Original Article

Effect of Propolis and Vitamin E on the Pituitary-gonad Axis and Gene Expression of Testosterone in Male Rats With Testicular Toxicity

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ABSTRACT

Background: The study was done at the College of Veterinary Medicine, University of Kufa. The primary objective was to examine the impact of propolis (Pro) and vitamin E (Vit E) on the reproductive hormones (follicle-stimulating hormone [FSH], luteinizing hormone [LH] and testosterone [T]), histopathological changes, and gene expression of *3β-HSD1* mRNA in male rats with testicular dysfunction induced by Bisphenol A (BPA).

Objectives: The primary objective of the current study was to examine the toxic effects of BPA on the reproductive organs and the role of Pro in the regulation of the hypothalamic-pituitary-gonadal axis, as well as gene expression in adult male rats, in comparison with Vit E This study focused on evaluating male reproductive hormones (FSH, LH and T), histopathological changes, and the expression of *3β-HSD1* mRNA.

Methods: The rats were randomly distributed into five groups, each consisting of ten male rats. Specifically, group 1 comprised rats receiving standard food and water, serving as the negative control group. In Group 2, rats were administered 0.2 mL of corn oil (the vehicle for BPA) through the intraperitoneal (IP) route, serving as the vehicle control group. In group 3, rats received BPA dissolved in corn oil at a dose of 50 mg/kg body weight, administered via IP injection three days a week for three weeks. In group 4, rats were protected with Pro at a dosage of 250 mg/kg body weight orally, administered through a gavage needle. This was followed by the IP injection of BPA at 50 mg/kg body weight dissolved in corn oil, conducted three days a week over three weeks. For group 5, rats received protection with Vit E at a dosage of 100 mg/kg body weight orally, administered through a gavage needle. This was followed by the IP injection of BPA at 50 mg/kg body weight dissolved in corn oil, administered three days a week over three weeks.

Results: BPA had significant adverse effects on male reproductive hormones (FSH, LH, and T), histopathological changes, and the expression of 3β-HSD1 mRNA. In contrast, Pro and Vit E groups positively influenced all these parameters.

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Conclusion: BPA exposure induced histopathological changes and affected male reproductive hormones (FSH, LH, and T) in male rats, as well as the gene expression of *3β-HSD1* mRNA (T). Pro and Vit E positively influenced the histopathological changes and male reproductive hormones (FSH, LH, and T) induced by BPA, restoring their normal architecture.

Keywords: Bisphenol A (BPA), Propolis (Pro), Spermatogenesis, Vitamin E, 3β-Hydroxysteroid Dehydrogenase types 1 (3β-HSD1)

Introduction

Testicular dysfunction in male rats can manifest in various ways and may be caused by a range of factors, including genetic, environmental, hormonal, and nutritional influences. Testicular dysfunction often refers to issues that affect the proper functioning of the testes, leading to impaired fertility or disruptions in reproductive health. Hormonal imbalances, genetic factors, nutritional deficiencies, and toxic exposures are some potential causes and aspects of testicular dysfunction in male rats (Usende et al., 2022; Sartorius & Handelsman, 2023; Agarwal et al., 2020).

Bisphenol-A (BPA) serves as a monomer in the production of polycarbonates and plays a crucial role as an intermediate in the manufacturing of epoxy resins, phenoxy resins, thermal receipts, dental sealants, medical devices, reusable containers for food and water, beverage containers, water supply pipes, flame retardants, and rubber manufacturing (Liu et al., 2021).

It operates as an endocrine-disrupting chemical, exhibiting estrogenic, anti-androgenic, and anti-thyroid activities that interfere with hormonal function (Rahman & Pang, 2019). The reproductive toxicity associated with BPA has been connected to the extensive use of plastic products, leading to frequent exposure of humans to BPA in their daily lives. The U.S. Center for Disease Control and Prevention (CDC) has identified measurable BPA levels in urine samples from 90% of the U.S. population (Lehmler et al., 2018). Acknowledged as a widely recognized endocrine disruptor that impacts male fertility, it is essential to clarify the mechanism by which BPA influences spermatogenesis (Liu et al., 2021).

3β-Hydroxysteroid dehydrogenase type 1 (*3β-HSD1*) is responsible for the formation of T, an enzyme involved in steroidogenesis, the process, by which steroid hormones are synthesized. In particular, *3β-HSD1* plays a role in the formation of T, which is an important male sex hormone (Gao et al., 2021). The synthesis of T begins with cholesterol, which serves as the precursor for all steroid hormones. Cholesterol is converted into pregnenolone, which is a precursor for various steroid hormones (Schade et al., 2020; Salih et al., 2019).

