Functional And Histological Effects Of Organic Selenium On Ovaries Of Female Rats Induced With Hypothyroidisms

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Abstract

The present study examined the association between hypothyroidism and reproductive dysfunction by the analysis of hypothyroidism effect on gonadotropin secretion in cyclic female rats, which is rectified by supplementation of these animals with selenium. Forty cyclic females were created to four groups (10 each). The control fed normally. First females were injected with 10 mg/kg propylthiouracil , Hypothyroidism was induced in the second and third groups using PTU, followed by treatment with selenium alone or with zinc at 7 mg and 10 mg/kg body weight, respectively. Blood samples were collected at late estrus to measure levels of thyroxin(T4), triiodothyronine(T3), thyroid–stimulating hormone(TSH), follicle-stimulating hormone(FSH), estradiol, and inhibin. Also, Morphological, histological changes, and ovarian function were examined. The hypothyroid group showed a decrease of T4, T3, FSH, and E2 levels and decreased ovary weight and diameter in parallel with the number of primary, secondary, and Graafian follicles; in the meantime, there were increased levels of TSH and inhibin concentrations. Treatment with selenomethionine alone or with zinc diminished some of the adverse consequences of the depressing action of the thyroid, leading to marked improvement or normalization of the hormone levels together with the results of the ovarian histological structure. It was identified that PTU caused hypothyroidism. In contrast, in the case when female white rats were treated with selenomethionine alone or with zinc, an effect was exerted to reduce the PTU depressive action on the system of female reproduction and the quantity of the sexual hormones.

Introduction:

Hypothyroidism decreases the levels of the thyroid hormones, whether thyroxine (T4) triiodothyronine (T3), or both, increasing the secretion of TSH. This leads to hyperplasia of the thyroid gland and, subsequently, its enlargement, known as goiter (1). Other element deficiencies, such as selenium, may cause endemic goiter—a condition found in iodine-lacking areas (2). It could also result from the reduced secretion of thyroid hormones brought about by thyroid inflammation and tumors. In other cases, there is dysfunction in either the hypothalamus or pituitary gland, which results in inadequate secretion of the thyroid-stimulating hormone (TSH), hence failure to stimulate the cells that secrete the thyroid hormone (3). Hypothyroidism among women is associated with irregular menstruation, anovulation, and infertility. In the case of men, hypothyroidism leads to abnormalities of sperm morphology, resulting in reduced fertility (4). Other trace elements are selenium and zinc. The elements are essential in the support of critical physiological functions. Selenium is essential for the balance of the antioxidant system and maintaining the protection of the organism under oxidative stress conditions, and, along with other elements, it plays a role

in the regulation of thyroid hormone metabolism. As far as fertility roles are concerned, several studies have indicated that this element is essential for male reproduction, required for normal testicular development, sperm production, and motility, and has a beneficial effect on oocyte growth, maturation, and reproduction in females. Besides these, selenium supports the hormones estradiol and progesterone. Ions of Zinc also serve intercellular communication and intracellular dynamics of events that ensure physiological means and programmed cell death. Zinc is also involved in the anabolic synthesis of bone tissues calcium homeostasis, and synthesis of the thyroid hormone TRH. It is also responsible for reproductive processes such as oocyte growth and maturation, fertilization, and embryonic and placental development (5).

Materials and Methods:

Experimental Animals:

This study was carried out in the animal house of the College of Education , University of Al-Qadisiyah. Forty female adult albino rats weighing 150 and 170 grams, sixty-five days old, were used. The animals were housed in specially designed plastic cages with metal mesh covers and a water drinking system dedicated to them, and their floors were covered with wood shavings. The animals were kept in cages, cleaned, and disinfected with antiseptics. Conditions in the laboratory were standard; animals were supplied with water and food prepared according to (6), ad libitum during the whole experiment.

Experimental Design:

After confirming the regularity of the estrous cycle for two consecutive cycles, hypothyroidism was induced in the animals by intraperitoneal injection of PTU at a dose of 10 mg/kg once daily for two weeks. The animals were then divided into four groups as follows:

Negative Control Group (C): Animals were fed regular and drinking water for eight consecutive estrous cycles

Treatment Group 1 (T1): Animals with induced hypothyroidism using PTU at a concentration of 10 mg/kg of body weight for eight consecutive estrous cycles

Treatment Group 2 (T2): Animals with induced hypothyroidism were administered selenomethionine at a dose of 7 mg/kg body weight via oral gavage for eight consecutive estrous cycles

Treatment Group 3 (T3): Animals with induced hypothyroidism were administered selenomethionine at a dose of 7 mg/kg body weight via oral gavage, along with zinc at a dose of 10 mg/kg body weight for eight consecutive estrous cycles

After 40 days had passed, the animals were sacrificed during the estrous phase, and samples were collected for hormonal assays.

