Research Article

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Sperm Characteristics in Hypothyroid Male Albino Rats Treated With *Vitex Agnus Castus* Extract

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Abstract

To evaluate the probable protective effect of *vitex agnus castus* extract on sperm characteristics in hypothyroid male albino rats. Forty adult male albino rats (200–250 g) were divided into 4 groups, 10 rats in each group. Negative control (First Group) got normal saline every day. After hypothyroidism, groups 2–4 received *vitex agnus castus* extract and/or levothyroxine (L-T4). (Second Group) received normal saline as a positive control, (Third Group) received *vitex agnus castus* extract (300 mg/kg body weight), and (Fourth Group) received L-T4 (0.1 mg/kg/day). HYPO (positive) increased TSH but decreased T3 and T4, while HYPO+ THYROXIN and HYPO+V.A.C decreased TSH and increased T3 and T4 compared to the HYPO (positive). THYROXIN and V.A.C decreased Malondialdehyde (MDA), but HYPO (positive) increased it. Catalase (CAT), Glutathione Peroxidase (GPX) and Superoxide dismutase (SOD) decreased in the HYPO (positive) group compared to the control treatment and increased in THYROXIN and V.A.C treatments compared to HYPO (positive) group. Indicators of sperm characteristics showed that the HYPO (positive) treatment caused a significant decrease in (sperm count, general motility, progressive motility, viability, and morphology) compared to the control, while both treatments of HYPO, whether with THYROXIN or V.A.C, increased all five indicators significantly despite the lack of difference between them and control in terms of impact. *Vitex agnus castus'* antioxidant characteristics make it a potential preventative measure against hypothyroidism's deleterious impact on sperm quality and antioxidant testicular levels.

Keywards: Sperm characteristics, Hypothyroidism, *vitex agnus castus*, Anti-oxidants, Antithyroid

خصائص الحيوانات المنوية في ذكور الجرذان البيضاء التي تعاني من قصور الغدة الدرقية والمعالجة بمستخلص عشبة كف مريم

الخلاصة

الهدف من هذه الدراسة هو تقييم التأثير الوقائي المحتمل لمستخلص عشبة كف مريم على خصائص الحيوانات المنوية في ذكور الجرذان البيضاء التي تعاني من قصور الغدة الدرقية. تم تقسيم 40 جرذ بالغ من الذكور (200-250 جم) إلى 4 مجموعات ، 10 فئران في كل مجموعة. حصلت مجموعة التحكم السلبي الاولى على محلول ملحي طبيعي كل يوم. بعد قصور الغدة الدرقية ، تلقت المجموعات 2-4 مستخلص عشبة كف مريم و / أو ليفوثيروكسين. تلقت المجموعة الثانية محلول ملحي عادي كعنصر تحكم إيجابي ، تلقت المجموعة الثالثة مستخلص عشبة كف مريم (300 مجم/كجم من وزن الجسم) ، وتلقت المجموعة الرابعة (L-T4 0.1 مجم/كجم/يوم). زاد مستوى TSH في مجموعة التحكم الإيجابية لكن انخفض مستوى كل من T3 وT4 ، على عكس المجموعتين الثالثة والرابعة حيث انخفض مستوى TSH لكن زاد مستوى كل من T3 وT4 . انخفض مستوى MDA في المجموعتين الثالثة والرابعة بينما زاد مستواه في المجموعة الثانية. انخفض CAT و GPX و SOD في مجموعة التحكم الايجابية مقارنة بمجموعة التحكم السلبية بينما زاد في المجموعتين الثالثة والرابعة مقارنة بالمجموعة الثانية (مجموعة التحكم الإيجابية). أظهرت مؤشرات خصائص الحيوانات المنوية أن مجموعة التحكم الإيجابية تسببت في انخفاض كبير في (عدد الحيوانات المنوية، والحركة العامة، والحركة التقدمية، والحيوية، والشكل) مقارنةً بمجموعة التحكم السلبية، بينما زاد كل من المجموعتين الثالثة والرابعة جميع المؤشرات الخمسة بشكل ملحوظ بالرغم من عدم وجود فرق بينها وبين مجاميع التحكم. تجعل الخصائص المضادة للأكسدة لعشبة كف مريم تدبيرًا وقائيًا محتملاً ضد التأثير الضار لقصور الغدة الدرقية على جودة الحيوانات المنوية ومستويات مضادة للأكسدة في الخصية.

