injected on day 3 of gestation in the pre-implantation period. Exposure of rats to nickel caused a significant decrease in maternal body weight on days 6 and 20 of gestation and an increase in absolute and relative thyroid weight compared to the controls. Nickel treatment was also led in decreased plasma triiodothyronine, thyroxine with a concomitant significant increase in thyroid-stimulating hormone levels when compared to control. These changes were confirmed by histological damages. However, Co-administration of selenium and/or zinc to nickel-treated rats restored the hormonal and histological aspects. Our findings suggested that selenium and zinc ameliorated nickel induced thyroid damage.

Keywords: Nickel, Preimplanted Rat, Thyroid Hormones, Selenium, Zinc

1. Introduction

Nickel is the 24th most abundant element in the earth's crust[1]. It has been extensively used in electroplating for manufacturing batteries and steel. Human exposure to nickel occurs primarily via inhalation, ingestion, and dermal[2]. This element is transported by blood and is retained by various tissues or excreted, mainly through urine, making thus the kidney the most target organ of nickel toxicity and carcinogenicity[3]. Nickel and nickel compounds have long been recognized to cause adverse health effects including hematotoxicity, neurotoxicity, hepatotoxicity, nephrotoxicity, genotoxicity, reproductive toxicity and an increase in the risk of cancer[4–6]. Indeed, nickel has been categorized as a carcinogenic heavy metal by the World Health Organization's International Agency for Research on Cancer[7]. The main hypothesized carcinogenic mechanisms of nickel are oxidative stress, genomic DNA damage, and epigenetic effects[8]. The thyroid gland regulates the body's metabolic rate and plays an exquisitely important role in human health[9]. Little is known about the toxic effects of nickel on the thyroid gland. Previous studies reported both structural and functional disturbances to the thyroid glands of rats and rabbits exposed with nickel[10–12]. However, as it appears through the available published literature, the co-effect of the two trace elements on nickel-caused thyrotoxicity is not yet studied.

Selenium is an essential trace element, which plays an important role in antioxidant defense systems. Furthermore, it is necessary for fertility, growth, and immunity in humans and animals[13]. Moreover, selenium was shown to be an integral component of various enzymes such as glutathione peroxidases, deiodinases, thioredoxin reductases, and other selenoproteins[14]. Several previous studies have shown that selenium could interact with other toxic ions in plants and animals[15,16]. It is well documented that selenium can prevent the harmful effects of nickel in pregnant rats[17,18].

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Zinc is a known fundamental component of the endogenous enzymatic antioxidant system and plays an essential role in cell membrane integrity and functions in many aspects of cellular metabolism[19,20]. It has encompassed a protective biological role against free radical damage through maintaining an adequate level of metallothioneins, being an essential component of Cu, Zn-superoxide dismutase (Cu, Zn-SOD), or as a protective agent of thiols and other chemical groups[19]. Indications of biological antagonism between zinc and nickel, as evidenced by the reduction of toxic effects of nickel have been reported earlier[21,22].

The present study was undertaken to evaluate the effect of subcutaneous exposure of nickel on the hormonal alterations and histological changes in the thyroid of the pre-implanted rats. We also examined the possibility that pretreatment with selenium and zinc could impair nickel toxicity.

2. Materials and Methods

2.1 Animals

All experimental protocols conducted on rats were performed in accordance with the standards established by the Institutional Animal Care and Use Committee at Batna University. Adult Female albino *Wistar* rats (Pasteur Institute, Algiers, Algeria) were housed under a lighting schedule of 12 h light: 12 h darkness at 23 ± 1°C with free access to food and water. Females (150–350 g b. wt) were caged with males overnight. In the morning, female rats with a positive vaginal lavage were accepted into the study and recorded as day 0 of gestation. The animals were tested under the experimental conditions on day 6 and day 20. Pregnant females were housed in cages with six rats per cage. The average duration of gestation in the breeding colony was 21 days[23].

2.2 Chemicals and Reagents

Nickel chloride hexahydrate (Ni:6H_2O), Zinc chloride (Zn) and elemental Selenium (Se) were purchased from Sigma-Aldrich (Chemie Gmbh., Taufkirchen, Germany). All other chemicals and reagents used in the study were of analytical grade.

