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profile of some reproductive hormones in female rats induced with hypothyroidisms and treated with organic selenium

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Abstract:

This research aimed to assess the adverse effects of hypothyroidism on body changes, the female reproductive system, and antioxidant status, and to examine how selenomethionine and zinc could counteract these effects in female white rats. The study involved 40 female white rats, randomly divided into four groups: a negative control group (C) given regular feed and water; a first treatment group (T1) injected with propylthiouracil (PTU) at 10 mg/kg body weight; a second treatment group (T2) with induced hypothyroidism treated with selenomethionine at 7 mg/kg body weight; and a third treatment group (T3) treated with PTU and given both selenomethionine at 7 mg/kg and zinc at 10 mg/kg body weight. At the end of the experimental period, the final body weight of the females was recorded, and they were sacrificed during the estrus phase. Levels of free thyroid hormones, growth hormone, Estradiol hormone, oxidative stress markers (GSH, MDA), and ovarian follicle counts were evaluated. Results showed that the hypothyroid group had decreased body weight and lower FT4, FT3, GH,E2 and GSH levels, while MDA levels significantly increased. This was accompanied by a notable reduction in ovarian activity, as indicated by decreased ovarian follicle counts. Treatment with selenomethionine alone or combined with zinc alleviated some negative effects of reduced thyroid activity, showing a positive trend compared to the untreated group. We conclude that hypothyroidism adversely affects reproductive functions, but administering selenomethionine alone or with zinc improves antioxidant activity, thyroid hormone levels, and reproductive activity in female white rats

Keywords: hypothyroidisms, selenium, Estradiol, body weight

Introduction

Thyroid hormones are crucial for the female reproductive system as they regulate metabolism and the development of ovarian, uterine, and placental tissues. Hypothyroidism can lead to reduced fertility, irregular menstrual cycles, anovulation, miscarriage, premature birth, preeclampsia, and mental retardation in children (1). Oocytes, granulosa cells, and ovarian tissue cells express receptors for thyroid hormones, indicating that T3 and T4 directly affect ovarian tissues. In conjunction with FSH, thyroid hormones stimulate ovarian follicle growth and enhance granulosa cell proliferation while reducing apoptosis. They also help prevent excessive androgen production and promote estrogen production (2). Growth hormone (GH) is a protein of 191 amino acids, secreted by somatotrophs in the anterior pituitary gland, regulated by growth hormone-releasing hormone (GH-RH) and somatostatin (SS). GH and insulin-like growth factor 1 (IGF-1) facilitate body growth by acting on major metabolic organs like the liver, muscles, and bones. GH receptors (GHR) are found in mouse oocytes and the entire reproductive system. IGF-1 stimulates pituitary gonadotropin biosynthesis and regulates puberty via direct action on GnRH neurons (3). GH interacts with GHR through cAMP and PKA (4), enhancing cell proliferation, preventing apoptosis, and supporting cumulus cell expansion, crucial for oocyte maturation. However, GH cannot fully prevent apoptosis in early antral follicles (5). Micronutrients like selenium and zinc are vital for physiological balance. Selenium protects against oxidative stress, enhances male fertility, and regulates thyroid function. It's crucial in enzymes such as glutathione peroxidase and thioredoxin reductase, found in various body tissues (6). The thyroid gland has the highest selenium concentration. Selenoproteins like GPXs and TRs neutralize H2O2, protect cell membranes, and repair molecular damage, providing antioxidant and anti-inflammatory effects. Selenium also regulates granulosa cell growth and 17β-estradiol synthesis in ovaries (7). Glutathione protects ovarian follicles from apoptosis and shields oocytes from oxidative damage during folliculogenesis, affecting oocyte quality (8). Zinc ions regulate key cellular functions like signaling, transcription, and replication. Zinc is crucial for thyroid function, aiding the deiodinase enzyme in converting T4 to active T3 and reducing metabolism (9). It's vital for female germ cell growth, fertility, and pregnancy, maintaining meiotic arrest in oocytes during follicular development. Zinc deficiency during pregnancy impairs placental integrity, fetal limb growth, and neural tube development (10). This study aims to explore the role of selenomethionine and zinc in mitigating hypothyroidism's negative effects in female rats.

Material and methods

Experimental Animals :

Forty Virgin cycling female rats, weighing between 150-170 grams and aged 65days, were used. The rats were housed in special plastic cages with metal mesh covers, equipped with a water drinking system, and lined with wood shavings. The cages were cleaned and disinfected regularly. The rats were kept under suitable laboratory conditions at a temperature of 20-25°C.

They were provided with water and food *ad libitum*, prepared according to Ward's (11) formula, throughout the experiment.