Induction of Hypothyroidism:

Hypothyroidism was induced using the drug PTU, administered at a daily dose of 10 mg/kg body weight, according to Ibrahim et al. (7).

Weight Parameters (Ovary Weight):

After excision and removal of fat and connective tissues, the organs were weighed using a sensitive balance.

Hormonal Assays:

Following blood sample collection, the blood was centrifuged to obtain serum for hormonal assays. The concentrations of the following hormones were determined using ready-made test kits manufactured by

ABO (British), Monobind-Inc (America), ABO (Swizerland) and Elabscience (America), according to the protocols provided by the manufacturers.

Histological Study:

Ovarian tissue sections were prepared after sacrificing the animals and excising the ovaries, followed by the removal of adhering fat. Tissue fixation and preparation of slides were carried out according to Humason et al. (8).

Statistical Analysis:

The results were subjected to statistical analysis to determine the significant differences between the means of the studied parameters. Comparisons were made using one-way analysis of variance (ANOVA1). Significant differences were identified at a probability level of 5%, and all statistical analyses were performed using SPSS software. The least significant difference (LSD) test was used to test for significant differences between means at a probability level of 0.05 (9).

Results:

Hormonal Study:

Thyroid Hormones (T4, T3, and TSH):

Table 1 shows the statistical analysis results of the levels of T4 and T3 hormones in the T1 group, these results confirmed a significant decrease ($P<0.05$) compared to the control group and the other experimental groups. However, these levels were significantly higher than those in the T1 group, but also significantly lower than those in the control group ($p<0.05$), whereas T2 and T3 groups presented a statistically significant increase ($p<0.05$) compared to the T1 group but they were still significantly lower than the control group. Furthermore, the results showed a significant or numeric elevation in the T3 group in comparison to the T2 group, while the second and third groups had no significant difference in the concentration of T4 hormone. In addition, the below table indicates a significant increase in TSH levels in the animals subjected to T1 compared to the other groups Table 2. The levels of TSH were significantly lower in the T2 and T3 groups than in the T1 group. The T2 group had a significant increase ($P<0.05$) in selenomethionine compared to the control treatment, and no difference was observed between the T3 and control group.

TABLE 1 presents the concentrations of TSH and thyroid hormones T4 and T3 in female rats with induced hypothyroidism and treated with selenomethionine alone or in combination with zinc.

Parameters / Groups	T4 (µg/dL)	T3 (ng/ml)	TSH (µIU/mL)
C	3.63 ± 0.41a	3.12±0.16a	4.65±0.05c
T1	1.99 ±0.18c	1.37±0.14c	10.91±0.57a
T2	2.76 ± 0.04 b	2.11±0.19b	8.98±0.35b
T3	3.02 ±0.037 ab	2.83±0.16a	5.56±0.49c
LSD Value	**0.654**	**0.478**	**1.21**

Values represent the mean ± standard error. Similar letters among means within columns indicate no significant differences at a probability level of (0.05). C: Represents the control group which received the regular diet and drinking water. T1: (First treatment) Induced hypothyroidism using PTU at a concentration of 10 mg/kg of body weight. T2: (Second treatment) Induced hypothyroidism using PTU and then dosed

with selenomethionine at a concentration of 7 mg/kg of body weight daily. T3: (Third treatment) Induced hypothyroidism using PTU and then dosed with selenomethionine at a concentration of 7 mg/kg and zinc at a concentration of 10 mg/kg of body weight daily.

Reproductive Hormones

The results of the study recorded in Table (2) showed a significant decrease in the levels of follicle-stimulating hormone (FSH) and estradiol in group T1 compared to the control group and the other groups. In contrast, there was a significant increase in the level of FSH in group T2 compared to T1. Group T3 showed a significant increase compared to T1 and T2, while no significant differences were found between T2 and the control group, as well as between T3 and the control group. The results also showed a significant increase in the level of estradiol in group T2 compared to T1, while it significantly decreased compared to the control. Conversely, no significant differences were recorded between T3 and the control group. Regarding inhibin hormone, the current study results showed that T1 achieved a significant increase in the concentration of inhibin hormone compared to the control and other groups. On the other hand, the results showed a significant decrease in the hormone concentration in groups T2 and T3 compared to T1, and a significant increase compared to the control. Group T2 showed a significant increase compared to T3.

TABLE 2 shows the concentrations of FSH, Estradiol, and Inhibin in female rats induced with hypothyroidism and treated with selenomethionine alone or with zinc.

