Protective role of selenium on thyroid morphology in iodine-induced autoimmune thyroiditis in Wistar rats

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Abstract. Excess iodine may induce and exacerbate autoimmune thyroiditis (AIT) in humans and animals. In order to assess the potential protective mechanisms of selenium (Se) in thyroid autoimmunity, the effects of inorganic Se (sodium selenite) administration on thyroid morphology and follicular cytology were investigated in adult Wistar rats with iodine-induced AIT. A total of 48 adult Wistar rats (24 females, 24 males) were allocated to one of four dietary regimens: C0, control; C1, only potassium iodine (KI); C2, concomitant KI and Se; C3, only KI initially, followed by Se administration. For AIT induction the rats were fed with 0.05% KI for 56 days. Se-treated rats received 0.3 mg/l sodium selenite in drinking water. Thyroid tissues were collected for pathologic diagnosis after 7 days in C0 group, 56 days in C1 and C2 groups, and 112 days in C3 group. In C1 group, moderate to severe thyroiditis was observed in 83% of males and 50% of female rats (P=0.223). In C3 group 16.7% of male rats developed moderate thyroiditis and none in C2 group, whereas no females were identified with moderate to severe thyroiditis in C2 or C3 group. Thus, the administration of Se was proven to have protective effects against thyroiditis cytology in both male and female Wistar rats.

Introduction

As most human autoimmune disorders, autoimmune thyroiditis (AIT) (chronic lymphocytic thyroiditis/Hashimoto's thyroiditis) results from a combination of genetic predisposition and environmental triggers (1). Clinical and epidemiologic evidence point to excessive iodine intake as the environmental agent responsible for the thyroid autoimmunity induction (2,3). The role of iodine in the homeostatic regulation of thyroid function was first demonstrated >50 years ago. However, the precise mechanism of regulation remains unclear (4). High doses of iodide suppress the functional activity of the thyrocytes (Wolff-Chaikoff effect), inhibiting the iodination of the thyroid protein fraction and decreasing the concentration of thyroid hormones in serum (5). It has been demonstrated that a single injection of a high dose of iodide inhibits the biosynthesis of thyroid hormones at several levels (6,7). The sensitivity to the stimulating action of thyroid-stimulating hormones decreases and the expression and activity of thyroperoxidase (the enzyme catalyzing iodination of thyroglobulin in the presence of iodide and hydrogen peroxide) are suppressed (8). Finally, the NADPH-oxidase reaction producing H_2O_2 (the limiting step in the iodide metabolism) is also suppressed (7,9). The necrotic effect is increased in case of selenium (Se) deficiency (10,11). However, thyroid cells have their own antioxidant system. Thus, in the case of iodine excess, the expression of antioxidative enzymes increases (12). Therefore, the first iodine-induced thyroiditis has been transient in most experimental animals, except for genetically modified animals prone to develop AIT, such as non-obese diabetic (NOD) mice. They present important areas of destroyed thyroid tissue which are replaced by inflammatory tissue (13).

Experimental autoimmune thyroiditis (EAT) has been used to simulate human autoimmune thyroid disease for decades (14). EAT can be easily induced in genetically susceptible strains of mice by excess iodine ingestion (15) or by immunization with mouse thyroglobulin (16).

However, iodine excess alone has also been used to induce EAT in insusceptible murine models, including Wistar rats (17,18), as well as in other animals (19). Iodine excess is a cheap and efficient method for EAT induction.

The aim of the present study was to assess the effects of inorganic Se supplementation on thyroid morphology in EAT induced by the administration of potassium iodide (KI) in Wistar rats.

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Key words: selenium, experimental autoimmune thyroiditis, iodine-induced thyroiditis in Wistar rats, iodine-induced thyroid dysfunction, selenium effect on thyroid pathology

Table I. Study group allocation and treatment regimens.

Factors	C0	C1	C2	C3
Sex (males/females)	6/6	6/6	6/6	6/6
KI administration	No	56 days of KI	56 days of KI	56 days of KI
Na-Se administration	No	No	56 days of Na-Se, concomitant with KI administration	56 days of Na-Se, after the KI administration
Total days of treatment/observation	7 days	56 days	56 days	112 days

KI, potassium iodine; Na-Se, sodium selenite.

Materials and methods

Animals. A total of 48 Wistar adult rats (24 females weighing 160±20 g and 24 males weighing 180±20 g) were used for the present study. The animals were obtained from the 'Victor Babes' National Institute of Research Development in the Pathology Domain and Biomedical Sciences (Bucharest, Romania). Wistar rats were housed under standard conditions at the Biobase for research animals of 'Grigore T. Popa' University of Medicine and Pharmacy (Iasi, Romania) and were fed with standard food. The rats were housed in clean and ventilated polyurethane cages; 2 rats were placed in each cage. All rats were maintained under standard conditions of temperature (20±40˚C), relative humidity of 55±10%, and light/dark cycles of 12/12 h consecutively. Access to food and water was *ad libidum*. The acclimatization of the rats lasted 7 days prior to the study initiation. The study was approved by the Ethics Committee of 'Grigore T. Popa' University of Medicine and Pharmacy.

EAT and Se administration. As AIT is more common in females (3:1) (20-22), it was investigated whether the same susceptibility of the female sex also occurs in the animal model of Wistar rats. The animals were randomized into groups according to four treatment regimens: C0, two control groups for each sex; C1, two (male and female) groups that received KI for 56 days (0.2 mg per animal in drinking water); C2, two (male and female) groups that received concomitant KI and sodium selenite (0.5 mg/kg body weight of sodium selenite administered in drinking water) for 56 days; and C3, two (male and female) groups that received KI for 56 days and afterwards sodium selenite for another 56 days (Table I).

Even though no dose-finding study was performed, a previous report (23) concerning sodium selenite administration in Wistar rats has shown consistent toxic effects of sodium selenite at a dose of >1 mg/kg body weight (using the same administration method: *ad libitum* in drinking water) (23,24).

Tissue collection and analysis. General anesthesia was performed with a combination of ketamine (60 mg/kg body weight) and xylazine (8 mg/kg body weight) administered intraperitoneally. Thyroid tissues were collected for pathology analysis, in accordance with the Council Directive 63/2010/EU on the protection of animals used for scientific purposes, after 7 days in control groups, 56 days in C1 and C2 groups, and 112 days in C3 groups. Tissue samples were harvested from the neck area containing the anterior muscular plan, the thyroid and parathyroid tissue and the tracheal tissue with the cartilage ring. In order to keep the thyroid intact, tissue samples were fixed in 10% formaldehyde at 24˚C for 24 h. The tissue samples were embedded in paraffin and cut into 4-μm thick sections. Next, the tissue samples were stained with hematoxylin and eosin (H&E) for 40 min, or van Gieson's (VG) stain for 15 min at room temperature 22-24˚C. The morphometric evaluation was performed using a light microscope, with x10 objective. For positive and negative control, thyroid and parathyroid tissues were immunohistochemically stained with synaptophysin, chromogranin, thyroglobulin and thyroid transcription factor (TTF1) at the 'Prof. Dr. Gioconda Dobrescu' Department of Pathology, 'Sf. Spiridon' County Hospital (Iasi, Romania). Parathyroid tissues showed diffuse negative immunoreactivity for chromogranin, synaptophysin and TTF1; parafollicular C cells were positive for chromogranin and synaptophysin, and thyroid tissue presented diffuse nuclear immunoreactivity for TTF1 and positive cytoplasmic immunoreactivity for thyroglobulin. Immunohistochemistry was performed at room temperature between 22-24˚C. The overnight staining technique was carried out at 4˚C with a maximum staining duration of 24 h.