2.3 Experiments

Pregnant rats were randomly divided into five groups of six animals each. Control and treated groups were used on day 3 of the pregnancy. Each animal was anesthetized with diethyl ether and was weighed before each experiment.

The first group was served as control received a subcutaneous (s.c) injection of sterile saline 0.9% NaCl. The second group nickel was dissolved in sterile saline (NaCl 0.9%) and was given at a dose of 100 mg/kg body weight. The chosen doses of nickel (Ni) administered s.c. to pregnant rats were based on the previous study[24]. The third group (Ni+Se) Se was given as an s.c. injection (0.3 mg/kg body weight) together with the dose (100 mg/kg) of Ni. This dose of Se was chosen based on our previous study Adjroud[24], as well as other previous studies[17]. The Fourth group (Ni+Zn) was given zinc as an s.c. at a dose of (20 mg/kg body weight), and Ni at a dose of (100 mg/kg). Rats in the five groups (Ni+Se+Zn) were treated with Ni, Se, and Zn.

2.4 Body and Organ Weight

At the end of the experiment, the rats were dissected; the thyroid glands were removed and weighed.

Absolute and relative organ weights were calculated as:

Absolute weight of organ (g) = mean of organ weight for each group.

Relative weight of organ (g/100 g body weight) = mean of organ weight for each group/body weight × 100.

2.5 Sample Preparation

Blood samples were collected in heparin from the jugular vein on day 6 (2 days after treatment) and day 20 of gestation. The plasma was separated by centrifugation of blood at 3000 rpm for 15 min and stored at −20°C until hormonal analysis.

Rats were then sacrificed on the day 20 of gestation under anesthesia and the thyroid glands were collected, weighed and fixed in the formaldehyde in order to carry out histological sections.

2.6 Hormonal Analysis

The quantification of triiodothyronine (T3), tetraiodothyronine (T4) and thyroid-stimulating hormone (TSH) were performed by Immunochemiluminescent tests (ECLIA) using the Roche Cobas e 411 analyzers with ECLIA Cobas kits (Roche Diagnostics, Germany). The values of T3 and T4 are expressed in pmol / L while the values of TSH are expressed as mUI/l.

2.7 Histopathological Analysis

For histopathological analysis, the thyroid tissues were dissected and the tissue samples were immediately fixed in formalin solution, embedded in paraffin. The paraffin sections were cut into 5 µm thick slices and stained with hematoxylin and eosin (H&E) for light microscopic examination.

2.8 Statistical Analysis

Data were analyzed using the Graphpad Prism statistical program (software version 7.0). All results are given as means ± Standard Error of the Mean (SEM).

Statistical analysis for comparison of mean values was performed by ANOVA followed by Dunnett post hoc test and the level of significance was set at $p<0.05$.

3. Results

3.1 Effects of Ni alone or in combination with Se and/or Zn on maternal body weight, absolute and relative thyroid weights

There was no mortality in any of the experimental groups. Administration of 100 mg/kg body weight of Ni by s.c. injection significantly reduced the maternal body weight at day 6 and day 20 of gestation compared with the control groups.

Treatment with Se and/or Zn along with Ni showed an improvement in body weight gain compared to the Ni group. The absolute and relative thyroid weights were significantly increased in Ni treated animals compared to the control and conversely, a significant decrease was noticed in the combined treatments in comparison with the Ni group (Table 1).

3.2 Effects of Ni alone or in combination with Se and/or Zn on triiodothyronine (T3) concentration

The subcutaneous administration of Ni declined the level of plasma T3 on days 6 and 20 of gestation compared to the control. Whereas, the administration of Se and/or Zn with Ni produced a significant increase in the value of T3 on day 20 of gestation compared to the Ni group (Figure 1).

3.3 Effect of Ni alone or in combination with Se and/or Zn on thyroxine (T4) concentration

Exposure to Ni caused a decrease of T4 levels on days 6 and 20 of gestation compared to the control. Moreover, The treatment of Ni-exposed rats with Se and Zn separately or in combination induced a significant increase in the value of T4 on day 20 of gestation compared to the Ni group (Figure 1).