Dehydroepiandrosterone (DHEA) formation: Pregnenolone is then converted into DHEA through a series of enzymatic reactions. 3β-HSD1 is one of the enzymes involved in this process.

Androstenedione formation: DHEA is further converted into androstenedione, another precursor in the pathway toward T (Elzenaty et al., 2022). Finally, androstenedione is converted into T, the primary male sex hormone. This conversion can occur in various tissues, including the testes and the adrenal glands.

The genes associated with T formation in male rats are as follows:

STAR (steroidogenic acute regulatory protein): The *STAR* gene codes for the steroidogenic acute regulatory protein, which plays a crucial role in cholesterol transport into the mitochondria, the first step in steroidogenesis (Tugaeva et al., 2020).

Cyp11a1 (cytochrome P450 family 11 subfamily A member 1): This gene encodes the enzyme P450scc, responsible for converting cholesterol to pregnenolone in the mitochondria (Kojima et al., 2010).

3β Hsd (3β-hydroxysteroid dehydrogenase): The *Hsd3b* gene family, including *Hsd3b1* and *Hsd3b2*, codes for 3β-hydroxysteroid dehydrogenase enzymes, such as 3β-HSD1, which catalyze the conversion of pregnenolone to DHEA (Lin & Papadopoulos, 2021).

Cyp17a1 (cytochrome P450 family 17 subfamily A member 1): This gene codes for the enzyme P450c17, which is involved in the conversion of pregnenolone and progesterone to androstenedione, a key precursor to T (Jayaraman et al., 2020; Khalid, 2024).

Hsd17b (17β-hydroxysteroid dehydrogenase): The *Hsd17b* gene family encodes enzymes that convert androstenedione to T. For example, 17β-hydroxysteroid dehydrogenase (*Hsd17b3*) is involved in this step (Liu et al., 2024).

Srd5a (5α-reductase): The *Srd5a* gene family codes for enzymes responsible for the conversion of T to dihydrotestosterone (DHT), which is a more potent androgen. The specific isoforms involved include *Srd5a1* and *Srd5a2* (Corti et al., 2022). In this study, we selected 3β-hydroxysteroid dehydrogenase (*3β Hsd1*) for gene expression.

Propolis (Pro) is a natural resinous substance produced by honeybees through the utilization of tree sap, bee saliva, and beeswax. Bees collect these materials from various plant sources, including tree buds, sap flows, and other botanical elements. They then mix these raw materials with their saliva and enzymes, transforming them into a sticky, resin-like substance known as Pro (Bae et al., 2022).

It has demonstrated efficacy in enhancing sperm quality in male Wistar rats with sexual impairment induced by paroxetine (Toutiaee et al., 2023; Al-Samarraae et al., 2023). The findings reveal a notable increase in plasma T levels, accompanied by improvements in both sperm count and motility (Polat et al., 2019). Pro is rich in flavonoids and phenolic acids, which act as antioxidants protecting sperm from oxidative damage caused by free radicals. This can improve sperm motility, viability, and morphology. Oral administration of Pro altered plasma levels of reproductive hormones. Pro increases the plasma levels of FSH, LH, and T (Polat et al., 2019).

Oxidative damage in testicular tissue has adverse effects on the reproductive system, and antioxidants are effective in preventing or reducing such damage (Aslankoc & Ozmen, 2019; Kaya et al., 2015; Tatli Seven et al., 2018). Furthermore, phenolic and flavonoid compounds, known for their antioxidant properties, play a pivotal role in safeguarding the reproductive system against the toxicity induced by BPA. These compounds act preventively, mitigating the adverse effects on testicular function, T levels, and semen quality caused by BPA (Gul Baykalir et al., 2016; Dadar et al., 2022).

Vitamin E (Vit E), a fat-soluble vitamin, plays a vital role in preserving cellular health and shielding cells from harm inflicted by free radicals. This vitamin exists in various forms, with alpha-tocopherol being the most biologically active compound among them. Vit E is known for its antioxidant properties, which help neutralize free radicals—unstable molecules that can damage cells and contribute to various chronic diseases, including heart disease and cancer (Elayapillai et al., 2017).