The sections were examined and photographed using Nikon Eclipse E600 (Nikon Corporation) and the Lucia Net program, equipped with the Nikon Digital Net Camera DN100 image capture system (Nikon Corporation) and morphometric analysis software (morphometric software LUCIA Net v.16.2®; Laboratory Imaging s.r.o., with NIS Elements 3.0®) at the 'Prof. Dr. Gioconda Dobrescu' Department of Pathology. The morphometric analysis of the thyroid tissues was made from 5 successive images captured from each lobe (digital pictures of 10 non-adjacent 10x fields). The interpretation of histopathological sections and the acquisition of images for all studied animals were performed by a single examiner (DGCA).

Morphopathology parameter assessment. The mean size of thyroid follicles was assessed by measuring the maximum diameter of ≥20 thyroid follicles per case in various areas of the thyroid gland. The mean size of the thyroid follicular epithelium was assessed by measuring the size of the follicular epithelium in fixed positions (at 12, 5 and 7 o'clock) in 20 follicles per case.

The morphological evaluation of thyroid follicles was performed in 20 thyroid follicles for each case and included

Table II. Scoring system used for the morphopathological evaluation of the AIT.

Parameter	Scoring system				Refs.
	0	1	2	3	
Inflammation	Normal morphology	Mild destruction of thyroid follicles with few lymphocytes attacking ~ 2 or 3 thyroid follicles	Focal foci with moderate destruction of the thyroid follicles in 10-40% of the total area	Severe destruction of thyroid follicles with inflammatory infiltration in >40% of the total area	(21,22,35), adapted
Vascular congestion	Normal morphology	Mild vascular distention in the capsular blood vessels	Moderate congestion in the capsular and intraglandular blood vessels in 10-40% of the total area	Severe congestion in the capsular and intraglandular blood vessels in >40% of the total area	(20,33), adapted
Resorption vacuoles	Normal morphology	Resorption vacuoles present in <10% of the follicular cavities	Resorption vacuoles present in 10-40% of the follicular cavities	Resorption vacuoles present in >40% of the follicular cavities	(20,33), adapted
Interfollicular space	Normal morphology	Interfollicular spaces observed in <10% of the glandular surface	Interfollicular spaces observed in 10-40% of the glandular surface	Interfollicular spaces observed in >40% of the glandular surface	(20,33), adapted
Interstitial collagen deposits	Normal morphology	Discreet collagenization of the thyroid capsule	Thickened capsule with fine pericapsular septae in the interstitial space	Important capsular fibrosis with thick pericapsular septae and perifollicular fibrosis	(20,33), adapted
Thyroiditis score	Normal thyroid morphology: 0-3 Mild thyroiditis: 4-6 Moderate thyroiditis: 7-9 Severe thyroiditis: 10-12				(20,33,36-38), adapted

AIT, autoimmune thyroiditis.

Table III. Comparison of thyroid morphology results between male and female rats in each treatment group.

	C0				C1				C2				C3			
	Males		Females		Males		Females		Males		Females		Males		Females	
Score	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Inflammation																
0 Normal morphology	6	100	6	100	6	100	4	66.7	6	100	6	100	5	83.3	6	100
1 Mild follicular destruction	-	-	-	-	-	-	1	16.7	-	-	-	-	1	16.7	-	-
2 Moderate follicular destruction	-	-	-	-	-	-	1	16.7	-	-	-	-	-	-	-	-
3 Severe follicular destruction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P-value		-				0.333				-					0.296	
Vascular congestion																
0 Normal morphology	6	100	6	100	-	-	-	-	-	-	-	-	3	50	5	83.3
1 Mild vascular distention	-	-	-	-	1	16.7	2	33.3	3	50	3	50	-		1	16.7
2 Moderate vascular congestion	-	-	-	-	1	16.7	2	33.3	3	50	3	50	1	16.7	-	-
3 Severe vascular congestion	-	-	-	-	4	66.7	2	33.3	-	-	-	-	2	33.3	-	-
P-value		-				0.513				-					0.212	
Resorption vacuoles																
0 Normal morphology	6	100	4	66.7	-	-	-	-	6	100	6	100	1	16.7	6	100
1 <10% of follicular cavities	-	-	2	33.3	2	33.3	3	50	-	-	-	-	3	50	-	-
2 10-40% of follicular cavities	-	-	-	-	2	33.3	2	33.3	-	-	-	-	1	16.7	-	-
3 >40% of follicular cavities	-	-	-	-	2	33.3	1	16.7	-	-	-	-	1	16.7	-	-
P-value		0.439				0.766				-					**0.036**	
Interfollicular space																
0 Normal morphology	6	100	6	100	-	-	-	-	6	100	6	100	4	66.7	6	100
1 <10% of glandular surface	-	-	-	-	3	50	4	66.7	-	-	-	-	-	-	-	-
2 10-40% of glandular surface	-	-	-	-	1	16.7	2	33.3	-	-	-	-	2	-	-	-
3 >40% of glandular surface	-	-	-	-	2	33.3	-	-	-	-	-	-	-	-	-	-
P-value		-				0.290				-					0.439	
Interstitial collagen deposits																
0 Normal morphology	6	100	5	83.3	-	-	1	16.7	-	-	-	-	1	16.7	5	83.3
1 Mild capsular collagenization	-	-	1	16.7	2	33.3	5	83.3	3	50	-	-	2	33.3	1	16.7
2 Thickened capsule	-	-	-	-	4	66.7	-	-	2	33.3	3	50	3	50	-	-
3 Important capsular fibrosis	-	-	-	-	-	-	-	-	1	16.7	3	50	-	-	-	-
P-value		0.296				**0.043**				0.122					**0.049**	
Final score of thyroiditis																
0-3 Normal thyroid morphology	6	100	6	100	-	-	-	-	4	66.7	1	16.7	2	33.3	6	100
4-6 Mild thyroiditis	-	-	-	-	1	16.7	3	50	2	33.3	5	83.3	3	50	-	-
6-9 Moderate thyroiditis	-	-	-	-	3	50	3	50	-	-	-	-	1	16.7	-	-
10-12 Severe thyroiditis	-	-	-	-	2	33.3	-	-	-	-	-	-	-	-	-	-
P-value		-				0.223				0.079					**0.049**	

Bold font indicates P<0.05.

Table IV. Association matrixes of morphopathological parameter results assessed within each study group.