3.4 Effect of Ni alone or in combination with Se and/or Zn on Thyroid-Stimulating Hormone concentration (TSH)

TSH levels were significantly increased in Ni exposed rats on days 6 and 20 of gestation as compared to controls. In contrast, there was a significant decrease in the value of TSH on days 6 and 20 of gestation in groups (Ni+Se), (Ni+Zn) and (Ni+Se+Zn) when compared with the Ni group (Figure 1).

3.5 Effects on the thyroid gland histopathology

Thyroid sections of control groups showed the normal histological structure. The thyroid gland has consisted of follicles of various sizes separated by interlobular

Table 1. Effects of subcutaneous Ni alone or in combination with Se and/or Zn on maternal body weight, thyroid weights (absolute and relative) in preimplanted rats

Treatment group	Body weight (g)			Thyroid weights	
	Day 3	Day 6	Day 20	Absolute (g)	Relative g/100g body weight
Control	233,6±12,17	237,3±12,04	281±14,14	0,12±0,007	0,050±0,004
Ni	193,1±12.27	192,1**±12.09	221,1***±4,69	0,15**±0,006	0,071***±0,002
Ni+Se	212,8±4,75	215,8±5.24	260,3$^{\#}$±10.55	0,14±0,007	0,054$^{\#\#}$±0,002
Ni+Zn	232,8±9,83	235,6$^{\#\#}$±9.32	267,1$^{\#\#}$±7,33	0,14±0,003	0,055$^{\#\#}$±0,002
Ni+Se+Zn	236,1±7.7	237,1$^{\#\#}$ ±8,02	275,5$^{\#\#\#}$ ±9.44	0,12$^{\#}$±0,009	0,047$^{\#\#\#}$±0,004

Values expressed as mean ± SEM, n = 6 rats/group (ANOVA, Dunnett).

* Significantly different from control, $^{**}p<0.01$, $^{***}p<0.001$.

$^{\#}$ Significantly different from Ni-treated group, $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$.

connective tissue. Each follicle was lined with a single layer of cuboidal epithelial cells and filled with homogenous colloid. Parafollicular cells have been observed.

However, treatment with Ni resulted in several histopathological changes, such as proliferation of thyroid follicles, the follicles became smaller, the amount of colloids decreased and the majority of follicles without colloids were dissociated by foci of edema, follicular degeneration with disintegration of the epithelium in the follicular lumen, vacuolation of the follicular epithelium, follicular cell hyperplasia and parafollicular cells.

Indeed, pathological lesions induced by Ni were remarkably reduced by the administration of Se or Zn (Figure 2).