Parameters / Groups	FSH (ng/ml)	E2 (pg/ml)	Inhibin (pg/ml)
C	4.03±0.04a	62.22±0.12a	607.71±21.8a
T1	1.33±0.0.08b	30.80±1.005b	915.78±1.86b
T2	3.71±0.03c	40.26±2.59c	747.8±7.45c
T3	3.88±0.12ac	56.38±0.35d	681.14±24.3d
LSD Value	**0.221**	**4.02**	**48.21**

Values represent the mean ± standard error. Similar letters among means within columns indicate no significant differences at a probability level of (0.05). C: Represents the control group which received the regular diet and drinking water. T1: (First treatment) Induced hypothyroidism using PTU at a concentration of 10 mg/kg of body weight. T2: (Second treatment) Induced hypothyroidism using PTU and then dosed with selenomethionine at a concentration of 7 mg/kg of body weight daily. T3: (Third treatment) Induced hypothyroidism using PTU and then dosed with selenomethionine at a concentration of 7 mg/kg and zinc at a concentration of 10 mg/kg of body weight daily.

Morphological and Histological-Functional Changes

Ovary Weight and Diameter

From Figures (1 and 2), we observe a significant decrease in ovary weight and diameter in group T1 compared to the control group and the other groups. On the other hand, groups T2 and T3 showed a significant increase compared to group T1 and a significant decrease compared to the control group. Group T3 outperformed group T2 in terms of ovary diameter.

Number of Ovarian Follicles

The results of the statistical analysis shown in Figure (3) indicated a significant decrease in the number of primary, secondary, and Graafian follicles in group T1 after treating the animals in this group with PTU

compared to the others. Conversely, the results indicated no significant differences between groups T2 and T1, as well as between groups T2 and T3. The results also indicated no significant differences between groups T3 and T1.

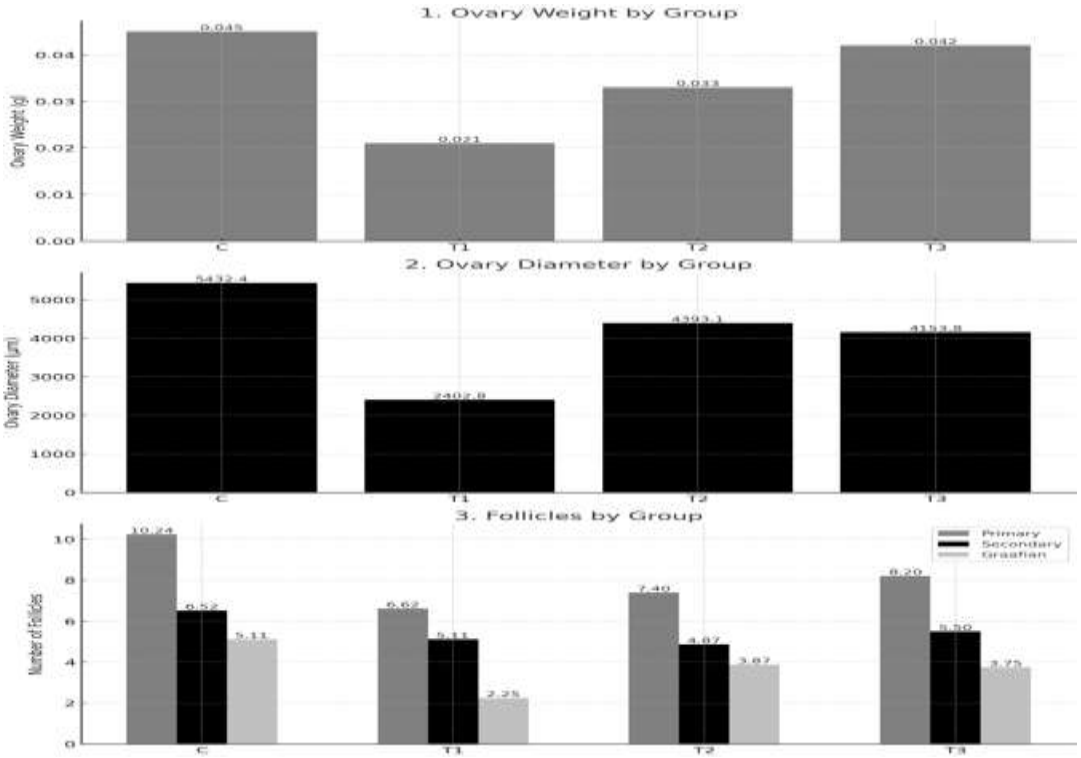

FIGURE (1,2,3) shows the weights, diameters of the ovaries and numbers of primary, secondary, and Graafian follicles in female rats induced with hypothyroidism and treated with selenomethionine alone or in combination with zinc.