Several studies have explored the role of Vit E in mitigating testicular toxicity, and the results have shown some promising effects.

Antioxidant properties: Vit E serves as a robust antioxidant, playing a key role in protecting cells, membranes, and other cellular structures from oxidative damage. By neutralizing free radicals, Vit E may help reduce oxidative stress in the testes (Amjad et al., 2020).

Sperm quality: Some research suggests that Vit E supplementation may have a positive impact on sperm quality. It may improve sperm motility, viability, and overall sperm function. These effects are thought to be related to the antioxidant properties of Vit E (Sabetian et al., 2021).

Material and Methods

Animals and housing

The present study was conducted at the College of Veterinary Medicine, University of Kufa, during the period from August 1, 2023, to March 6, 2024. The study comprised two experiments involving a total of seventy adult male rats, 12 weeks old, weighing between 200 and 250 grams. The rats were kept for an adaptation period of three weeks in the animal house of the College of Veterinary Medicine, University of Kufa. The animals were housed in cages, with four rats in each cage, under optimal conditions (12-hour light/dark cycle, 22±2 ˚C). The animals had ad libitum access to food and water during the experiments.

Experiment animals

Seventy adult male rats, each weighing between 200 and 250 grams, were employed for this study. The experimental procedures commenced on August 1, 2023, following a two-week acclimatization period. The animals were randomly divided into different cages, with each cage containing six animals. Throughout the experimental period, all animals had unrestricted access to food and water.

Experimental design

The male rats in the experiment were randomly divided into five groups, with each group comprising ten male rats, as follows:

Group 1 consisted of rats that were given standard food and water, serving as the negative control group.

Group 2 included rats that were administered 0.2 mL of corn oil (the vehicle for BPA) via the intraperitoneal (IP) route, serving as the vehicle control group.

Group 3 received BPA dissolved in corn oil at a dose of 50 mg/kg body weight, administered via IP injection three days a week for three weeks (Othman et al., 2014).

Group 4 received protection with Pro at a dosage of 250 mg/kg body weight orally, administered through a gavage needle. This was followed by the IP injection of BPA at 50 mg/kg body weight dissolved in corn oil, administered three days a week for three weeks (Singla et al., 2014)

Group 5 received protection with Vit E at a dosage of 100 mg/kg body weight orally, administered through a gavage needle. This was followed by the IP injection of BPA at 50 mg/kg body weight dissolved in corn oil, administered three days a week over three weeks (Amraoui et al., 2018). All animals were sacrificed after three weeks and the blood samples were collected for further assessment.

Animal preparation

The animals were anesthetized using ketamine (90 mg/kg body weight) and xylazine (40 mg/kg body weight). Following anesthesia, the bilateral testes were collected for histological examination and homogenized for gene expression analysis, and blood samples were obtained before the animals were subsequently euthanized.

Blood sample collection

Ten rats from each group were sacrificed upon completion of the therapy. Blood samples were taken from the heart's inferior vena cava of each sacrificed rat by a sterile syringe and placed in a plain tube without anticoagulant. Serum was extracted and stored in micro-Eppendorf tubes at -20 ˚C after 15 minutes of centrifugation at 3000 rpm to conduct laboratory analyses for biochemical tests. The testes were also collected for pathohistological examination.

Histopathological examination of testicular tissue

The histopathological examination of testicular tissue involved excising the testis, longitudinally opening it, and preserving it in a 10% formalin solution until histological sections were prepared. Following established protocols (Goncalves et al., 2010), tissue sections were meticulously prepared. Upon the immediate removal of tissue samples from the organs, specimens were fixed in 10% buffered formalin for 48 hours at room temperature. Subsequent procedures included graded dehydration in alcohol concentrations, clearing in two stages of xylene, and embedding in liquid paraffin at 56 ˚C for 2 hours. The tissues were sliced to a thickness of 5 micrometers using a microtome. The subsequent step included dewaxing and staining with Hematoxylin and Eosin. Tissue sections were examined using X4, X10, and X40 objectives of light microscopy, providing a detailed assessment of the histological features.