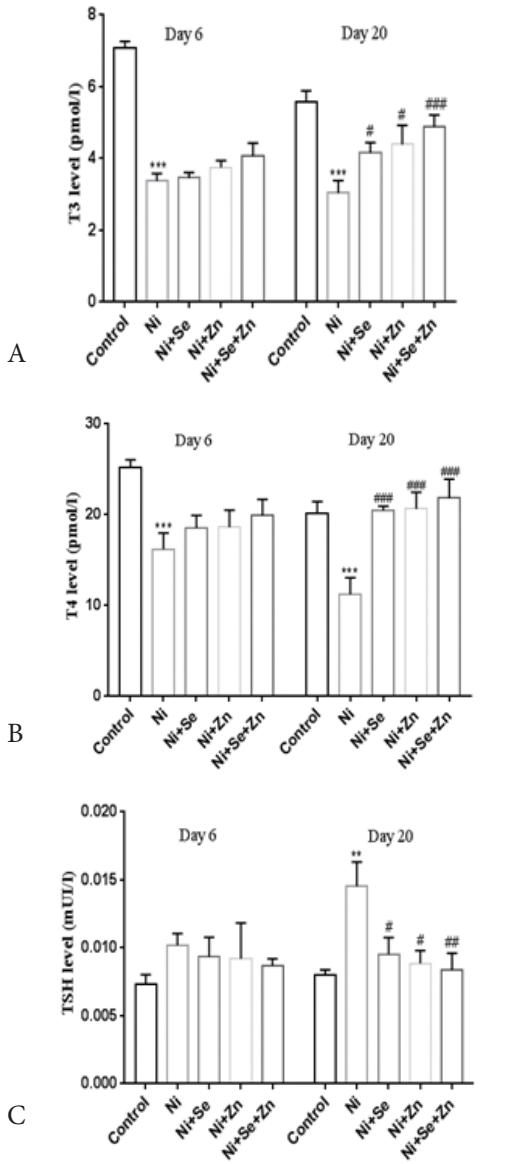
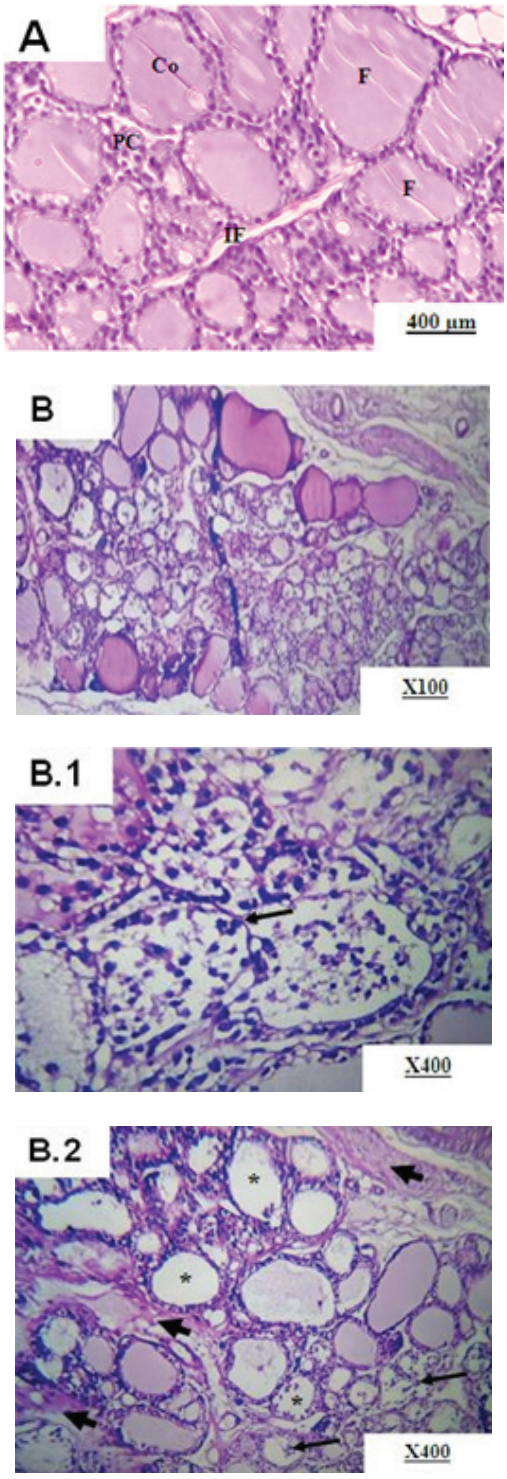


Figure 1. Effects of subcutaneous Ni alone or in combination with Se and/or Zn on thyroid hormonal levels in preimplanted rats. (A) triiodothyronine (T3), (B) thyroxine (T4), and (C) Thyroid Stimulating Hormone (TSH).

The results were expressed as Mean ± SEM (N = 6).
Values were considered significantly different at **p<0.01, ***P<0.001 versus control group and #p<0.05, ##p<0.01, ###p<0.001 versus Ni group.