Experimental Design:

This study was conducted at the animal house of the College of Education, University of Al-Qadisiya. After confirming regular estrous cycles over two consecutive cycles, hypothyroidism was induced in the animals by injecting 10 mg/kg PTU (propylthiouracil) intraperitoneally once a day for two weeks. The animals were then divided into four groups:

1. Negative Control Group (C): 10 animals given standard diet and drinking water for eight consecutive estrous cycles

2. Treatment Group 1 (T1): 10 animals with induced hypothyroidism for eight consecutive estrous cycles

3.Treatment Group 2 (T2): 10 animals with induced hypothyroidism, were given selenomethionine at a dose of 7 mg/kg body weight orally for eight consecutive estrous cycles.

4. Treatment Group 3 (T3): 10 animals with induced hypothyroidism, given selenomethionine at a dose of 7 mg/kg body weight and zinc at a dose of 10 mg/kg body weight orally (1 ml per animal) for eight consecutive estrous cycles.

After 40 days had passed, the animals were sacrificed in the estrous phase, and samples were collected for physiological blood tests and the study of ovarian follicle development and numbers.

Vaginal Smear Examination :

To ensure regular estrous cycles, vaginal smears were performed using the pipette smear technique. 100 microliters of normal saline (NaCl 0.9%) were gently injected into the vagina using a micropipette. After washing the vagina, part of the fluid was drawn back into the pipette. The collected fluid was then placed on a clean glass slide for microscopic examination. The samples were examined immediately under 10X and 40X magnification.

- A smear with many cornified cells in clumps indicates the estrus phase.

- A large number of small leukocytes indicates the metestrus phase.

- A mixture of leukocytes, epithelial cells, and cornified cells indicates the diestrus phase.

- Predominantly epithelial cells indicate the proestrus phase (12).

Induction of Hypothyroidism :

Propylthiouracil (PTU) was used to induce hypothyroidism with a daily dose of 10 mg/kg body weight (13). The dose was dissolved in distilled water, and each animal (except the control group) received a 1 ml intraperitoneal injection daily for two weeks. Blood tests confirmed hypothyroidism, showing decreased T4 and T3 levels and increased TSH levels.

Blood Sample Collection :

Blood samples were centrifuged at 3000 rpm for 15 minutes to separate the serum, which was then stored in Eppendorf tubes at -20°C for laboratory tests.

Studied Parameters:

Weight Parameters :

Weight Gain (g): Calculated by subtracting the initial average weight from the final average weight after the experiment.

Hormonal and Antioxidant Markers:

ELISA technique was used to measure serum concentrations of FT3, FT4, GH, E2 , GSH and MDA according to the manufacturer's instructions (ABO, British; ABO, Switzerland; Biosolar, China).

Statistical Analysis:

Analyzed using SPSS to find significant differences ($p<0.05$) between group means, including mean, standard error, and one-way ANOVA (14).

Result

Rate of Body Weight Changes (g):

Table 1 shows a significant decrease ($P<0.05$) in weight gain for the T1 group compared to the control (C) and other groups. Hypothyroid rats treated with selenomethionine alone or with zinc also had significantly lower weight changes than the control. However, the T3 group showed a significant increase ($P<0.05$) compared to the T2 group.

Table 1: Rate of Body Weight Changes in Female Rats with Induced Hypothyroidism Treated with Selenomethionine Alone or with Zinc.

Parameters / Groups	Initial weight (g)	Final weight (g)	Rate of weight changes

C	156±0.84a	212.4±2.1a	56.4±2.26a
T1	157.9±1.15a	88.4±2.04d	-69.5±2.16d
T2	157.5±1.36a	181.3±1.86c	23.8±1.86c
T3	156.6±1.8a	201.5±1.41b	44.9±2.75b
LSD value	**3.84**	**5.38**	**6.55**

The values denote mean values with their corresponding standard errors. Matching letters among the means in each column indicate no statistically significant differences at a significance level of 0.05.

C: Control group received standard feed and water. T1: Hypothyroidism induced with PTU (10 mg/kg body weight). T2: Hypothyroidism induced with PTU, then administered selenomethionine (7 mg/kg body weight daily). T3: Hypothyroidism induced with PTU, then administered selenomethionine (7 mg/kg body weight daily) and zinc (10 mg/kg body weight daily).

The hormonal study:

Statistical analysis in Table (2) indicated a significant decrease ($P < 0.05$) in levels of FT4, FT3, estrogen, and growth hormone in group T1 compared to the control and other experimental groups. Meanwhile, groups T2 and T3 showed a significant increase ($P < 0.05$) compared to T1 and achieved a significant decrease ($P < 0.05$) compared to the control. Results also demonstrated a significant increase ($P < 0.05$) in group T3 compared to T2.

Table 2 shows the concentrations of thyroid hormones and some sex hormones in female rats induced with hypothyroidism and treated with seleniummethionine alone or with zinc.

