

EFFECT OF PROPYLTHIOURACIL-INDUCED HYPOTHYROIDISM ON REPRODUCTIVE EFFECIENCY OF ADULT MALE RATS

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ABSTRACT

This study is designed to evaluate the effect of hypothyroidism on reproductive efficiency of adult male rats. Hypothyroid state was induced by administration of anti-thyroid drug propylthiouracil (PTU) (15 mg/kg b.w) orally by gavage for 45 days. Twenty adult male rats of (146-200gm) body weight were used in this study and divided into two groups: **control group** and **PTU treated group**. At the end of the experimental period, the rats were euthanized via chloroform . Blood was collected in order to determine serum levels of some hormones, including thyroid stimulating hormone (TSH), thyroxin (T4), triiodothyronine (T3), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone(T). Semen analysis was also done. The results of hormones assay showed that there was a significant increase ($P \leq 0.05$) in the TSH while there were a significant decrease ($P \leq 0.05$) in the serum concentrations of T4, T3, T, FSH and LH. Semen analysis showed that there was no significant difference in the sperm count while there was a significant decrease ($P \leq 0.05$) in the individual motility whereas; there was a significant increase ($P \leq 0.05$) in the abnormal and dead sperm. Thus PTU-induced hypothyroidism can causes impairment of reproductive efficiency of adult male rats.

INTRODUCTION

About 93 per cent of the metabolically active hormones secreted by the thyroid gland is thyroxine, and 7 per cent triiodothyronine, However, almost all the thyroxine is eventually converted to triiodothyronine in the tissues, so that both are functionally important (1). Thyroid hormone level can severely affect reproductive functions including fertility, pregnancy and postnatal development in humans and rat (2). PTU

is a thiouracil-derived drug which has been used to treat hyperthyroidism by decreasing the amount of thyroid hormone produced by the thyroid gland (3). PTU inhibits the synthesis of thyroid hormones; specifically, it interferes with conversion of T_4 to T_3 (4). Various investigators have shown that PTU induced hypothyroidism (5, 6). Hypothyroidism is the most frequent thyroid disease, the incidence of which is influenced by both sex and age (7). A researcher showed that Short-term hypothyroidism has no significant effect on male reproduction in adults; while severe, prolonged hypothyroidism may impair the reproductive function (8). Abnormal supply of thyroid hormones may trigger alterations in the number of sertoli cells and consequently alter testis size and number of sperm produced leading to reproductive impairment (9).

Spermatogenesis is generally divided into three distinct stages: (i) mitosis of spermatogonia (ii) meiosis to make haploid germ cells (iii) maturation of spermatids to spermatozoa, disturbance at any step could affect the process of spermatogenesis and the spermatozoa may become defective (10). Recent identification of thyroid hormone receptors (TRs) directly on the testes and finding that thyroid hormone affects the growth and development of the male testes has accelerated research in this field (11, 12).

MATERIALS AND METHODS

This study was carried out on 20 adult male albino rats (*Rattus norvegicus*), weighing (146-200) gm, at the beginning of the study; the rats were housed in the animal house of College of Veterinary Medicine /university of Basrah. They were housed in plastic cages with metal covers measuring (15×35×50), containing bedding of fine wood which was changed twice weekly. They were maintained under light dark cycle (14/10) hours, at a (25±2) C°. All rats were supplied food and water *ad libitum*.

Animal were divided into two groups; control group administrated distal water and treated group administrated PTU 15mg/kg b.w orally by gavage for 45 days. At the end of experiment, the rats were euthanized via chloroform. Testes and epididymis were removed and weighed with an electronic balance. The tail of epididymis was kept in concave watch glass contain 5 ml normal saline to be used for semen analysis. The sperm were counted according to the method of (13). The sperm individual

motility was recorded according to (14). Abnormal and dead sperms percentage were recorded in the same stained slide by eosin-nigrosin stain according to (13) .

Blood was collected from the posterior vena cava using 5 ml needles. Following collection, the blood was kept in glass plain tube then centrifuged at 3000 for 15 minutes. Serum was collected from the top clear layer after centrifugation and kept in Eppendorf tubes and stored at -20 C° until the measurement of hormones by using enzyme linked immune sorbent assay (ELISA) kit manufactured by Monobind Inc. USA.