Histological Changes

Histological sections study of the control group showed the ovarian stroma with normal follicular growth in the form of primary follicles, secondary follicles, and Graafian follicles. On the other hand, feeding resulted in a significant reduction in primary and secondary follicles and also Graafian follicles compared to the control, the second, and the third group, caused by hypothyroidism, verifying the presence of the effect of hypothyroidism lowering fecundability as previously reported. The transfer of embryos resulted in a slight reduction compared to the control group, but it was nonsignificant. The second group was given selenomethionine alone while the third group received zinc together with this one. As indicated in Figure 4, the second and third groups demonstrated a significant elevation of primary, secondary, and Graafian follicles.

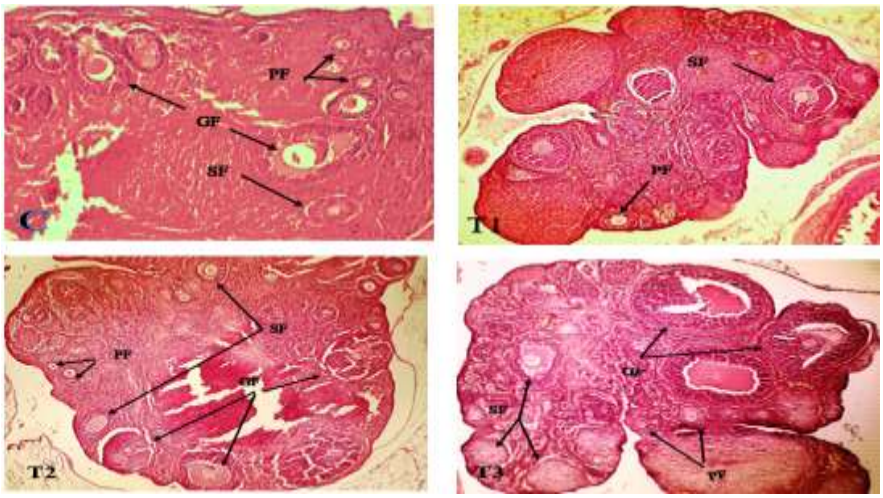

FIGURE 4 (C,T1, T2, T3) A histological section taken from the ovaries of the T1: (First treatment) Induced hypothyroidism using PTU at a concentration of 10 mg/kg of body weight. T2: (Second treatment) Induced hypothyroidism using PTU and then dosed with selenomethionine at a concentration of 7 mg/kg of body weight daily. T3: (Third treatment) Induced hypothyroidism using PTU and then dosed with selenomethionine at a concentration of 7 mg/kg and zinc at a concentration of 10 mg/kg of body weight daily. shows the presence of primary follicles (PF), secondary follicles (SF), and Graafian follicles (GF) (Eosin-Hematoxylin X100).

Discussion:

The decrease in T3 and T4 levels may be due to PTU inducing hypothyroidism and reducing hormone formation (10). PTU lowers thyroid hormone production by inhibiting thyroid peroxidase and 5-deiodinase, key enzymes in hormone synthesis. If hormone production does not decrease, the improvement in thyroid hormone levels may be due to selenomethionine increasing T4 to T3 conversion and reducing TSH levels (11). According to Pirola et al. (12), 5-deiodinase is a selenium-dependent enzyme, and selenium is found in high concentrations in the thyroid gland. Brigelius-Flohe and Flohe (13) state that selenium is essential for forming selenoproteins like GPX, SelP, and TrxR, which are vital for thyroid cell growth, development, and function. These selenoproteins are crucial for the synthesis, metabolism, and protection of thyroid hormones from oxidative stress. Consequently, selenium deficiency leads to fewer 5-deiodinase enzymes, resulting in lower T3 levels and causing hypothyroidism. Zinc plays a crucial role in thyroid metabolism and the conversion of T4 to T3. The increase in thyroid hormones due to zinc may be attributed to its impact on 5-deiodinase activity through its antioxidant properties. Beserra et al. (14) found similar results, noting that zinc supplementation improved thyroid function in nine patients with low zinc levels (15). Zinc metabolism and thyroid hormones are closely linked (16), and zinc deficiency might significantly affect thyroid hormone development in patients with low FT3 levels, normal T4, and mild to moderate zinc deficiency. Oral zinc supplementation for 12 months resulted in increased FT3 and TT3 levels and normal TSH levels in the blood. The reduction in follicle-stimulating hormone (FSH) levels may be due to the adverse impact of PTU treatment on the hypothalamic axis, which secretes gonadotropin-releasing hormone (GnRH) that regulates anterior pituitary hormones, including FSH. Inhibition of GnRH secretion leads to reduced secretion of pituitary hormones, resulting in decreased FSH levels, impairing follicle development and maturation, and preventing ovulation. This aligns with findings from Saran et al. (17), who noted that hypothyroidism leads to decreased FSH and E2 levels. However, another study found that FSH levels did not differ in hypothyroid rats compared to the control group (18). Thecal cells regulate steroid activity in granulosa cells and produce androstenedione, which is converted to estrogen by aromatase in granulosa