Introduction

Hypothyroidism, the most frequent form of pathologic hormone insufficiency, is a kind of thyroid dysfunction (1). The disruption of the neuroendocrine axis, which includes the hypothalamic-pituitary-gonadal axis, due to hypothyroidism is a novel and essential component that affects testicular function (2).

Some researchers have found a weak link between hypothyroidism and diminished sperm quality, whereas others have found no such association (3, 4). Numerous studies looked for a connection between thyroid and reproductive function, which may reflect the crucial role of thyroid hormone in human and rodent gonad development (5, 6). Thyroid hormones have been shown to have an effect on testicular function in mammals by acting on and altering the function of the testis (7). Researchers proved that short-term hypothyroidism has no effect on male fertility, whereas long-term hypothyroidism has been linked to impaired reproductive function (8). However, with hypothyroidism, the quantity of Sertoli cells is affected, as the shape and mobility of the sperm, resulting in a reproductive defect (9).

The hypothalamic-pituitary-gonadal axis, normal morphology of reproductive system cells, and reproduction can all be negatively impacted by thyroid diseases, as has been described in previous research (10).

Many studies have findings altered the traditional concept of the testis as an organ insensitive to thyroid hormone, indicating that thyroid hormone may have real impacts on the testis (11).

Physiologically, thyroid hormones are recognized to play a role in reducing the oxidative stress generated by Reactive oxygen species (ROS) (12). Hypothyroidism, oxidative stress, and possibly dysfunctional testes and infertility have all been linked to thyroid hormones absence (13). Male hypothyroidism is accompanied by anomalies in the gonads, or hypogonadism, and a decrease in serum testosterone (14). The shape and movement of sperm can be affected by hypothyroidism as well (15).

Many years ago, reports of a lack of metabolic response from the testes to thyroid hormones sparked debate over the effect of thyroid illness on male fertility (16). In addition to their role as a major regulator of cellular metabolism, thyroid hormones also play a crucial role in oxidative stress (17).

The chaste tree, or *Vitex agnus castus*, is a member of the family Verbenaceae and can be found all along the Anatolian coastline (18). Because of its history of usage in treating gynecological issues, including ovarian insufficiency, uterine hemorrhage, premenstrual syndrome, fibroid cysts, infertility, and acne, it has been called a "women's herb" for more than 2500 years, ever since the time of Hippocrates (19-22). Long employed in Anatolian folk medicine as a diuretic, digestive, antifungal, and treatment for anxiety, premature birth, and stomachache, among other conditions (18, 23). Extracts of ripe fruits have demonstrated significant cytotoxic effect against human cancer cells in recent years (18, 21, 24-25).

Over a long period of time, studies of *vitex agnus castus'* pharmacological effects have been extensive. However, there is a dearth of research examining its effect on sexual hormones, and none at all examining its function in sperm features or the antioxidant enzymes and MDA in the testis. Therefore, the aim of this study is to determe the effect of *vitex agnus castus* methanolic extract on sperm characteristics and antioxidant enzymes and lipid MDA changes in the testis of hypothyroid male rats induced by the antithyroid drug.

Materials and Methods

Animals

Healthy male Sprague-Dawley albino rats were

obtained from the animal house of Faculty of Veterinary Medicine-University of kufa and kept in a strict hygienic environment. They had free access to food and water (26-28).

Ethical Approval

This study was approved by the ethics committee of the Faculty of Veterinary Medicine, University of kufa and conforms to the Guide for the Care and Use of Laboratory Animals.

Animal groups

Total four groups of (200-250 g) male rats were formed containing 10 rats in each group. Groups 2 to 4 were rendered hypothyroidism according to (29) by giving them a water supply containing 0.05% 6-propyl-thiouracil (PTU) for 30 days. The whole experiment period is 30 days:

Group 1: Negative Control (c-negative), saline for 30 days.

Group 2: Positive Control (HYPO (positive)), saline for 30 days.

Group 3: *vitex agnus castus* extract (HYPO+V.A.C), 300 mg/kg B.W given orally by gavage).

Group 4: L-T4 (HYPO+ THYROXIN), 0.1 mg/kg/day intra-peritonellly (31).