Males	C1	C2	C3	C0	Females	C1	C2	C3	C0	Males vs. females
Vascular congestion scoring										
C1	-				C1	-				0.513
C2	**0.049**	-			C2	0.301	-			-
C3	**0.020**	**0.029**	-		C3	**0.025**	**0.011**	**-**		0.212
C0	**0.001**	**0.002**	0.050	-	C0	**0.007**	**0.002**	0.500	-	-
C1+2+3				**0.001**	C1+2+3				**0.049**	0.407
Resorption vacuoles scoring										
C1	-				C1	-				0.766
C2	**0.007**	-			C2	**0.001**	-			-
C3	0.661	**0.036**	-		C3	**0.001**	-	-		**0.036**
C0	**0.007**	-	**0.036**	-	C0	0.050	0.439	0.439	-	0.439
C1+2+3				**0.001**	C1+2+3				0.050	**0.036**
Interfollicular spaces scoring										
C1	-				C1	-				0.290
C2	**0.007**	-			C2	**0.002**	-			-
C3	**0.025**	0.439	-		C3	**0.002**	-	-		0.439
C0	**0.007**	-	0.439	-	C0	**0.002**	-	-	-	-
C1+2+3					C1+2+3					
Interstitial collagen deposits scoring										
C1	-				C1	-				**0.043**
C2	0.393	-			C2	**0.004**	-			**0.043**
C3	0.497	0.439	-		C3	**0.003**	**0.040**	-		0.122
C0	**0.002**	**0.007**	**0.014**	-	C0	**0.003**	**0.040**	-	-	0.296
C1+2+3				**0.001**	C1+2+3				0.050	0.050
Final thyroiditis scoring										
C1	-				C1	-				0.223
C2	**0.025**	-			C2	0.105	-			0.079
C3	**0.049**	0.393	-		C3	**0.002**	**0.019**	-		**0.049**
C0	**0.007**	0.439	**0.049**	-	C0	**0.002**	**0.019**	-	-	-
C1+2+3				**0.046**	C1+2+3				**0.033**	0.474

Bold font indicates P<0.05.

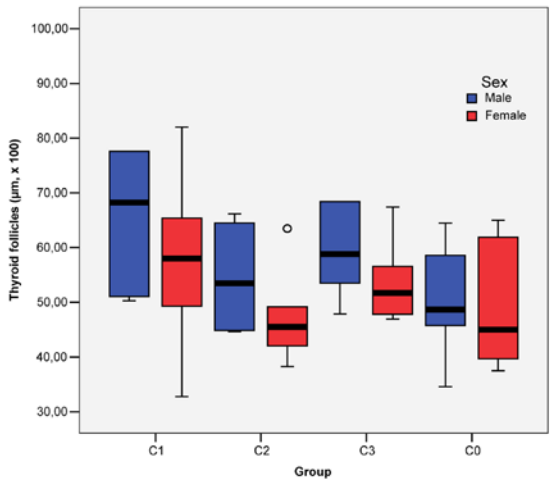
a scoring system from 0 to 3 for each of the following parameters: Presence of inflammation, vascular congestion, resorption vacuoles, interfollicular space and interstitial collagen deposits (Table II). A final thyroiditis score was calculated as the sum of the inflammation score, vascular congestion, fibrosis and resorption vacuoles scores, and the results corresponded to either normal thyroid morphology (final score 0-3), mild thyroiditis (final score 4-6), moderate thyroiditis (final score 7-9) or severe thyroiditis (final score 10-12) (Table II).

Statistical analysis. All statistical analyses were performed using SPSS v24.0 software (IBM Corp.). Skewness and kurtosis (-2<P<2) tests, the tests of normality in frequentist statistics, were used to examine the distribution of continuous variables. For multiple comparisons of normally distributed data, two-way ANOVA was performed with Tukey's HSD post hoc test. If the normality assumption was not satisfied, Kruskal-Wallis test and Dunn-Bonferroni post hoc test were carried out. Associations between categorical variables were assessed by Chi-square test.

The results are presented in Tables III and IV and Figs. 1 and 2. Specifically, Figs. 1 and 2 present the data on thyroid morphofunctional parameters, i.e., the mean size of thyroid follicles and follicular epithelium. Table III presents the comparison of the thyroid morphology results between male and female rats in each treatment group. Association matrixes of the morphopathological parameters assessed within each study group are presented in Table IV (vascular congestion, resorption vacuoles, interfollicular space, interstitial collagen deposits).

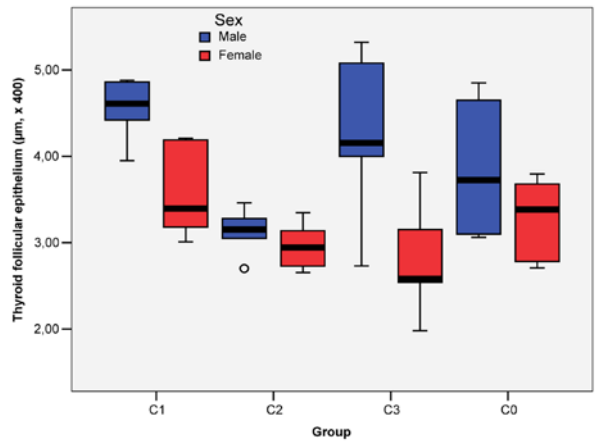
Results

Mean size of thyroid follicles. Regarding the size of thyroid follicles, the normality tests revealed the following aspects:



	P-values			P-values			P-values			P-values	
C1	C2	0.149	C2	C1	0.149	C3	C1	0.877	C0	C1	0.099
	C3	0.877		C3	0.491		C2	0.491		C2	0.997
	C0	0.099		C0	0.997		C0	0.377		C3	0.377

Figure 1. Comparison of the mean value of thyroid follicles (μm, x100) evaluated by sex between the study groups (median values are indicated by a bold line for each group).



	P-values			P-values			P-values			P-values	
C1	C2	**0.001**	C2	C1	**0.001**	C3	C1	0.104	C0	C1	0.177
	C3	0.104		C3	0.220		C2	0.220		C2	0.133
	C0	0.177		C0	0.133		C0	0.993		C3	0.993

Figure 2. Comparison of the mean value of thyroid follicular epithelium (μm, x400) evaluated by sex between the study groups (median values are indicated by a bold line for each group). Values in bold correspond to P<0.05.

The analysis of the entire study group showed that the mean value (56.48±17.05 μm x100) was far different than the median value (52.40 μm x100); Skewness (skw = 2.105) and Kurtosis (krt = 6.605) test results >2 suggested that the assumption of normality was not satisfied for the entire range of values; however, in C0, C1, C3, for both male and female subgroups, continuous values were confirmed.

The male rat thyroid morphology (Figs. 1, and 3A and B) showed that C1 group had higher mean value of thyroid follicles than C0 group (73.82 vs. 50.13 μm x100) and C2 group (73.82 vs. 53.74 μm x100). In C3 group, the mean value was higher than that recorded in the control group C0 (65.86 vs. 50.13 μm x100). The lowest mean value of the thyroid follicles was registered in the control group C0 (Fig. 1).