cells under the influence of FSH from the pituitary gland. Therefore, the decrease in FSH levels in hypothyroid rats leads to impaired follicle growth, reduced aromatase activity, increased hyperandrogenemia, and decreased estrogen levels (19). Liu et al. (20) found that hypothyroidism is linked to the inhibition of cytochrome enzyme expression, preventing the synthesis of sex hormones, including estrogen. Additionally, the decrease in the number of healthy follicles in ovarian cells observed in the first treatment group led to a greater reduction in sex hormone levels. The results showed a significant increase in inhibin hormone concentration in the first treatment group, possibly due to the decreased level of pituitary FSH, indicating dysfunction in the hypothalamic-pituitary-gonadal axis. Razavi et al. (21) demonstrated that selenium supplementation increases E2 concentration and follicle growth in rabbit ovaries. Selenium's antioxidant effect increases the proliferation of thecal and granulosa cells, protecting them from oxidative damage and enhancing the hypothalamic-pituitary-gonadal axis, increasing gonadotropin hormone levels (22). The zinc group outperformed others due to zinc's role in neutralizing free radicals and its involvement in the enzyme superoxide dismutase, which regulates GnRH secretion, affecting FSH levels and follicle growth and development in the ovary. This was evident during the histological examination. The increase in blood estradiol levels originates from developing follicles in the ovary, as indicated by the presence of growing follicles observed during the histological examination. The increased FSH concentration activates several intracellular chemical reactions, including the activation of the P450 aromatase gene, which determines the E2 produced from the developing ovarian follicle (23). In the current study, the T2 and T3 groups showed a clear improvement in thyroid function, evidenced by decreased TSH levels compared to the control group. Additionally, the concentration of sex hormones and gonadotropins improved in these groups compared to the control. This improvement indicated a close association between thyroid dysfunction and hypothalamic-pituitary-gonadal axis disorders. PTU treatment significantly affected the histological structure of the ovaries, resulting in a significant decrease in ovary weights in the T1 group. Hypothyroidism affects FSH levels, disrupting the negative feedback mechanism and impacting the ovary since thyroid hormones stimulate metabolic processes and energy production in ovarian tissue cells, affecting ovarian tissue growth and differentiation (24). The number of ovarian follicles decreases, leading to reduced ovary weights. FSH promotes follicular growth, granulosa cell activity, and the formation of thecal layers, essential for development (25). Groups T2 and T3 showed improved ovary weights due to selenomethionine increasing FSH levels, stimulating follicular growth, and estrogen secretion, crucial for protein synthesis and ovarian maturation (26). Zinc treatment improved histological structure and hormone levels by preventing free radical damage and enhancing steroid hormone biosynthesis. This explains the rise in primary, secondary, and antral follicles in T2 and T3 compared to T1, which had reduced follicles due to hormonal imbalance from the drug (27). Histologically, T2 and T3 showed follicular growth, likely due to FSH stimulation reactivating the reproductive system until ovulation (28). Selenomethionine also promoted thyroid hormone secretion, working synergistically with the ovary. Thyroid hormones, especially T3, stimulate granulosa cells in ovarian follicles with FSH, increasing LH-hCG receptors and enzymes like 3 β-hydroxysteroid dehydrogenase and aromatase, promoting steroid hormone synthesis (29). Increased FSH and E2 secretion activates the cAMP system, leading to estradiol production (30). The significant decrease in ovarian diameters in T1 may be due to fewer follicles, leading to reduced estradiol secretion and inhibited cell division. T2 and T3 showed increased ovarian diameters compared to T1 but decreased compared to the control group, with no significant difference between T2 and T3, likely due to the correlation between ovarian diameter and follicular content (30).

Conclusion:

From the study, we can, therefore, conclude that propylthiouracil can induce hypothyroidism with adverse effects on thyroid function. The condition had adverse effects on some reproductive hormones and the structural composition of ovarian tissue. However, the study found that there may be an ameliorating effect from selenium alone or in combination with zinc.

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