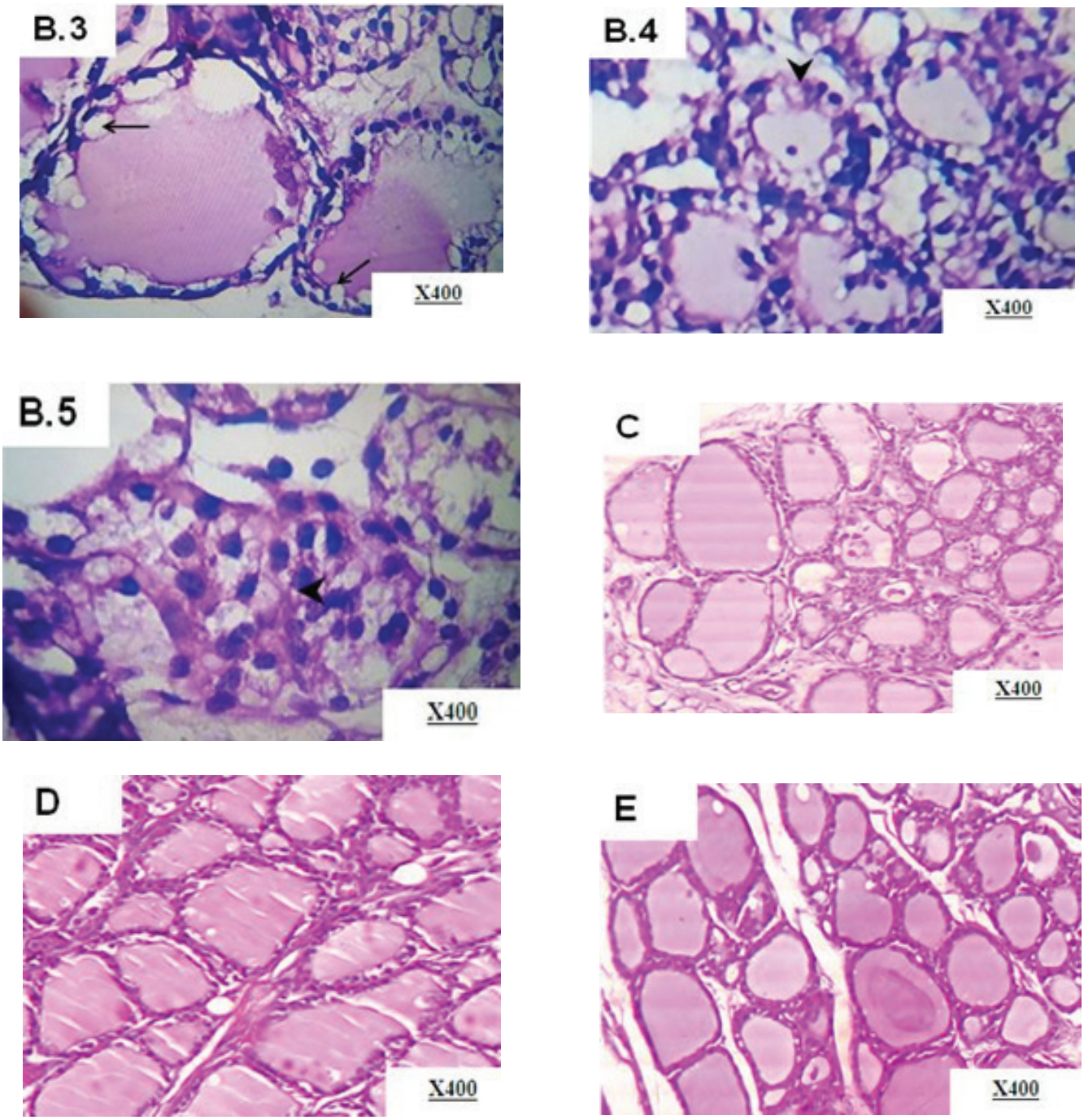


Figure 2. Thyroid gland histological sections of preimplanted rats.

(A) Control: normal thyroid architecture showing normal follicles (F) separated by interlobular connective tissue (IF) and filled with colloid (Co), parafollicular cells (PC).

(B, B1, B2, B3, B4, B5) Ni thyroid showed proliferation of the thyroid follicles (B), follicular degeneration (small arrows), the majority of follicles without colloids (*), odematous Fluid (big arrows) (B1, B2), Vacuolation of the follicular epithelium (arrows) (B3), follicular cell hyperplasia and parafollicular cells (arrow head) (B4, B5).