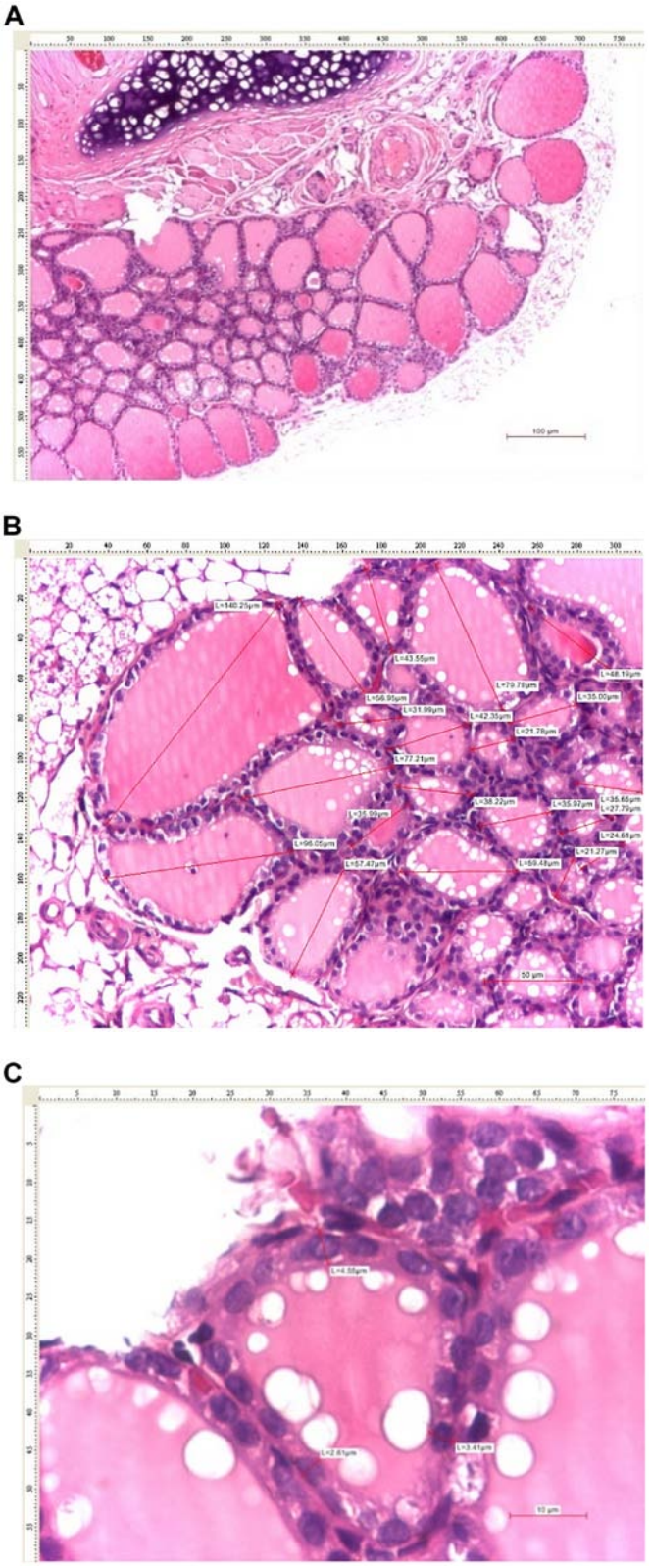


Figure 3. Thyroid images of male rats with KI administration. (A) Thyroid tissue (H&E, x4), (B) measurements of the maximum diameter of 20 thyroid follicles (H&E, x10) and (C) measurements of follicular epithelium heights (H&E, x40). KI, potassium iodine; H&E, hematoxylin and eosin.