Preparation of methanolic extract of *Vitex agnus castus*

The methanolic extract of the *Vitex agnus castus* was prepared according to (30), the methanolic extract of Vitex's aerial component was made by macerating 25 g of powder in 100 ml of methanol at room temperature for 24 h with 15 min of stirring at the start. After mixing, it was filtered and taken up twice in 50 methanol. A rotary evaporator (E100) evaporated all filtrates at 65 °C in the same vessel. Finally, the extract was oven-dried for one day at 37 °C and stored at 4 °C in a hermetically sealed flask. Green paste is extracted.

Blood collection, and sample analysis

At the end of the experiment, i.e. 31th day, blood samples from 10 different rats of each group were collected, and serum was isolated from the blood to determine the hormone levels in the serum. Serum levels of triiodothyronine (T3) (mouse/rat ELISA Kit) (Abnova, Taiwan), and thyroxin (T4) and (TSH) (rat ELISA Kit) (MyBioSource, Inc. USA) were determined by colorimetric method according to the manufacturer instructions.

After blood collection, semen was collected from left caudal epididymis was used for general motility, progressive motility, viability, morphology, and sperm count (concentration), whereas the semen which collected from the right caudal epididymis was homogenized in Tris buffer (PH = 7.4) to make 15% (g/ml) homogenate, and 100 µl of this homogenate was used for measurement of oxidative stress markers (32) MDA, CAT, GPX and SOD levels, all were determined by ELISA kit Elabascience Biotechnology Inc. China.

Statistical analysis

The results are presented in a mean-standard-deviation format (SD), and analysis was performed using ANOVA (version 5). The significance level used was ($p < 0.05$) (33).

Results and Discussion

Thyroid gland hormonal tests results:

From the first table and figure, it is noted that the thyroid hormones showed different responses according to the effect of the treatments, as it was noted that the HYPO (positive control) caused a significant increase in TSH, but it caused a significant decrease in both T3 and T4, while the treatments of HYPO+ THYROXIN and HYPO+V.A.C it caused a significant decrease in TSH compared to the HYPO (positive control), and a significant increase compared to the negative control but the percentage of the increase did not reach the limit it reached in the HYPO (positive control) treatment. HYPO+

THYROXIN and HYPO+V.A.C show significant increase in both T3 and T4 compared to the HYPO (positive control). As for the treatment of HYPO+V.A.C, it caused a significant increase in T3 compared to the negative control, but the treatment of HYPO+ THYROXIN had no role in T3 as its value did not differ from the negative control treatment. While a significant decrease in T4 hormone was observed when using thyroxin when compared to negative control, the decrease also increased significantly when using *vitex agnus castus*.

Antioxidants tests results:

It is noticed from table (2) and figure (2) that there was a significant increase in MDA in the HYPO (positive control) group compared to the negative control treatment, while THYROXIN and V.A.C caused a significant decrease in MDA if compared with the negative control.

As for CAT, a significant decrease in the HYPO (positive control) group compared to the negative control treatment and a significant increase in THYROXIN and V.A.C compared with the HYPO (positive control) group, it is noted that the use of all treatments caused a significant decrease in comparison with the negative control treatment, and the decrease was slight despite its significant in the THYROXIN and V.A.C treatments.

Both THYROXIN and V.A.C caused a significant increase in GPX when compared to the negative control, but the increase was less significant when treated with V.A.C, while HYPO (positive control) had the opposite effect as it caused a significant decrease in GPX when compared to the negative control, the results of SOD followed the same path as GPX, but V.A.C May exceed THYROXIN.

Sperm characteristics analysis results:

It is noticed from the previous five indicators of sperm characteristics in table (3) and figures (3) and (4) that the HYPO (positive control) treatment caused a significant decrease in all indicators compared to the negative control treatment, while both treatments of HYPO, whether with THYROXIN or V.A.C, caused an increase in all five indicators compared to the negative control treatment and significantly despite the lack of difference between them in terms of impact.

Groups	T3	T4	TSH
G1	5.26±0.299 b	7.35±0.381 a	3.03±0.013 c
G2	1.05±0.019 c	2.07±0.034 b	7.42±0.157 a
G3	5.91±0.416 a	6.26±0.125 c	4.03±0.017 b
G4	5.14±0.201 b	6.48±0.272 b	3.99±0.156 b

Table (1): Thyroid hormones (T3 and T4), and TSH levels in the experimental groups. Each letter represents a statistically significant difference between the groups at the (p≤0.05) level.