Statistical analysis: it was performed by T test. Data were expressed as mean±SEM. Statistical significance was set at $P \leq 0.05$. The SPSS (Statistical Packages for the Social Sciences) program (V.14) were used.

RESULTS

Table (1) shows that there was a significant increase ($P \leq 0.05$) in the serum concentration of TSH in PTU treated group compared with control group, while, there was a significant decrease ($P \leq 0.05$) in the serum concentration of T_4 , T_3 , T, FSH, and LH of PTU treated group compared with control.

Table (1): Effect of PTU on serum concentrations of TSH, T_4 , T_3 , T, FSH and LH in adult male rats (M±SD) (n=10)

Treatments Parameters	Control group	PTU group
TSH	0.10±0.02 b	0.40±0.22 a
T_4	5.46±1.32 a	1.79±0.51 b
T_3	1.58±0.46 a	0.81±0.33 b
T	5.27±2.49 a	0.90±0.72 b
FSH	1.93±0.29 a	0.72±0.27 b
LH	6.41±1.39 a	2.03±0.45 b

- Value with different letters within a row refer to significant difference at ($P \leq 0.05$).

Table (2) shows there was no statistical difference in the sperm count in the PTU group compared with control group. While, there was a significant decrease ($P \leq 0.05$) in the individual motility of sperm in the PTU treated group compared with control. Also a significant increase ($P \leq 0.05$) in the abnormal and dead sperm of PTU treated group compared with control group.

Table (2) Effect of PTU on sperm characteristic of adult male rats ($M \pm SD$) (n=10)

Parameters	Treatments	Control group	PTU group
Sperm count		187.00 \pm 30.95 a	175.17 \pm 13.68 a
Individual motility		80.83 \pm 3.76 a	62.17 \pm 8.84 b
Dead sperm		13.67 \pm 2.58 b	21.00 \pm 3.52 a
Abnormal sperm		13.17 \pm 2.31 b	20.50 \pm 2.42 a

- Value with different letters within a row refer to significant difference at ($P \leq 0.05$).

DISCUSSION

The results of the present study indicate that there was a significant increase ($P \leq 0.05$) in serum concentration of TSH in rats treated with PTU (30mg/kg b.w) for 45 days period compared with control group while there were a significant decrease ($P \leq 0.05$) in the thyroid hormones (T_4 and T_3) concentrations of PTU treated rats. This agreed with (15) who reported that PTU dramatically reduced thyroid hormones. However, the present results refer to disruptor effect of PTU on hypothalamus-pituitary-thyroid (HPT) axis. Because of serum concentrations of thyroid hormones (T_3 , T_4) and TSH are commonly used as reliable indicators of the thyroid function in humans and experimental animals (16).

PTU effect may be by inhibiting the thyroid hormone synthesis and blocking the transformation of T_4 to T_3 (17). The synthesis of T_4 by thyroid peroxidase (TPO)

involves two independent steps: iodination of tyrosine and phenolic coupling of the resulting iodotyrosine residues (18). So, PTU acts by inhibiting the enzyme TPO, which adds iodide to tyrosine residues on the thyroxine hormone precursor thyroglobulin (19). The prohormone T_4 is then converted to its biologically active form T_3 by iodothyronine deiodinase (ID-I), which is present in highest amounts in liver, kidney, thyroid and pituitary (20). Only about one-seventh to one-half of the circulating T_3 , however, is of thyroid origin, and the remainder is produced through peripheral deiodination of T_4 , on the other hand, type I deiodinase is strongly inhibited by the anti-thyroid drug, PTU (21). After the discovery that ID-I is responsible for the activation of thyroxine, it has been reported that PTU, reacts with the selenenyl iodide intermediate (E-SeI) of ID-I to form a selenenyl sulphide as a dead end product, thereby blocking the conversion of T_4 to T_3 during the monodeiodination reaction (20).

However the lowered serum thyroid hormones lead to increase serum TSH by positive feedback on pituitary gland. Increased TSH secretion causes overgrowth of thyroid gland in rats treated with PTU. This result is in agreement with (22) who mentioned that a parallelism exists between thyroid hypertrophy and pituitary TSH hypersecretion, due to a decrease in thyroid hormone levels. The TSH stimulative effect on thyroid follicular cell function is modulated by the action of various molecules such as neuropeptides, peptides derived from parafollicular cells and growth factors (23).

