



## Effect of aqueous rosemary extract on some sexual hormones in male rats with high thyroxine level

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### Abstract

The main purpose of this research is to evaluate the high level of thyroxine affects on each of the luteinizing hormone (LH), testosterone, follicle-stimulating hormone (FSH) of rats and to estimate the potential effect of the administration of aqueous rosemary extract and propylthiouracil against testicular toxicity induced by levothyroxine in male rats. Negative control group rats were treated with distilled water. Three groups of male rats were treated subcutaneously with 0.5 mg/kg levothyroxine for 12 days: Since day 13<sup>th</sup> to the 24<sup>th</sup> day of the experiment first group was regarded as a positive control group that received distilled water, the second group was given propylthiouracil at a dose level of 10 mg/kg b.wt, and the third was given aqueous extracts of rosemary 10 mg/kg b.wt. The results revealed that treatment with aqueous extract of rosemary lead to a significant decrease in the levels of serum testosterone while a highly significant decrease in testosterone, FSH, and LH levels in serum revealed after treatment with propylthiouracil. Thus, it can be concluded that the effect of propylthiouracil and rosemary aqueous extract resulted in dialectical hormonal results in which the favor was to propylthiouracil.

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### Introduction

Levothyroxine (LT<sub>4</sub>), synthetic thyroxine administrated orally, is the favorable treatment for patients with hypothyroidism. Moreover, it could be administered for the repression of pituitary production of thyroid-stimulating hormone in cases of not poisonous multinodular goiter and diverse forms of neoplasia in thyroid (1). However, Levothyroxine has a limit curative index, subjected patients to the risk of iatrogenic hyper- or hypothyroidism at dosage just 25% more than normal, or that are more than the most favorable dose, depending on patients' serum level of TSH (2). Data from many investigations showed that Levothyroxine induce hyperthyroidism "high level of thyroxine" in male rats at 0.5mg/kg i.p or subcutaneously for successive 12 days (3,4).

Testosterone is the major secretive production of the testis, besides the 5 $\alpha$ -dihydrotestosterone (DHT), 17-

hydroxyprogesterone, progesterone, pregnenolone, androstosterone, and androstenedione (5,6). testosterone is a first-rate endocrine agent, a piece of evidence available as a local organizer of spermatogenesis (7). Also, it is testosterone that determines the former phase of testicular migration and the growth of male exterior genitalia (8). FSH and LH are hormones of glycoprotein secreted from the pituitary gland which regulate growth, maturation, and roles of the gonad (9).

Rosemary (*Rosmarinus officinalis* L.) (general name, rosemary; family Labiatae), is a common domestic herb grown in abundant parts of the world. It is used for cosmetics, flavouring food, and as a drink (10-12). Rosemary is known to be a rich origin of effective metabolites (13) and is used in popular medicines (14). Archaeologists and anthropologists have found proof that herbs of rosemary have been used as a medical herb, culinary in cooking and for beauty in Mesopotamia, ancient Egypt, India and China

(15). *Rosemary officinalis* enhanced the immunity (16), and considered as an antimutagenic agent (17). Internal use of rosemary for dyspeptic troubles and external use for rheumatic cases and hypotonic-circulatory disorders. As well it is used in conventional medicine for migraine, headache, digestive symptoms, amenorrhea, dysmenorrhea, oligomenorrhea, states of exhaustion, poor memory, and dizziness. Externally, it is used for poorly healing wounds as a poultice, also in eczema, for injuries of the mouth and throat as an analgesic, for myalgias used topically, intercostal sciatica and neuralgia. However, it must not be used throughout the pregnancy period. There were no side effects or health prohibitions are known in synchronism with the suitable administration of fixed curative doses. Allergies have been noticed on occasions. Extremely large amounts of rosemary leave misapplied for the abortion, which could lead to spasm, gastroenteritis, vomiting, kidney irritation, uterine bleeding, deep coma and death in humans (18). This study showed the effect of both and rosemary (*Rosmarinus officinalis*) and Propylthiouracil serum of luteinizing hormone, testosterone, follicle-stimulating hormone levels with experimental hyperthyroidism.

## Materials and methods

### Chemicals

L-Thyroxine (T4), propylthiouracil (PTU) taken up from Sigma Chemical Co., USA. The testosterone, FSH, LH and T4 hormones were managed by using commercially available kits supplied by Calbiotech Inc., USA.