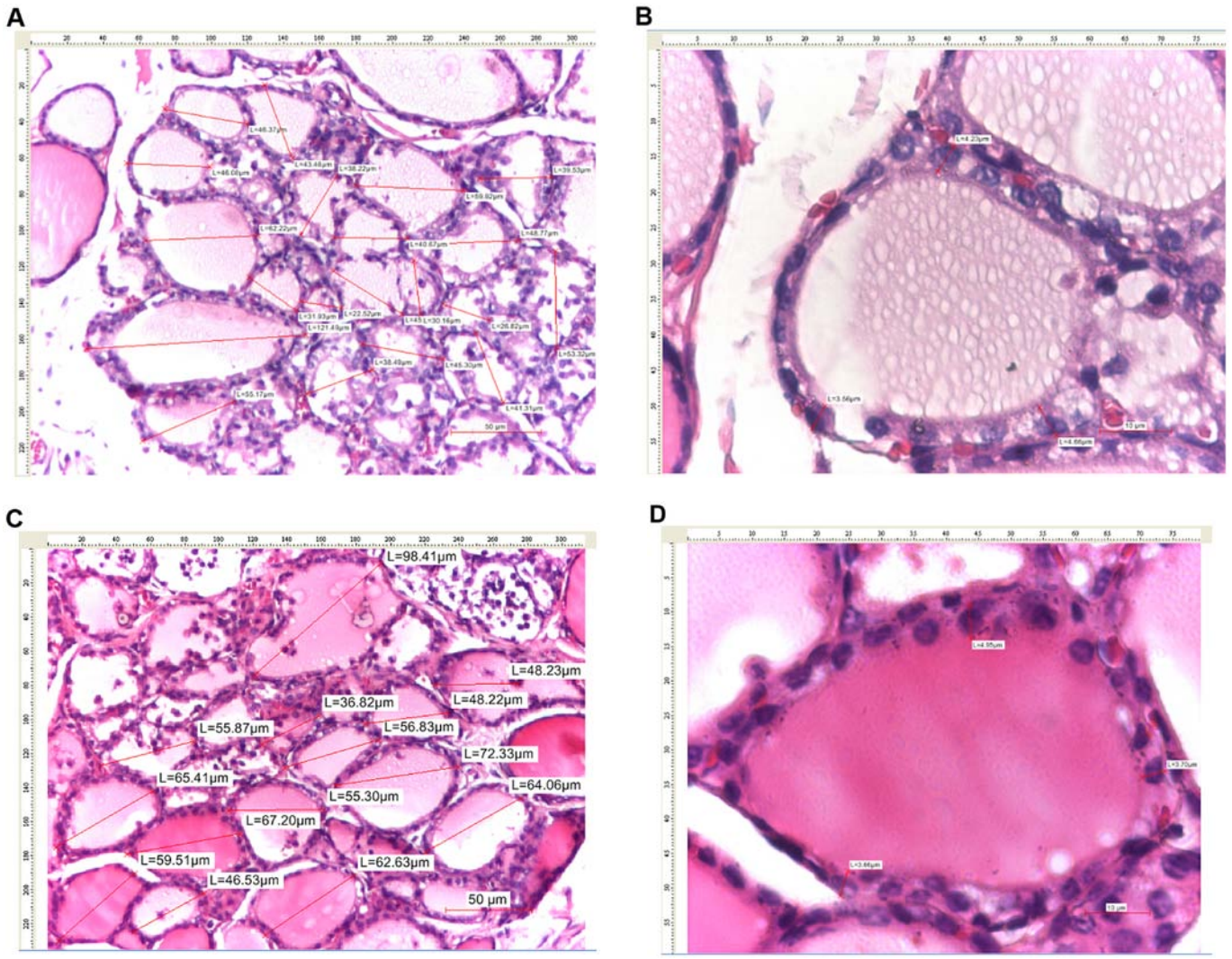


Figure 4. Thyroid images of female rats with concomitant KI and sodium selenite administration. Measurements of (A) the maximum diameter of thyroid follicles (H&E, x10) (20 thyroid follicles were measured in total) and (B) follicular epithelium heights (H&E, x40). Comparison with the control group: (C) maximum diameter of thyroid follicles (H&E, x10) (20 thyroid follicles were measured in total) and (D) follicular epithelium heights (H&E, x40). KI, potassium iodine; H&E, hematoxylin and eosin.

In female rats, the highest mean value of thyroid follicles was recorded in C1 group (57.56 μm x100) and the lowest in C2 group (47.32 μm x100; Figs. 1 and 4A and C, 7B, and 8B).

The results of two-way ANOVA showed no statistically significant differences in the mean size of thyroid follicles analyzed by sex and intervention group (Fig. 1).

Mean size of the thyroid follicular epithelium. The values for the size of follicular epithelium in the entire study group were homogeneous, thus significance tests could be applied for these continuous variables: The mean value (3.55±0.80 μm x400) was close to the median value (3.29 μm x400); Skewness (skw = 0.472) and Kurtosis (krt = -0.649) test results were comprised in the interval [-2, +2].

In male rats, the highest mean value of the thyroid follicular epithelium was recorded in C1 group (only KI administration; 4.56 μm x100) and the lowest mean value in C2 group (3.13 μm x100) (Figs. 3C and 5B).

In female rats, the highest mean value of the thyroid follicular epithelium was recorder in C1 group (3.56 μm x100) and the lowest mean value was recorded in C3 group (2.77 μm x100) (Figs. 2, 4B and D, 7C and E, and 8C).

Two-way ANOVA results showed statistically significant differences in the mean size of thyroid follicular epithelium analyzed by sex and intervention group only between C1 (only KI adminsitration) and C2 (concomitant KI and sodium selenite administration) groups (Fig. 2).

Inflammation assessment. The results on thyroid inflammation revealed that there were no significant differences between sex or treatment regimens in the study groups (C1, P=0.333; C3, P=0.296; Table III).

Vascular congestion. In male rats, significant differences were found between groups: In C1 group, severe vascular congestion was observed in 66.7% of male rats; in C2 group, 50% of rats had moderate vascular congestion; whereas in C3 group, 50% of male rats had normal vascular morphology (P=0.001; Table III).

In female rats, significant differences were also identified between groups: In C1 group, the same percentage of

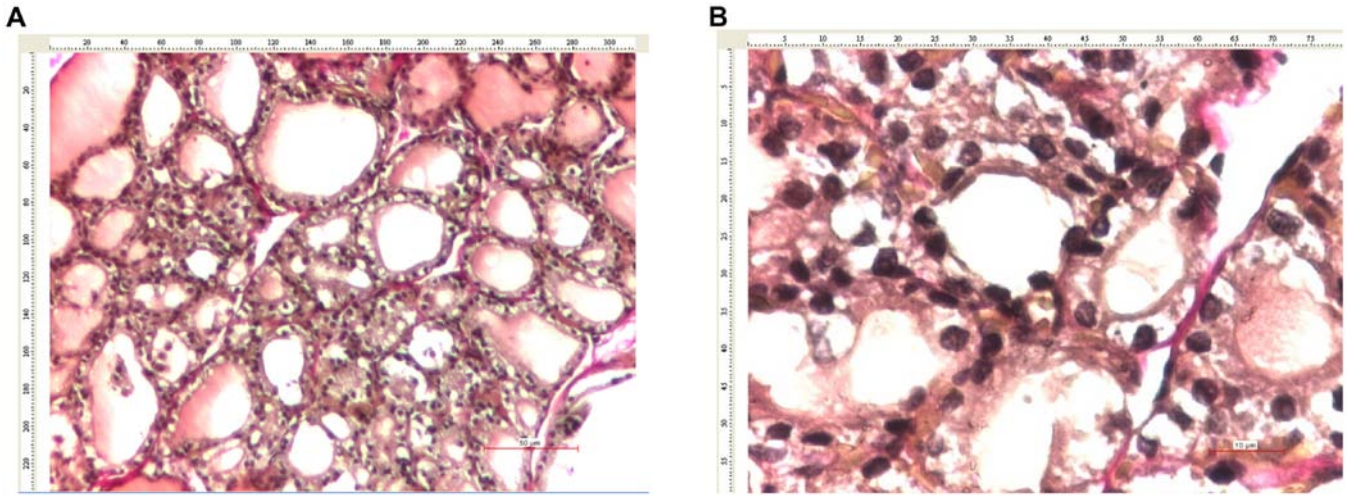


Figure 5. Images of KI-induced thyroiditis in male rats. (A) Fibrosis, inflammation, vascular congestion and resorption vacuoles (VG, x20) and (B) follicular epithelium details (VG, x40). KI, potassium iodine; VG, van Gieson's stain.