Gene expression assay

Upon treatment, the testes of all male rats were excised and stored at -80 ˚C until quantitative polymerase chain reaction (qPCR) analysis was conducted. RNA extraction was performed using the Easy-spin™ (DNA-free) total RNA extraction Kit (Intron/Korea, Catalog No.: 17221) as per the manufacturer's protocol. Fresh tissue samples (50-100 mg) were lysed with 1 mL of lysis buffer (easy-BLUETM reagent), followed by vigorous vortexing and the addition of 200 µL of chloroform. After centrifugation, the upper fluid was transferred to a new tube and mixed with binding buffer. The mixture was then loaded onto a column, washed with washing buffers A and B and centrifuged to dry the column membrane. Elution buffer was added directly onto the membrane to elute the RNA, which was incubated and then centrifuged to collect the eluate for downstream analysis.

Preparation of primers

Following the primer synthesizer company's instructions, the lyophilized primers were reconstituted in ddH_2O to achieve a final concentration of 100 pM/µL, constituting a stock solution stored at -20 ˚C. A working primer concentration of 10 pM/µL was prepared from the stock primers for use in subsequent experiments (Table 1).

Statistical analysis

The data underwent an analysis of variance, and significant differences at $P \leq 0.05$ were assessed using one-way ANOVA with the statistical software SigmaStat, version 4.0.

Results

This investigation aimed to determine the positive effect of Pro and Vit E on animals, in which we created a testicular dysfunction by BPA, to shed light on their potential impact on the complex processes that control

Table 1. Primers used in the study

Organism	Target Gene	Primer Name	5'-3' Sequence	PCR Product	Reference	Accession Number
Rattus rattus	*Hsd3b1*	F	CCCTGCTCTACTGGCTTGC	189 bp	Ji et al., 2021	XM_032897634.1
		R	TCTGCTTGGCTTCCTCCC			
R. rattus	*GAPDH*	F	ATGACTCTACCCACGGCAAG	89 bp	Kunst et al., 2013	NM_017008
		R	CTGGAAGATGGTGATGGGTT			

male reproductive health. The evaluation included a range of assessments, such as male reproductive hormones (FSH, LH, and T), gene expression analysis of 3β-HSD1 mRNA, and histopathological examination.

The impact of Pro and Vit E on serum pituitary-gonadal-axis hormones in adult male rats with testicular dysfunction

FSH

In Figure 1, a significant (P≤0.05) reduction is observable in serum FSH concentration in the BPA group compared to the control group. Conversely, the protected groups (BPA plus Pro and BPA plus Vit E) exhibited a substantial (P≤0.05) rise in serum FSH concentration compared to the BPA Group, although this increase is not significantly different (P>0.05) from the control groups. Importantly, there was no significant difference between the BPA plus Pro and BPA plus Vit E groups.

LH

Figure 2 indicates a significant (P≤0.05) reduction in serum LH concentration in the BPA group compared to the control groups. In contrast, the protected groups (BPA plus Pro and BPA plus Vit E) demonstrated a significant (P≤0.05) increase in serum LH concentration compared to the BPA group. The protected group BPA plus Vit E showed a significant compared to the control groups, while the protected group BPA plus Pro also exhibited a significant (P≤0.05) increase in serum LH concentration compared to the control groups. Finally, there was no significant difference between the BPA plus Pro and BPA plus Vit E groups.

Testosterone (T)

Figure 3 shows a significant (P≤0.05) decline in serum T concentration in the BPA group compared to the control groups. In contrast, the protected groups (BPA plus Pro and BPA plus Vit E) exhibited a significant (P≤0.05) increase in serum T concentration compared to the BPA group. However, there was also a significant (P≤0.05) decrease in serum T concentration in the protected groups, particularly in the BPA plus Pro group compared to the control groups. Additionally, there is a significant (P≤0.05) decrease in serum T concentration in the BPA plus Vit E group compared to both the BPA plus Pro group and the control groups. Finally, there is no significant difference between the control groups.

Gene expression of 3β-HSD1 mRNA in testis

The expression of 3β-HSD1 mRNA in the testis of male rats with testicular dysfunction is presented in Table 2 and Figure 4. The 3β-HSD1 mRNA expression significantly (P<0.0001) decreased in the BPA group compared to the control groups. Conversely, the 3β-HSD1 mRNA expression significantly (P<0.0001) increased in all protected groups (BPA + Pro and BPA + Vit E) compared to both the BPA and control groups.