(C) Ni+Se, (D) Ni+Zn, (E) Ni+Se+Zn: co-treatment with Se and/or Zn presented displaying remarkable improvement in the histological appearance with a marked reduction in nickel-induced thyroid damage.

4. Discussion

Nickel is one of the environmental pollutants and is known to be a potential toxicological hazard for living organisms because of its carcinogenic, teratogenic, genotoxic and immunotoxic effects[18, 25–28]. In this study, we investigated the effect of Ni on the morphology and levels of thyroid hormones in preimplanted *Wistar* albino rats. We also evaluated whether co-administration of micronutrients such as Se and Zn prevents thyroid damage caused by Ni. Previous studies have shown that a change in body weight or organ after chemical exposure may indicate toxicity[29,30].

Our study shows that the administration of Ni on day 3 of gestation in the preimplantation period induces a low weight on days 6 and 20 of gestation in the pregnant rats treated with Ni compared to the control. This result is agreeing with those of Adjroud[18], and Siani et al.[31] The decrease in maternal body weight is due to both decreased diet consumption and water intake and increased degeneration of lipids and proteins as a result of nickel toxicity[18,32].

However, the association of Ni with Se and/or Zn showed an improvement in maternal body weight on days 6 and 20 of gestation compared to Ni administered alone which may be due to the protective effect of Se and/or Zn against Ni toxicity. Our results are in agreement with those of Käkelä et al.[17] and Adjroud[18] who observed that Se could counteract the deleterious effects of Ni on reproduction in rats. A similar protective action of Zn to improve the body weight of Ni-treated animals has been reported by Djemli et al.[22] Moreover, Amara et al.[33] and Rao et al.[34] explain the animal's body weight gain by an increase in appetite.

On the other hand, in our research, there was an increase in absolute and relative thyroid weight in pregnant rats treated with Ni compared to the control. This increase is probably due to the accumulation of Ni in the thyroid gland or to tissue hypertrophy caused by Ni. Indeed, other studies using heavy metals such as lithium and cadmium report an increase in thyroid gland weight in male rats[35,36].

In this study, we reported that pretreatment of rats with Se and/or Zn was susceptible to improve the Ni-induced toxicity on the absolute and relative thyroid weights in preimplanted rats. The same results were observed in rats treated with cadmium in combination with Se and Zn[36].

The results of the present study clearly demonstrated that Ni caused thyroid gland hypofunction as revealed by the significant decrease in the plasma levels of both T3 and T4, which was accompanied by a significant increase in the plasma levels of TSH on days 6 and 20 of gestation compared to the control. Similarly, Cheng et al.[10] reported that exposure to nickel sulfate in *Wistar* male rats induced a decrease in T3 and T4 levels. Ni has been reported to reduce iodine uptake by the thyroid[37]. Levels of T3 and T4 in serum are indicators of the thyroid function and any change in their levels reflects a disturbance in their synthesis and/or secretion, as well as disorders in the extrathyroidal metabolism[38].

In agreement with our results, it was reported that serum T3 and T4 levels were lowered and the serum TSH levels was increased in rats administrated with chromium and lithium[39–42].

A possible explanation for the thyroid dysfunction in our study could be due to the oxidative stress of Ni in the thyroid gland (Data not shown). Indeed, Other heavy metals such as chromium can cause thyroid toxicity by increasing cellular oxidative stress and decreasing antioxidant activity[42,43].

In the present study, the decrease in plasma levels of T4 in pregnant rats treated with Ni may indicate that Ni affects the production and/or secretion of T4 by follicular cells. The studies carried out by Yoshizuka et al.[44] indicated that cadmium affects the synthesis and release of T4 by oxidative phosphorylation disorders in the mitochondria of follicular cells. In addition, the relatively low level of T3 may be due to a deficiency in deiodinase synthesis. According to Matović et al.[45] cadmium inhibits peripheral hepatic deiodination from T4 to T3 by binding to 5-monodiodinase sulfhydryl groups, a seleno-enzyme containing a selenocysteine active site.