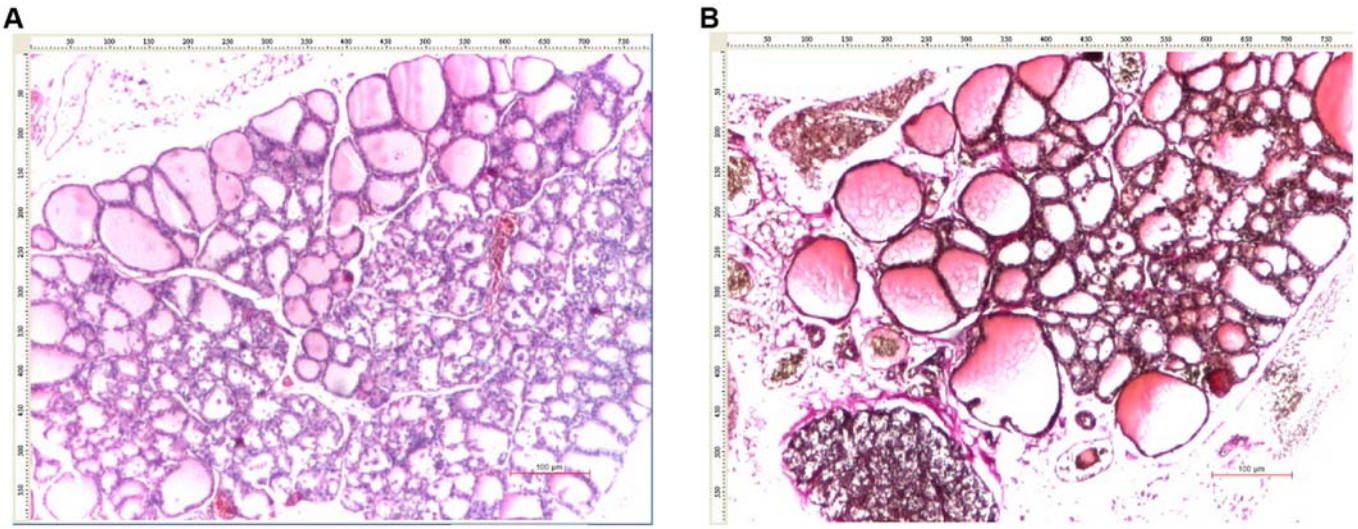


Figure 6. Thyroid images of female rats with concomitant KI and sodium selenite administration. Thyroid tissue stained with (A) H&E (x4) and (B) VG (x4). KI, potassium iodine; H&E, hematoxylin and eosin; VG, van Gieson's stain.

cases (33.3%) presented severe, moderate and mild vascular modifications; in C2 group, 50% of female rats had moderate vascular congestion; whereas in C3 group, 83.3% of the cases had normal vascular morphology (P=0.049). However, within each of the study groups, no statistically significant differences in terms of sex were confirmed (C1, P=0.513; C3, P=0.212; Tables III and IV).

Resorption vacuoles. In male rats, significant differences were confirmed between the groups: In C1 group, resorption vacuoles assessment revealed equal percentages of cases (33.3%) with score 1 (<10%), 2 (10-40%) and 3 (>40%); in C2 group, all cases had normal morphology; and in C3 group, 50% of the male rats had resorption vacuoles <10% (P=0.001; Table III).

In female rats, significant differences were also found between groups: In C1 group, 50% of female rats had resorption vacuoles <10%; and in C2 and C3 groups, all cases had normal morphology (P=0.05; Tables IV and 7D). Significant sex differences were observed only in C3 group (P=0.036; Table III).

Interfollicular space. All rats (regardless of sex) in C1 group (only KI administration) presented interfollicular spaces. The interfollicular space score for C1 male rats (treated only with KI) was significantly different than that in C2 group (concomitant KI and Se administration, P=0.007), C3 group (subsequent KI and Se administration, P=0.025) and C0 group (control, P=0.007) (Table IV). Scores of 2 (10-40% of glandular surface) and 3 (>40% of glandular surface) were particularly recorded in 50% of male rats (one rat with score 2 and two rats with score 3), whereas all the female rats presented only scores of 1 (<10% of the glandular surface) and 2 (Table III).

Interstitial collagen deposits. In male rats, 66.7% of the cases in the C1 group had moderate fibrosis; in C2 group, 50% of male rats had mild collagen deposits and only 33.3% moderate fibrosis; whereas in C3 group, only 33.3% of cases had mild collagen deposits and 50% moderate fibrosis (P=0.001; Table III).

In female rats, significant differences between groups were also confirmed: In C1 group, 83.3% of the rats had

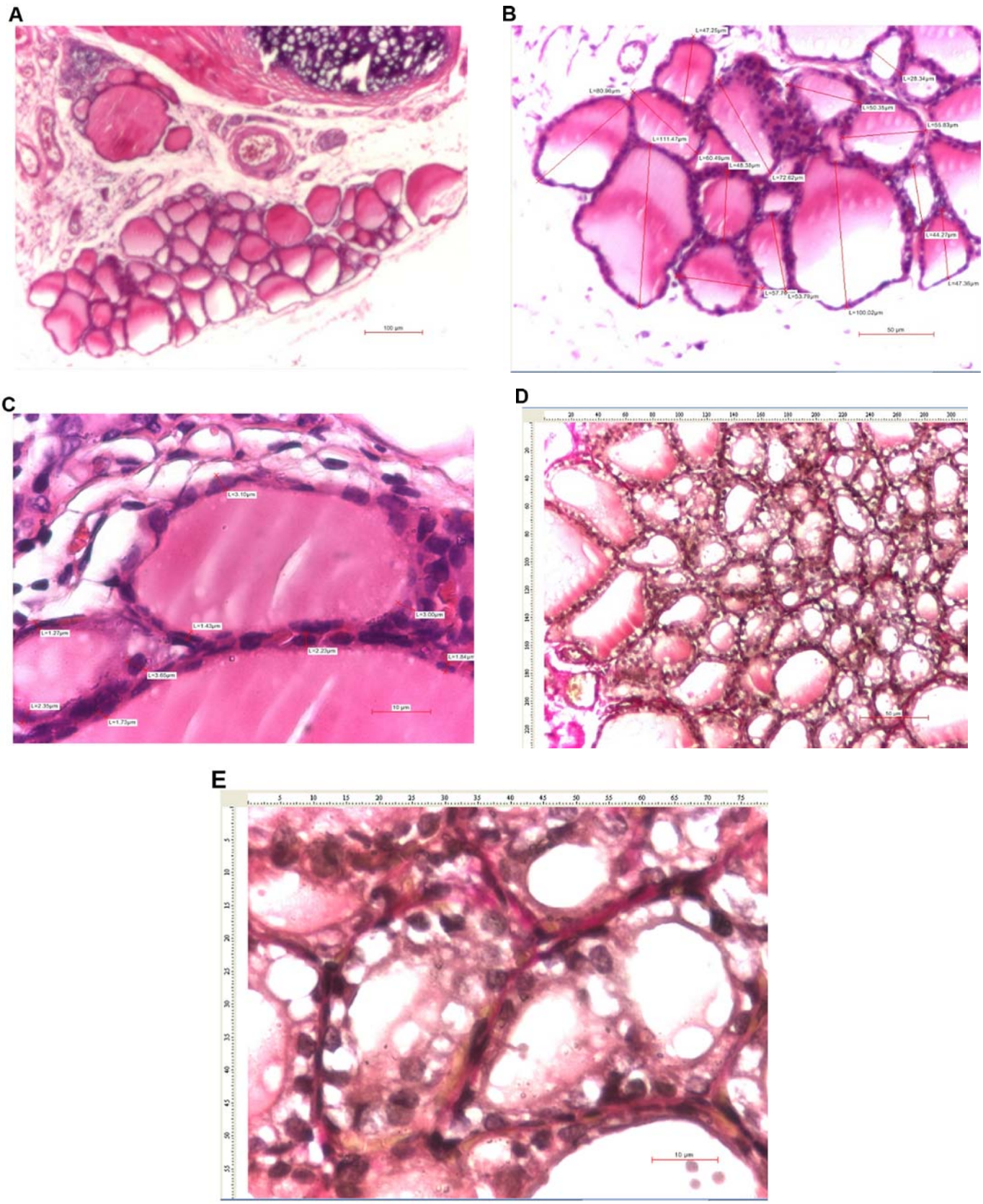


Figure 7. Thyroid images of female rats with sequential administration of KI and sodium selenite. (A) Thyroid tissue (H&E, x4), (B) measurements of the maximum diameter of thyroid follicles (H&E, x10) (20 thyroid follicles were measured in total), (C) measurements of follicular epithelium heights (H&E, x40), (D) resorption vacuoles (VG, x20) and (E) follicular epithelium details (VG, x40). KI, potassium iodine; H&E, hematoxylin and eosin; VG, van Gieson's stain.