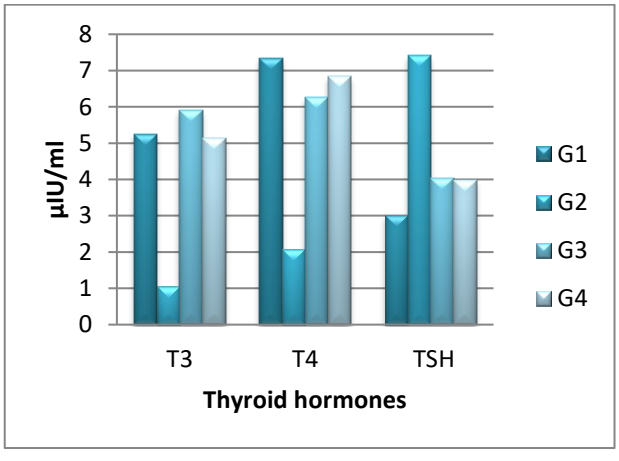

Figure (1): Thyroid hormones (T3 and T4), and TSH levels in the experimental groups.

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Table (2): Oxidative stress biomarkers levels in the right testis of the experimental groups.

Groups	MDA (nmol/L)	CAT (U/ml)	GPX (µmol/L)	SOD (ng/mL)
G1	9.03±0.166 b	562.19±1.93 a	71.61±0.893 c	10.19±0.155 c
G2	16.13±0.721 a	227.79±4.04 c	38.14±0.345 d	4.30±0.215 d
G3	7.28±0.227 c	548.87±6.78 b	77.53±2.02 b	11.05±0.02 a
G4	6.91±0.158 c	544.35±8.7 b	81.13±1.96a	10.77±0.28 b

Each letter represents a statistically significant difference between the groups at the ($p \leq 0.05$) level.

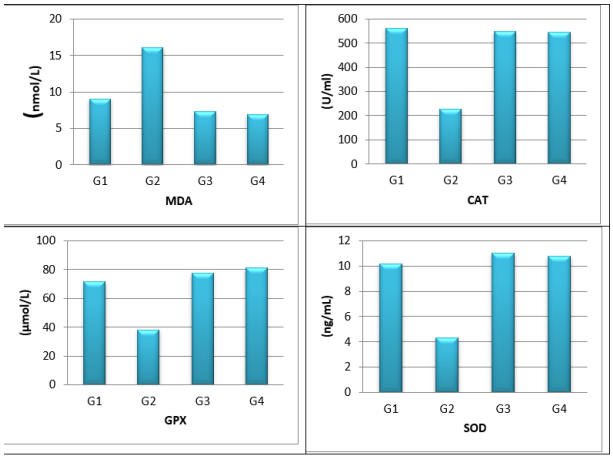

Figure (2): Oxidative stress biomarkers levels in the right testis of the experimental groups

Table (3): Sperm characteristics in the left testis of the experimental groups.

Groups	General Motility	Progressive Motility	Viability	Morphology	Concentration
G1	80.4±1.14 b	80.8±2.58 b	87.86±2.93 b	92.16±0.71 a	3960±230.2 b
G2	41.4±6.87 c	24.6±5.7 c	60.4±1.76 c	83.12±0.93 b	1080±238.7 c
G3	89.8±1.78 a	88.2±2.38 a	92.54±1.65 a	92.62±1.52 a	7042±467.2 a
G4	87.8±2.68 a	87.2±3.11 a	91.46±2.26 a	91.3±2.07 a	7080±665.7 a

Each letter represents a statistically significant difference between the groups at the ($p \leq 0.05$) level.

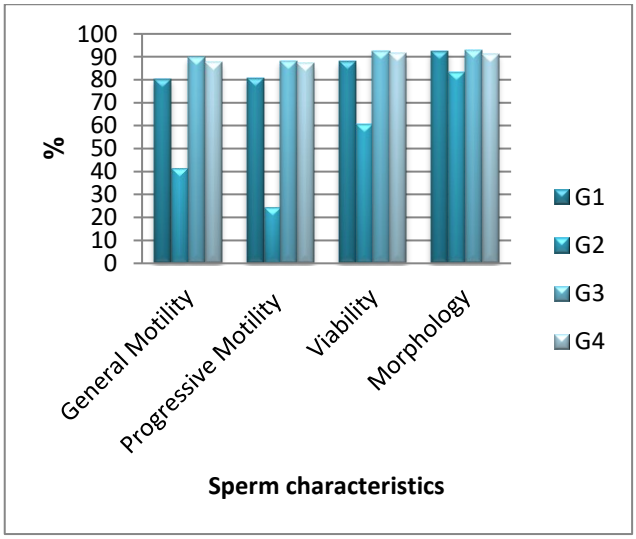

Figure (3): Sperm general motility, progressive motility, viability, and morphology in the left testis of the experimental groups