Moreover, the results of the present study indicate that the treated rats with PTU have shown a significant decrease ($P \leq 0.05$) in the serum concentration of main androgen (T) and pituitary hormones FSH and LH as represented in table (1) compared with control group. These results referred to pituitary hypogonadism induced by hypothyroidism. The results coordinated with (24).

However, PTU may decrease production of sex hormone-binding globulin (SHBG) by the liver, thereby decreased SHBG lead to increase free T which inhibits the release GnRH secretion from anterior pituitary by negative feedback mechanism and therefore decreased of FSH and LH levels, decreased LH leads to decrease T. In men with hypothyroidism total androgen level is reduced due to multiple factors such as decreased SHBG (24). In normal males, 2% of testosterone is free (unbound), and 44% is bound to a high-affinity SHBG, and the remainder is bound to albumin and

other proteins. Free- and albumin-bound portions make up the measure known as bioavailable testosterone (25, 26).

According to the results of present study, the decreased in the T level maybe due to decrease in SHBG these results agreed with (27). (28) showed that PTU acts directly on Leydig cells to inhibit steroidogenesis by reducing the function of P450 side-chain cleavage (P450scc) enzyme and the steroidogenesis acute regulatory (StAR) protein. And decrease in production of testosterone from Leydig cells (29).

The effect of PTU on semen analysis in the present study may refer to the disruptor effect of PTU on hypothalamus-pituitary- gonadal (HPG) axis and then impairment of spermatogenesis process this may be due to the decrease in the level of T hormone in PTU treated rats. Similarly (30) mentioned that without T, conversion of round spermatids to spermatozoa during spermiogenesis is impaired. In the other hand, decreased level of thyroid hormones caused by PTU as mentioned previously in the results of the present study may affect the spermatogenesis. Since, thyroid hormone receptors (TRs) are located on the Sertoli cells in the seminiferous tubules, and it is believed that T₃ binds directly to these receptors (31). Sertoli cells are first somatic cells to differentiate in the testis and they support and nurture sperm during spermatogenesis (32). Therefore, disturbances in the thyroid function could affect spermatogenesis and male fertility (33). It is clear that hypothyroidism causes impairment to HPG axis which causes a reduction in reproductive efficiency.

تأثير قصور الدرقية المستحدث بواسطة البروبيل ثيورايل على الكفاءة التناسلية لذكور

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الخلاصة

صممت هذه الدراسة لتقييم تأثير قصور الغدة الدرقية على الكفاءة التناسلية لذكور الجرذان البالغة. حيث استحدثت قصور الغدة الدرقية بواسطة اعطاء عقار البروبيل ثيورايل المضاد للدرقية (15مغم/كغم من وزن الجسم) عن طريق الفم بواسطة التغذية الانبوبية لمدة 45 يوما. وقد أجريت هذه الدراسة على 20 من الجرذان الذكور البالغين (146-200 غم) مقسمة الى مجموعتين : مجموعة سيطرة ومجموعة عولجت بالبروبيل ثيورايل. في نهاية فترة التجربة تمت التضحية بجميع الجرذان, جمعت عينات الدم مباشرة بعد التضحية في انابيب اختبار خالية من مضادات التخثر للحصول على مصل الدم لقياس مستوى الهرمونات التالية المشتملة

على الهرمون المحفز للدرقية ,هرموني الدرقية, هرمون المحفز للجريب,الهرمون اللوتيني و الشحمون الخصوي. وكذلك اجراء تحليل المنى.

وأظهرت نتائج فحص الهرمونات زيادة معنوية في مستوى الهرمون المحفز للدرقية و انخفاض معنوي في مستوى بقية الهرمونات. في حين, أظهر تحليل المنى عدم وجود فرق معنوي في عدد النطف و انخفاض معنوي في الحركة الفردية, و زيادة معنوية في الحيوانات المنوية المشوهة والميته. وبالتالي قصور الدرقية المستحدث بواسطة البروبيل ثيوراسيل يسبب انخفاض قيمة الكفاءة التناسلية لذكور الجرذان البالغة.

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