Parameters / Groups	FT4 (ng/dL)	FT3 (pg/dL)	Estradiol (pg/ml)	GH (ng/ml)
C	35.76±1.25a	2.46±0.32a	50.38±0.35a	11.3±0.22a
T1	17.54±0.56d	1.23±0.14c	30.80±1.005c	7.08±0.38d
T2	23.76±0.20c	1.72±0.18bc	40.26±2.59b	9.81±0.36b
T3	28.94±0.42b	2.24±0.23ab	52.22±0.12a	8.26±0.40c
LSD value	**2.08**	**0.667**	**4.02**	**1.01**

The values denote mean values with their corresponding standard errors. Matching letters among the means in each column indicate no statistically significant differences at a significance level of 0.05.

C: Control group received standard feed and water. T1: Hypothyroidism induced with PTU (10 mg/kg body weight). T2: Hypothyroidism induced with PTU, then administered selenomethionine (7 mg/kg body weight daily). T3: Hypothyroidism induced with PTU, then administered selenomethionine (7 mg/kg body weight daily) and zinc (10 mg/kg body weight daily).

Ovarian activity

From the statistical analysis results in Table 3, it is observed that treatment of white female rats with PTU caused a significant decrease in the number of primary and secondary follicles, as well as Graafian follicles, compared to the control treatment. However, when treating rats induced with hypothyroidism (T3&T2), a significant improvement in the number of ovarian follicles is noted compared to the first treatment. Nevertheless, the number of follicles decreased for the second and third treatments compared to the control treatment.

Table (3) shows the numbers of primary and secondary follicles and Graafian follicles for female rats in which hypothyroidism was induced and treated with selenomethionine, selenomethionine, and zinc

Parameters / Groups	Primary follicles	Secondary follicles	Graafian follicles
C	10±4.44a	6.5±2.07a	5.12±0.83a
T1	6.37±1.84b	2.25±0.71c	1.62±0.51c
T2	7±0.75b	4.87±2.29b	3.87±1.35b
T3	8.1±2.1ab	5.75±0.88ab	3.75±0.46b
LSD value	**2.71**	**1.68**	**0.89**

The values denote mean values with their corresponding standard errors. Matching letters among the means in each column indicate no statistically significant differences at a significance level of 0.05.

C: Control group received standard feed and water. T1: Hypothyroidism induced with PTU (10 mg/kg body weight). T2: Hypothyroidism induced with PTU, then administered selenomethionine (7 mg/kg body weight daily). T3: Hypothyroidism induced with PTU, then administered selenomethionine (7 mg/kg body weight daily) and zinc (10 mg/kg body weight daily).

Antioxidant indicators:

From the result of figure 1, the results of the current study indicated a significant decrease ($P<0.05$) in GSH levels in group T1 compared to the control and the other experimental groups. Conversely, groups T2 and T3 showed a significant increase ($P<0.05$) in GSH levels compared to T1, although they decreased significantly ($P<0.05$) compared to the control. Regarding MDA levels, there was a significant increase ($P<0.05$) in group T1 compared to the control and the other experimental groups. Additionally, groups T2 and T3 exhibited a significant decrease ($P<0.05$) compared to group T1, despite showing higher levels than the control, and MDA levels were lower in the third group compared to the second group.

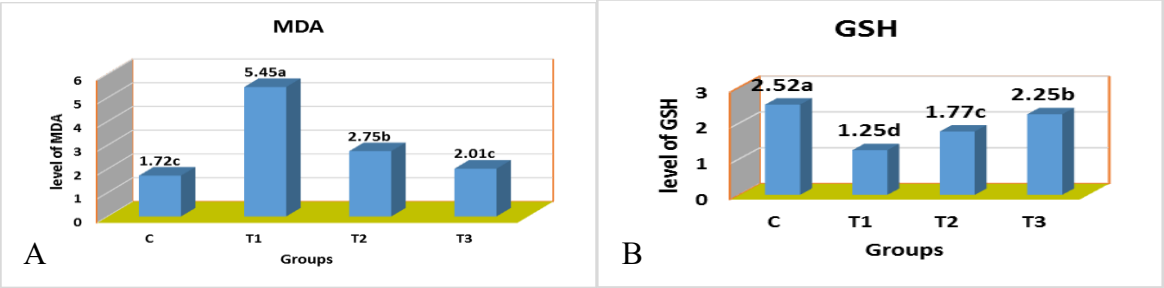

Figure 1 (A& B) shows the concentration of malondialdehyde (MDA) and glutathione (GSH) in female rats induced with hypothyroidism and treated with selenium-methionine alone or with zinc.