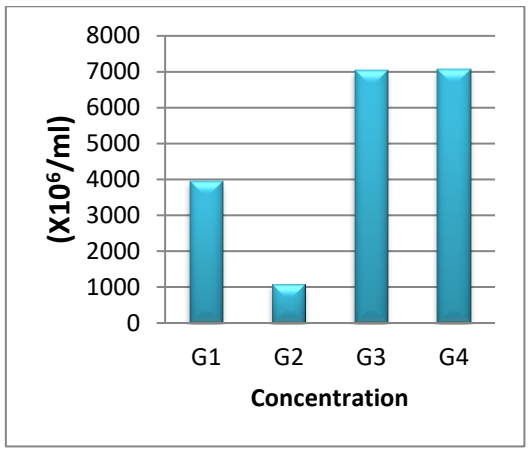

Figure (4): Sperm concentration in the left testis of the experimental groups

Animal studies suggest that thyroid hormone fluctuations impair sexual activity and fertility. The thyroid stimulating hormone (TSH) and thyroxin hormones (T4) and (T3) levels were utilized to diagnose healthy thyroid function in humans as well as animals. Significant decreases in T4 and T3, as well as an increase in the TSH serum level, indicated the induction of hypothyroidism in this investigation. However, the thyroid function was improved by given V.A.C and thyroxin to hypothyroid rats in which the level of T3, T4 and TSH were closer to the control group.

In the present study, the levels of content in testicular tissue homogenate CAT, GPX and SOD were significantly decreased in the HYPO (positive) group, while the level of MDA was significantly increased; this suggests that the antioxidant defenses of the testicular tissue were overwhelmed by oxidative stress. These results agree with those of (34-36) who indicated that hypothyroidism is associated with a drop in CAT and SOD.

Also, Sahoo et al. who administered propylthiuracil 0.05% in the drinking water to induce hypothyroidism in rats reported a substantial drop in SOD and CAT levels after 30 days of administration (37). Choudhury et al. also noted that a decrease in SOD and CAT is accompanied by an increase in MDA, both of which are indicative of oxidative stress (34). Loss of the testicular defense mechanism against reactive oxygen species (ROS) produced by cellular metabolism is accompanied by a decrease in antioxidant defense when thyroid hormone levels are low (35, 37). However, there has been a lack of research assessing the impact of hypothyroidism on adult testes and highlighting the role that the testicular antioxidant system plays in this condition (37). Reduced forms of oxygen and their reaction products with other molecules are known as reactive oxygen species. The mitochondria are the main biological generator of reactive oxygen species. Many biological components, including unsaturated fatty acids, sulphydryl proteins, and nucleic acids, are susceptible to oxidation by ROS under physiological conditions (38). Most reactive oxygen species in semen come from sperm and a few leukocytes seen in contaminated seminal plasma (39). When ROS levels exceed than anti-oxidant levels in cells, oxidative stress occurs (38).

Vitex agnus-castus L. (VAC) is an essential Verbenaceae plant used in traditional herbal medicine. (40). Premenstrual syndrome, atypical menstrual cycles, amenorrhea, mastodynia, hyperprolactinemia, premenstrual dysphoric disorder, and lactation difficulties are only few of the conditions that this herb is used to treat. (41). Flavonoids, diterpenes, iridoid glycosides, and a volatile oil make up the bulk of its chemical composition. Vitex has shown effectiveness in treating reproductive disorders in some diabetic patients. (42).

It has not yet been determined how exactly Vitex functions. The levels of sex hormones may be controlled by Vitex by pharmacological and physiological processes. The capacity of apigenin to bind to the estrogen receptor makes

it the most potent phytoestrogen. (43). In other hand, Linoleic acid, an estrogenic molecule found in Vitex, can bind to estrogen receptors and activate specific estrogen-inducible genes. (44).

Many different chemical components make up Vitex agnus-castus L., and these include: Flavonoids (orientin, kaempferol, penduletin, luteolin, artemetin, vitexin, and casticin), diterpenoids (vitexlactam, vitexilactone, viteagnusin I, and rotundifuran), and iridoids (agnuside, agnusoside, agnucastosid A/B, and aucubin) and vitexilactone, rolundifuran, ketosteroids, diterpenoids (vitexlactam, vitexilactone, viteagnusin I, and rotundifuran) (45–47) Vitex possesses antioxidant and antiapoptotic activities; vitexilactone isolated from V. agnus-castus decreased caspase-3 and apoptosis marker expression in Sprague-Dawley rats (48).