discrete collagen deposits; in C2 group, 50% had important collagen deposits; and in C3 group, 83.3% had normal morphology (P=0.05; Table IV). Concerning the morphology of interstitial collagen deposits, significant sex differences

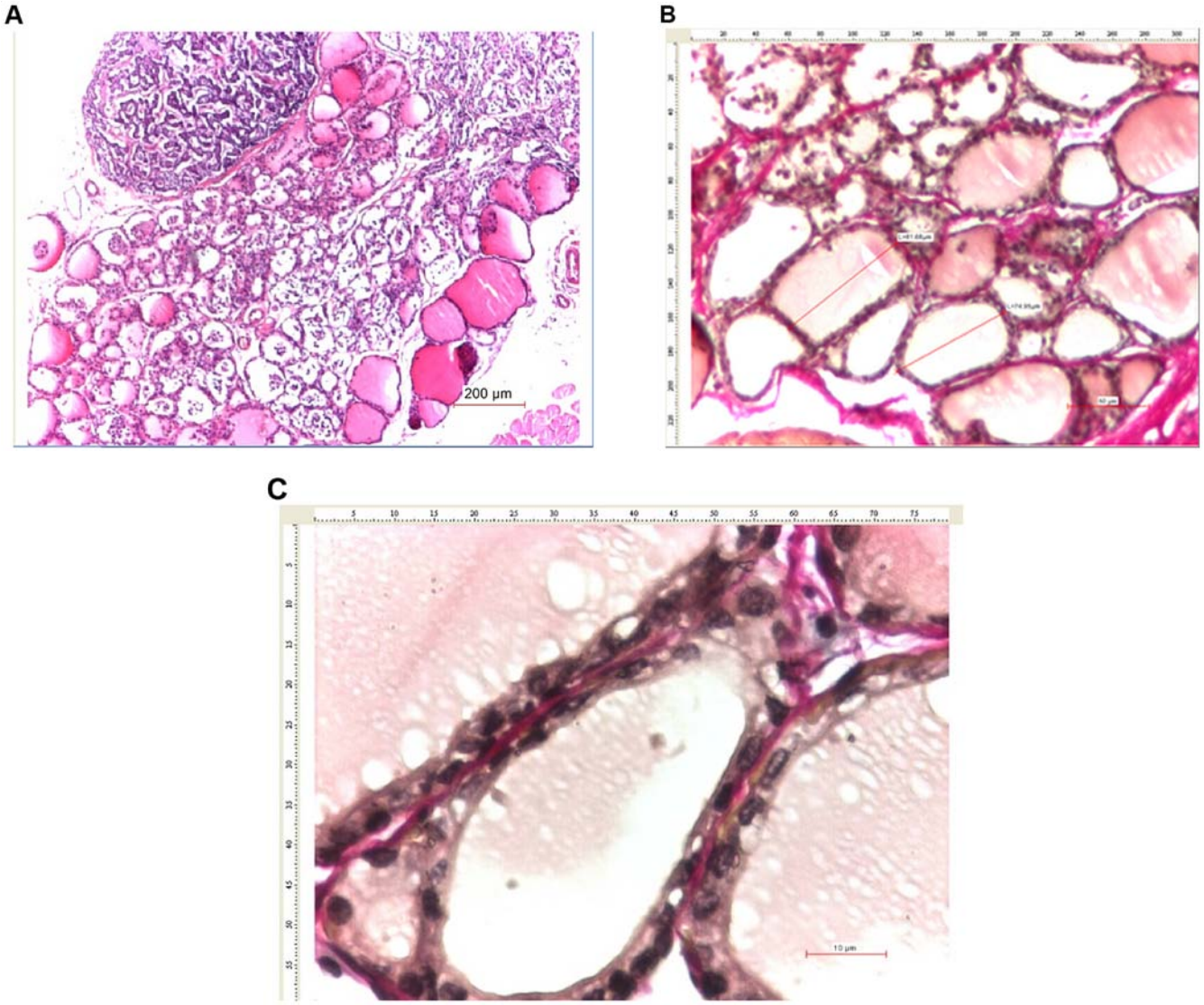


Figure 8. Thyroid images of female control rats. (A) Thyroid and parathyroid tissue (H&E, x4), (B) measurements of the maximum diameter of thyroid follicles (VG, x10) (20 thyroid follicles were measured in total) and (C) follicular epithelium details (VG, x40). H&E, hematoxylin and eosin; VG, van Gieson's stain.

were observed only within C1 (P=0.043) and C3 (P=0.049) groups (Table III).

Thyroiditis final score. In males, significant differences between treatment regimens were confirmed: In C1 group (only KI administration), 50% of the rats developed moderate thyroiditis and 33.3% severe thyroiditis; in C2 group (concomitant KI and Se administration), 33.3% of the male rats developed mild thyroiditis; and in C3 group (subsequent KI and Se administration), 50% of cases had mild thyroiditis and 16.7% moderate thyroiditis (P=0.046; Table IV and Fig. 5A).

Female rats demonstrated significant differences in overall thyroid morphology: In C1 group, 50% of cases developed moderate thyroiditis; in C2 group, 83.3% of female rats had mild thyroiditis; whereas all cases in C3 group had normal morphology (P=0.033; Table IV and Figs. 6A and 7A).

Regarding final thyroiditis score, significant sex differences were recorded only in C3 group where all females had normal thyroid morphology, similar to the female control group (P=0.049; Table III and Fig. 8A).

Discussion

In the present study, the protective role of Se on thyroid morphology in iodine-induced AIT in Wistar rats was confirmed. Se effect was more evident in female rats, as the subsequent administration of Se after iodine exposure determined minimum modifications on the thyroid morphology, preserving the normal aspect of the thyroid gland.

Iodine plays an important role in the induction and modulation of thyroid autoimmunity. Several studies have assessed the prevalence of thyroid antibodies and autoimmune hypothyroidism in patients which are located in iodine-replete versus in iodine-deficient areas (25-27).

After iodine prophylaxis in iodine deficient areas, a 4-fold increase in the prevalence of anti-thyroid antibodies has been reported (28). According to a Danish survey, following the administration of 500 μg/day iodine dose for 6 months, AIT occurred in 20% of the healthy individuals that were included in the study (29).

The same results were obtained using NOD.H2^{h4} mice as experimental animal models for genetically determined AIT.

Iodine enrichment in these mice showed increased incidence and severity of the disease in a dose-dependent manner (1). Several possible mechanisms have been described by which iodine could trigger AIT. Iodine exposure leads to increased iodination of thyroglobulin and, therefore, to increased antigenicity (immunogenicity) by creating new iodine-containing epitopes or by discovering cryptic epitopes. This facilitates antigen exposure (antigen processing or antigen presentation) and, thus, increases T cell receptor binding and activation of T cells, respectively (1).