### Preparation of extracts

Dried leaves of Rosemary were purchased from a local supermarket in Kufa, Iraq. the leaves were grind and store in the dark. Briefly, we made the extract by stirring of powder 10 g/100 ml in distilled water for 30 minutes at 50 °C succeeded by fast refinement throughout a rough piece of cloth, then, through the refinery sheet. The definitive nominate was freeze-dried (lyophilized) and thereafter applied in the experiment (19).

### Experimental animal design

Twenty-four male albino rats 170-200 gram weight were housed in the animal house under the steady status of temperature 24±2°C for three weeks prior to the beginning of the experiment, the animal house under laboratory conditions 12/12 hours light - dark cycle (20,21) and being preserved on a standard water and diet ad libitum (22,23). The animals were treated according to the specific guidelines of the veterinary medicine faculty and the study was approved by the Animal Ethics Committee of the University of Kufa. The experimental rats were divided as the following (in which every group has six rats). Group 1 animals were received 0.1 ml/day distilled water orally by gavage and considered as negative control group. Group 2 animals were

treated subcutaneously for successive 12 days with Levothyroxine 0.5mg/kg. From the 13<sup>th</sup> to 24<sup>th</sup> day the animals were received 0.1ml/day distilled water by gavage orally in which considered positive control group. Group 3 animals were treated subcutaneously for successive 12 days with Levothyroxine 0.5 mg/kg. From the 13<sup>th</sup> to 24<sup>th</sup> day the animals were received 10 mg/kg propylthiouracil orally by gavage. Group 4 animals were treated subcutaneously for successive 12 days with Levothyroxine 0.5 mg/kg. From the 13<sup>th</sup> to 24<sup>th</sup> day the animals were received 10 mg/kg aqueous leaves extract of rosemary (*Rosemarinus officinalis*) orally by gavage.

### Blood collecting

At the end of the experiment, all rats were subjected to light ether anesthesia, then sacrificed for the collection of blood, then serum separation was performed for thyroxine hormone to confirm the state of hyperthyroidism, serum testosterone level, serum follicle stimulating hormone level, and serum luteinizing hormone level.

### Statistical analysis

The data were subjected to analysis of variance and the significance differences at  $P \leq 0.05$  which were determined by analysis of variance (ANOVA), one-way by using the statistical soft ware's sigma statistical (24).

## Results

T4 level had significantly increased in positive-control group as compared with the negative-control group as presented in (Table 1). Data in Table 2 showed that levels of serum testosterone, LH and FSH were significantly increased in the positive-control group as compared with the negative-control group. The serum testosterone level of the experimental groups was significantly decreased in PTU treated group while highly significant decreased in aqueous rosemary extract treated group in comparison with the negative control group. Serum levels of LH and FSH in the experimental groups were significantly decreased in PTU treated group while significantly increased in aqueous rosemary extract treated group as compared with the negative-control group, in other words there were insignificance differences between the LH and FSH serum levels in rosemary treated group and positive-control group.

Table 1: Effect of Levothyroxine on serum thyroxine in male rats

	Thyroxine (T4) µg/dl (Mean± SE)
Negative control	58.91±2.22B
Positive control	76.74±1.93A
LSD	8.279

Capital letters denote differences between groups,  $P < 0.05$ .

Table 2: Effect of aqueous rosemary extract and PTU on LH, testosterone, and FSH in male rats with a high level of thyroxine induced by levothyroxine

	Mean± SE		
	Testosterone (ng/dl)	FSH (mul/ml)	LH (mul/ml)
Negative control	0.715±0.027C	0.897±0.011B	0.724±0.024B
Positive control	4.950±0.371A	0.975±0.008A	0.903±0.038A
PTU	0.095±0.013D	0.756±0.017C	0.644±0.012C
Rosemary	3.497±0.146B	0.965±0.012A	0.830±0.004A
LSD	0.616498	0.039409	0.0739415

Capital letters denote differences between groups, P<0.05.

## Discussion

In this experimental project, we found that propylthiouracil administration decreased serum testosterone, LH and FSH levels which seem to have more effective role than rosemary in comparison their data with untreated control group. This is definitely related with role of PTU in decreasing T4 level and limited the side effect of high level of thyroxine represented with raise serum level of LH and FSH. Hyperthyroidism is related to main changes in testosterone metabolism, the harmonious findings have been determined that the LH and FSH level responses to gonadotropin-releasing hormone (GnRH) administration are increased in men hyperthyroidism (25). This oddity of the hypothalamic-pituitary-gonadal axis correlates with the concentrations of serum T4 and regain the normal correction of the hyperthyroidism by treatment with thyroid hormone inhibitors as the use of PTU in our study. The concentration of sex hormone-binding globulin (SHBG) in serum or plasma is increased (26) that is associated with the rise in total testosterone level (27). The changes in the concentration of total testosterone resulted in a studied blood outcome rate for testosterone in patients with hyperthyroidism that is not significantly diverse from normal individuals (28). Though the abnormalities of the hypothalamic-pituitary testicular-axis have been authenticated in thyrotoxicosis (29), unequivocal clinical proof of hypo or hyperandrogenic is generally not found.