C: Control group received standard feed and water. T1: Hypothyroidism induced with PTU (10 mg/kg body weight). T2: Hypothyroidism induced with PTU, then administered selenomethionine (7 mg/kg body weight daily). T3: Hypothyroidism induced with PTU, then administered selenomethionine (7 mg/kg body weight daily) and zinc (10 mg/kg body weight daily).

Discussion

The study investigates the effects of propylthiouracil (PTU), an antithyroid drug, on body weight in female rats. The results showed a significant decrease in body weight in PTU-treated rats compared to controls and other treatments. This weight loss is attributed to reduced energy consumption and increased fat levels, leading to elevated leptin, a hormone regulating appetite, in hypothyroid conditions. Previous studies support this, noting decreased food intake with reduced thyroid activity (15). In hypothyroid conditions, protein autolysis also occurs, further contributing to weight loss (16). Conversely, the administration of selenomethionine and zinc, both individually and combined with PTU, mitigated the negative effects of PTU. Selenium, through glutathione peroxidase, prevents lipid peroxidation, adjusting metabolism and increasing food intake (17). Elevated estrogen levels also contribute to weight gain by promoting protein synthesis and muscle hypertrophy (18). Rats treated with selenomethionine and zinc showed significant weight gain compared to PTU treatment alone, though less than controls. Zinc likely increases appetite and feed intake, aiding gradual weight improvement, and its antioxidant properties enhance protein metabolism The combined effect of zinc and vitamin E in cell membranes boosts metabolic activities and overall health, leading to weight gain (19).This study's results are consistent with Ibrahim et al. (13), showing that PTU exposure in rats significantly decreased serum FT4 and FT3 levels and increased serum TSH levels in the first treatment (T1). The reason for this decline may be due to PTU's role in reducing thyroid hormones by inhibiting the enzymes peroxidase and 5-deiodinase, crucial for thyroid hormone synthesis (20). Selenomethionine supplementation increased FT4 and FT3 levels, likely due to selenium's role in enhancing T4 to T3 conversion (21). Selenium, essential for selenoproteins in the thyroid, supports hormone synthesis and protects against oxidative stress, Selenium depletion reduces 5-deiodinase activity, leading to lower T3 and hypothyroidism (22). Zinc also increased

thyroid hormone levels, consistent with findings by (23). Zinc's antioxidant properties enhance deiodinase activity and T4 to T3 conversion (24). Zinc improved thyroid function and hormone levels in patients with zinc deficiency (25). Zinc and thyroid hormone metabolism are closely linked, and addressing zinc deficiency can normalize hormone levels (26). The reduction in growth hormone (GH) levels in animals treated with propylthiouracil (PTU) is likely due to the inhibition of growth hormone-releasing hormone (GHRH), leading to decreased GH secretion and reduced GH stores in the pituitary gland during hypothyroidism. This finding aligns with (27), who noted that thyroid hormones influence GH synthesis and metabolic functions. Lower estrogen levels may also contribute to decreased GH, as GH interacts with estrogen and is essential for various reproductive functions, including follicle development and steroid synthesis (28). GH may stimulate steroid synthesis in granulosa cells or enhance gonadotropin activity, promoting estradiol production (29). Kraemer et al. (30) highlighted that insulin-like growth factors (IGF1) support granulosa cell growth and differentiation. Reactive oxygen species (ROS) from oxidative stress can damage oocytes, and reducing oxidative stress may be a mechanism by which GH improves oocyte quality (31). In this study, the second and third treatments improved thyroid function, indicated by lower TSH levels, and enhanced antioxidant status and GH levels, suggesting a link between thyroid dysfunction and hypothalamic-pituitary-gonadal axis disruption.This study observed a notable decrease in GSH levels and an increase in MDA levels in rats treated with PTU, consistent with previous research (32) The reduction in GSH is attributed to oxidative stress induced by PTU, which raises ROS levels and depletes GSH by converting it to its inactive form, GSSG (33) PTU also reduces NADPH, crucial for GSH regeneration (34), and impairs GSH synthesis by increasing gamma-glutamyl transpeptidase activity (35).The combination of selenomethionine and zinc with PTU treatment lowered MDA and increased GSH levels compared to PTU alone. Selenium, a strong antioxidant, protects cells from ROS damage and is vital for the GPX enzyme, which reduces lipid peroxidation and safeguards cell membranes (36).Selenium also enhances the activity of glutathione reductase, converting GSSG back to GSH (37). Zinc boosts the activity of aconitase, leading to increased production of L-glutamate, a GSH precursor, thus elevating GSH levels (38).

Conclusion:

The results of this study conclude that daily treatment of female rats with hypothyroidism using selenomethionine alone or in combination with zinc improves reproductive function. This improvement is evidenced by an increase in the number of ovarian follicles and enhanced activity of hormones responsible for ovarian function, in conjunction with thyroid hormones.

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