In order to counteract the ROS-related damage, cells produce many anti-oxidant molecules such as Catalase (CAT), Glutathione Peroxidase (GPX) and Superoxide dismutase (SOD), in the present study, *vitex agnus castus* extract proved itself as an antioxidant enhancer against the oxidative stress caused by hypothyroidism. It reduced the MDA level in testicular tissue indicating improvement to the oxidative stress. In addition, it maintained the CAT, GPX and SOD levels near their levels in the control group and prevented their exhaustion by the stress of hypothyroidism.

In the present study, propylthiouracil administration significantly decreases general motility, progressive motility, viability, morphology, and sperm count as a result to the induction of hypothyroidism. These findings were concomitant with many other studies focused on the impact of hypothyroidism in sperm characteristics in general. The sperm of hypothyroid patients exhibited a marked reduction in their progressive motility (49). Scientists reported that both the number of sperm and their ability to move around were drastically reduced in those with hypothyroidism (34, 36, 50-54), and a rise in the number of dead sperms (34-36, 50, 53). Men with hypothyroidism also had poorer sperm motility, but not considerably less than controls (54).

However, researchers (55, 4), proposed that thyroid hormones were hypothesized to influence sperm morphology and motility. Reproductive impairment may result from an inadequate supply of thyroid hormones, which may change the size and amount of sperm in the testes (56). Reduced antioxidant defense mechanisms against free radicals produced by normal cellular metabolism are linked to hypothyroidism (35, 37). A rise in cell death and dysfunction are due to free radical buildup (57) High levels of polyunsaturated fatty acids, such as docosahexaenoic acid, in the sperm plasma membrane are easily damaged by reactive oxygen species, reducing the tail's flexibility and motility (39). Reduced production of ATP, the fuel for sperm movement, is another consequence of ROS (58).

On the other hand, a significant elevation of MDA level in the hypothyroid group. This finding corroborated the findings of other researchers who found that oxidative stress and lipid peroxidation caused by free radical accumulation contributed to cellular damage and testicular dysfunction in hypothyroidism, as evidenced by a decrease in sperm count and an increase in the proportion of immotile sperm (53). Romano et al., 2017 also reported that a low level of thyroid hormone has been shown to decrease sperm viability (49). An imbalance between elevated oxidative stress (from sources like lipid peroxidation) and weakened antioxidant defenses (from enzymes like catalase and superoxide dismutase) may be to

blame for declining sperm vitality (34, 37). Krassas et al., 2008 indicated that sexual dysfunction and/or morphological testicular deterioration are common side effects of thyroid disease (11). It was determined that sperm morphology was the only criterion significantly influenced by hypothyroidism in men, and that hypothyroidism had a negative effect (10).

In the present study, the increased prevalence of sperm with morphological abnormalities in persons with an altered thyroid state may be due to the effect of thyroid hormones on the cytoskeleton of sperm. Most research corroborates to suggest that male hypothyroid individuals have defects in sperm morphology, these abnormalities resolve or regulate when euthyroidism is restored (10). Previous studies showed that changes in thyroid hormone levels negatively affected mitochondrial GSH regulation, antioxidant defense, and their relative contribution to adult rat testes (59). After supplementation with *vitex agnus castus* extract, however, a statistically significant enhancement was observed in each of the five indicators of sperm qualities that were the subject of this investigation. This may help verify the hypothesized failure of the antioxidant system in the testes of propylthiouracil-treated rats and the essential antioxidant role of the methanolic extract of *vitex agnus castus* in counteracting the toxic effects of propylthiouracil on sperm.

Conclusions

According to the information that was gathered, we can conclude that *vitex agnus castus* methanolic extract act as an antioxidant, and can ameliorate the toxic effects of hypothyroidism on sperm count, general motility, progressive motility, viability, and morphology in adult male rats. In addition to its ameliorative effect on the thyroid gland and thyroid hormones which in turn enhances testicular function. Furthermore, it can correct antioxidant values in the testes that are affected by the oxidative stress on testicular tissues as a pathway for propylthiouracil induction of its harmful effect.

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Conflict of interest

The authors declare no conflict of interest.

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