On the other hand, the role of rosemary might be contributed to the various constituents of it. Rosemary consists abundant of biologically active components; including antioxidants items rosmarinic acid, carnosic acid, betulinic acid, ursolic acid, rosmanol, and rosmaridiphenol. Thus, rosemary extract appears as a cytoprotective factor when exposed to the free radical scavenging activity which leads to widespread damage to cellular compounds as membrane lipids and highly raised the normal cell viability and the activity of antioxidant enzymes GSH and SOD and significantly decreases MDA contents (30). Rosemary extract is able to donate electrons to reactive radicals, altered them to more stabilized and on reactive kinds, subsequently preventing them from coming biomolecules for instance

polyunsaturated fatty acids, lipoproteins, amino acids, DNA, proteins, and sugars, in liable biological systems (31).

The rosemarinic acid and phenolic compounds were believed to be the most significant constituents of rosemary, which identified by their anti-free radical's activity, these constituents are fully absorbed from the skin and gastrointestinal tract. (32). There are several vital actions of rosemary leaves, like reluctance to all types of cancers, infections, bacteria, and its antioxidant activity for the existence of caffeic acid and its derivatives. The vital actions of leaves extract of rosemary are corresponding with known anti-oxidants components, such as rosmarenic, arnosic, carnosol, ursolic acids, butylated hydroxytoluene, and butylated hydroxyanisole, without ally hazard to carcinogenic or the cytotoxicity of the artificial antioxidants (33-35). In the present work, the administration of rosemary leaves aqueous extract after high level of thyroxine induced by levothyroxine had a poor effect on testosterone serum level which is firmly due to the low dose of rosemary extract 10 mg/kg b.w, whereas the favor was to the propylthiouracil.

## Conclusion

Although the poor effect of rosemary leaves extract on serum testosterone, we must as well be mindful of its ameliorative effects if utilized in higher dosage. Thus, further studies must be done in the future.

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## Conflicts of interest

No conflicts.

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بواسطة الليفوثيروكسين في ذكور الجرذان. تم معاملة جرذان المجموعة الضابطة السلبية بالماء المقطر أما الثلاث مجموعات الأخرى من ذكور الجرذان تم معاملتها تحت الجلد باستخدام ٠,٥ ملغم/كغم من الليفوثيروكسين لمدة ١٢ يوماً، منذ اليوم الثالث عشر إلى اليوم الرابع والعشرين من التجربة، تم اعتبار المجموعة الأولى كمجموعة ضابطة إيجابية عوملت بالماء المقطر، وأعطيت المجموعة الثانية بالبروبيلثيوراسيل بجرعة بلغت ١٠ ملغم/كغم من وزن الجسم، والمجموعة الثالثة تم معاملتها بالمستخلص المائي لإكليل الجبل بجرعة بلغت ١٠ ملغم/كغم من وزن الجسم. أظهرت النتائج أن العلاج بالمستخلص المائي لإكليل الجبل يؤدي إلى انخفاض معنوي في مستويات هرمون تستوستيرون في مصل الدم بينما أظهرت المعاملة باستخدام البروبيلثيوراسيل انخفاض معنوي كبير في مستويات التستوستيرون والهرمون اللوتين وهرمون المنبه للجريب في مصل الدم. يمكن الاستنتاج أن تأثير البروبيلثيوراسيل والمستخلص المائي لإكليل الجبل أدى إلى النتائج الجذلية للهرمونات والتي كانت لصالح البروبيلثيوراسيل.

## تأثير مستخلص إكليل الجبل المائي على بعض الهرمونات الجنسية في ذكور الجرذان ذات المستوى المرتفع للثايروكسين

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### الخلاصة

الهدف الرئيسي من هذا البحث هو تقييم تأثير المستوى المرتفع من هرمون الثايروكسين على هرمون اللوتين، التستوستيرون، والهرمون المنبه للجريب في الجرذان وتقدير التأثير المحتمل عند إعطاء مستخلص إكليل الجبل المائي والبروبيلثيوراسيل ضد سمية الخصية المستحدثة