Moreover, the observed increase in serum TSH is a response to the decrease in serum T4 and T3 levels in rats with Ni exposure. The results of Ambali et al.[46] have shown that the increase in serum TSH in rats poisoned with potassium dichromate could be an attempt by the body to stimulate the thyroid gland to increase the synthesis and secretion of T3 and T4 to compensate for the apparent deficit in the system.

Another important point is that the reduction in T4, T3 levels observed in our study may be related to the alteration of transcriptions expression of genes related to thyroid hormone synthesis. In fact, Exposure to hexavalent chromium down-regulated TRβ and Dio2 mRNA expression and up-regulated Dio3 mRNA level *in Bufo gargarizans (B. gargarizans)* embryos[47]. According to Sun et al.[48] extra-thyroidal disruption of thyroid function can

also happen through inhibition of the thyroid hormone receptor (TRβ) gene expression, which was shown in Chinese toad tadpoles larvae exposed to different cadmium concentrations. Moreover, bisphenol A (BPA) exposure led to significant down-regulation of tshβ, trα, trβ, and dio2 genes at the highest experimental dose of 10 mg/L in rats[49].

Essential elements such as selenium and zinc are known to reduce the absorption and retention of toxic elements and prevent their toxic effects[50,51]. In our study, the administration of Se and/or Zn in rats treated with Ni subcutaneously protects thyroid function from Ni intoxication, as indicated by significant restoration of T3, T4 and normalization of TSH concentration. Recent studies have shown that selenium has protective effects against thyroid damage induced by certain heavy metals such as chromium and lead[43,52], which is well in agreement with our results. In fact, Se is an important micronutrient in animals and humans, and Se-containing enzymes such as glutathione peroxidase and thioredoxin reductase exert protective effects on the reproductive system by eliminating free radicals and preventing oxidative damage[53,54]. Adjroud[18] observed that Se plays a beneficial role in preventing Ni hepatotoxicity in pregnant rats.

Several experimental studies have shown that Zn protects against lead and lithium-induced thyroid dysfunction in male or female rats[55,56] which was similar to our observation. Zn is well known as an essential trace element that affects several vital processes such as immune function, cell proliferation, the defense against oxidative stress and free radicals generation and activation of antioxidant[57]. It is known to induce the synthesis of metallothionein (MT), a protein capable of binding heavy metals and recovering hydroxyl radicals[58]. In addition, Hammouda et al.[36] have demonstrated the beneficial effects of selenium and zinc in the treatment of cadmium thyrotoxicity in rats.

Consequently, these hormonal changes could be correlated with histological alterations. In fact, the actual study suggests that Ni produced various pathological perturbations in the thyroid such as follicular degeneration with disintegration of the epithelium in the follicular lumen, proliferation of thyroid follicles, the follicles became smaller, the amount of colloids decreased and the majority of follicles without colloids were dissociated by foci of edema and vacuolation of the follicular epithelium, follicular cell hyperplasia and parafollicular cells. Similar changes in thyroid tissue following exposure to nickel chloride in rabbits have been documented in other studies[12]. According to Al-Mogairen[11] the subcutaneous administration of Ni in rats induced mild proliferation of the thyroid follicles, the follicles became smaller in size, the amount of colloid was diminished and lined by plump cuboidal epithelium.

However, pathological lesions induced by Ni have been remarkably reduced by the administration of Se or Zn which might be attributed to their antioxidant roles. Accordingly, these findings are in line with those obtained by Mohamed et al.[40] and Hassanin et al.[43], who found that Se attenuated the chromium-induced histological alterations in the thyroid in rats. In addition, according to Sidhu et al.[59], zinc has a protective effect against the harmful effects of nickel on the liver structure of rats.

5. Conclusion

In conclusion, this study demonstrates that exposure to Ni provoked a deleterious effect on the hormonal function and histological structure of the thyroid gland of preimplantated rats. Ni can be considered as a thyroid function disruptor in preimplantated rats.

However, Co-administration of Se and Zn attenuates the toxicity of this metal in pregnant rats, objectified by an improvement of hormonal and thyroid histoarchitecture.

6. Acknowledgement

This work was supported by the DGRSDT/ MESRS (code: N ° E2212600).

7. Conflict of Interest

The authors declare that there are no conflicts of interest.

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