Secondly, increased iodine exposure determines the increase of reactive oxygen species (ROS) in thyrocytes. ROS may increase the expression of intracellular adhesion molecule 1 in the thyroid follicular cell, which subsequently attracts immunocompetent cells to the thyroid gland. The binding of ROS to the phospholipidic membrane may induce injury to the thyroid and the release of auto-antigens (28). In addition, iodine excess promotes apoptosis of thyroid follicular cells by inducing the expression of TRAIL (TNF-related apoptosis-inducing ligand) necrosis factor and its receptor, death receptor-5, in the thyroid. There is also *in vitro* evidence of the iodine's influence on the immune system cells as it may increase dendritic cell maturation, and increase the number of T cells, as well as the production of immunoglobulins (28).

In the present study, the EAT in adult male and female Wistar rats was induced by KI administration in the drinking water. Most studies in the literature, which sought to induce AIT, used genetically modified animal models, NOD.H2^{h4} (1,13) and BB/W (Bio Breeding/Worcester) rats (30,31). NOD mice and BB/W rats are animal models generally used for the study of type 1 diabetes. NOD.H2^{h4} is a genetically animal model predisposed to develop AIT over time. Iodine administration increases the prevalence of AIT, earlier occurrence, and a more severe form of disease (32). A similar study was performed on BB/W rats by looking at the effect of excess iodine on thyroid function and on immunological phenomena that trigger AIT. The results of the study showed an increase in the number of dendritic cells and lymphocyte infiltrate in animals receiving additional iodine in drinking water (31). Both the increase in the number of dendritic cells and the lymphocyte infiltrate are possible mechanisms involved in triggering AIT.

In a study on NOD.H2^{h4} mice of different ages, it was observed that the prevalence and severity of AIT increased with age in genetically predisposed animals (32). In the present study, only young adult Wistar rats were used, with no genetic predisposition to influence the onset or the form of the disease. This was also preferred in order to have a homogenous group and to avoid possible age-induced changes in the experiment.

Regarding the presence of thyroid inflammation in both male and female Wistar rats, no significant changes between the treatment regimens were described, the results being similar to those in the control group. The absence of inflammation could be explained by the short period of iodine administration or by insufficient iodine quantities. Similar studies that obtained inflammatory changes had administered iodine up to 12 weeks (32-34).

The evolution of follicular epithelioum size shows potential benefits of Se treatment as favourable statistically significant differences were observed between the measured parameter in the group treated with concomitant sodium selenite and KI administration, in comparison with the group administered with only iodide.

Significant changes were observed in the groups treated with KI and Se compared with the KI treated groups, with forms of thyroiditis less aggressive in both males and females treated with Se. In rats with sequentially KI and sodium selenite administration, the same favourable outcomes were not obtained as in the case of the concomitantly treated groups; this effect was only observed in males. The females initially treated with KI and subsequently with Se had a surprising evolution, the results being almost identical to those of the control group, as they no longer had AIT. Experimental studies have shown that the antigen that initiates AIT in animal models is thyroglobulin, regardless of species (studies in mice, rats and birds) (35). The thyroglobulin allografts affect the susceptibility to thyroiditis. Moreover, modulating genes related to the X-chromosome have been highlighted, which could explain the different responses in AIT not only in animals, but also in humans (36). The effectiveness of Se supplementation proved to be different depending on the time of treatment initiation and sex.

Autoimmune thyroid disease is highly prevalent, with the highest female-to-male ratio among all autoimmune diseases (37). There is a large body of evidence that moderate amounts of estrogen may enhance immunologic reactivity to self-antigens (38,39). However, as AIT is frequently diagnosed after menopause, the X-chromosome seems to be the source of enhanced susceptibility rather than sex steroid levels. For example, X-chromosome inactivation has been associated with autoimmune thyroid disease (40). However, there have been reports in men that confirm a connection between estradiol levels (or estradiol to testosterone ratio) and thyroid autoimmunity (41,42).

In males, Se supplementation has been shown to be more effective with concomitant administration of KI. Males have been presented with less aggressive forms of the disease than those who had received successive administration (initially with KI and subsequently with Se). Se administration, in both concomitant and then successively treated groups, contributed to milder forms of AIT, compared with the group not supplemented with Se.

Moreover, an extremely important aspect was observed in the groups of female rats in which, unlike male rats, Se supplementation proved to be very effective. In the group of females treated successively, the thyroid morphological aspect was identical to the morphological appearance of the control group, which did not show AIT, thus advocating the remission of the KI-induced disease. Concomitant administration resulted in a significant improvement in thyroid morphology.

Overall, the results of the present study revealed the effectiveness of Se supplementation in both co-administration with KI and sequential administration in female and male sex alike. Significant sex differences were recorded in the groups initially treated with KI and subsequently with Se ($P=0.049$): While in females histological appearance of the thyroid was normal in the whole group, in males only 33% had normal thyroid, the rest having mild (50%) or medium (17%) thyroiditis. In the rest of the study groups, the differences were not statistically significant (Table III).

This was especially observed in females due to the hormonal features involved in the AIT pathogenesis. It is known that estrogen increases (while androgen decreases) the response of the hypothalamic-pituitary-adrenocortical axis to stress, and activation of this axis is more pronounced in women than in men, which explains the higher incidence of autoimmune thyroid disease in women (43).

There is a number of limitations in the present study. A low number of Wistar rats was used in each study group, although valid for statistical analysis following previous scientific research protocols. In addition, a Se dose-finding study was not performed; however, no clinical signs of Se toxicity were observed during the study at the administered dosages. An additional limitation is represented by the fact that all histopathological sections and the acquisition of images of the studied animals were performed by a single examiner and no Cohen's kappa could be established to confirm the rater's reliability.

The present study showed the effective results of Se supplementation on restoring the normal thyroid morphology in iodine induced AIT in Wistar rats.

In conclusion, the impact of induction of AIT on Wistar rats (induced by KI administration) is higher in males than in females, although the latter are more prone to the disease. Males develop more severe forms; the difference is primarily due to the modulating role of estrogens.

Se supplementation has been shown to be effective, resulting in improved forms of AIT. The timing of Se administration has also been proven to be important and concomitant administration of KI and sodium selenite is associated with the return of thyroid morphology to normal in most cases.

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Availability of data and materials

All data generated or analyzed during the study are included in this published article.

Authors' contributions

CP, DGCA, IV, OB and ILS were involved in the conception and design of the study. CP, DGCA, IV and OB acquired the data. DGCA was involved in the analysis and interpretation of the histological data. The statistical analysis and overall interpretation of data was performed by CP, DGCA, IV, IA and ILS. IV, IA, DGCA and CP drafted the manuscript. CP, DGCA, IV and IA revised critically the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of 'Grigore T. Popa' University of Medicine and Pharmacy (Iasi, Romania).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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