Histopathological examination of testes

Normal histology of testicular architecture is observed, with seminiferous tubules (black arrow) and Leydig cells (yellow arrow) present in the intra-seminiferous tubule spaces, as shown in Figures 5, 6, and 7. In contrast, the testes of male rats treated with BPA, which experienced testicular toxicity, exhibit histopathological changes, including complete necrosis of spermatogenic cells in the seminiferous tubules, leading to the complete absence of affected seminiferous tubules and the formation of spaces in the testicular parenchyma. Debris from necrotic cells was observed in the spaces of affected seminiferous tubules, as illustrated in Figures 8 and 9. Another histopathological section of the testes from male rats treated with BPA showed necrosis of spermatogenic cells in all seminiferous tubules; however, some spermatogonia were still observed in these tubules. Notably, one of the seminiferous tubules exhibited severe necrosis affecting all spermatogenic cells. However, Sertoli cells (red arrow) did not show any necrosis in the affected seminiferous tubules, as depicted in Figures 10 and 11.

Table 2. Effect of Pro and Vit E on the expression of 3β-HSD1 mRNA in adult male rats with testicular dysfunction

Groups	Significance Status	Adjusted P
Control vs corn oil	Yes	0.0132*
Control vs BPA	Yes	0.0002***
Control vs BPA + Pro	Yes	<0.0001****
Control vs BPA + vit E	Yes	<0.0001****
Corn oil vs BPA	Yes	<0.0001****
Corn oil vs BPA + Pro	Yes	<0.0001****
Corn oil vs BPA + vit E	Yes	<0.0001****
BPA vs BPA + Pro	Yes	<0.0001****
BPA vs BPA + vit E	Yes	<0.0001****
BPA + Pro vs BPA + vit E	Yes	0.0027**

*, **, ***, ****Significant difference between groups.

In contrast, male rats treated with pro exhibited no significant occupied lesions in the testis and showed normal spermatogenesis in some seminiferous tubules, as shown in Figures 12 and 13. Moreover, the testis of male rats treated with Vit E showed necrosis of spermatogenesis cells of seminiferous tubules, affecting less than 50% of the seminiferous tubules. In these cases, debris from necrotic cells was aggregated in the center of the seminiferous tubule lumen, and the necrosis of spermatogenic cells led to a reduced population of spermatogenic cells, as shown in Figures 14.

Discussion

Effect of Pro on FSH, LH, and t levels in male rats with testicular dysfunction induced by BPA

The administration of BPA for 21 days resulted in a decrease in the concentrations of FSH, LH, and T hormones in the BPA group compared to the control groups. These results are in agreement with other studies (Wisniewski et al., 2015; Bordbar et al., 2023). BPA has been found to compromise spermatogenesis by inhibiting reproductive hormones and triggering apoptosis in germ cells through the activation of the Fas/FasL signaling pathway (Jin et al., 2013; Wang et al., 2014). Sertoli cells, existing within the seminiferous tubules, play a supportive and nutritive role (O'Donnell et al., 2022). They play a crucial role in the proliferation and differentiation of germinal cells, thereby contributing significantly to the process of spermatogenesis (Shah et al., 2021; Liu et al., 2020). Therefore, the apparent loss of these supportive cells in BPA-protected rats may result in the deficiency of supportive functions, leading to the loss of spermatogenic cells.

It has been observed that Sertoli cells function as targets for pituitary-derived FSH and T, playing a role in transmitting signals for the paracrine regulation of spermatogenesis (Smith & Walker, 2014; Oduwole et al., 2018) Consequently, the observed depletion of Sertoli cells in the present study following BPA treatment may be attributed to a reduction in FSH and T levels. T secretion takes place in Leydig cells within the testicular interstitium, responding to the stimulation by LH. (Urriola-Muñoz et al., 2014). Hence, the absence of LH stimulation in the BPA-protected groups could explain the diminished presence of Leydig cells, interstitial tissue atrophy, and the subsequent decrease in T production. The observed decrease in serum LH levels depicted in Figure 2 aligns with the findings reported by some researchers (Nakamura et al., 2010; Gharravi et al., 2006; Akingbemi et al., 2004). This phenomenon might be elucidated by BPA's capacity to disrupt LH receptor-ligand binding, leading to the uncoupling of LH from its receptor. This potential interference could contribute to the reduced stimulation of steroidogenesis by LH, as reported by Biswas et al. (2020).

Alternatively, an elevated release of prolactin following BPA exposure, as noted by Oguazu et al. (2021) could also contribute to these observed changes. Hyperprolac

tinemia has been demonstrated to induce reproductive dysfunction (Edinoff et al., 2021). This dysfunction is not mediated through a direct action on the testis but rather

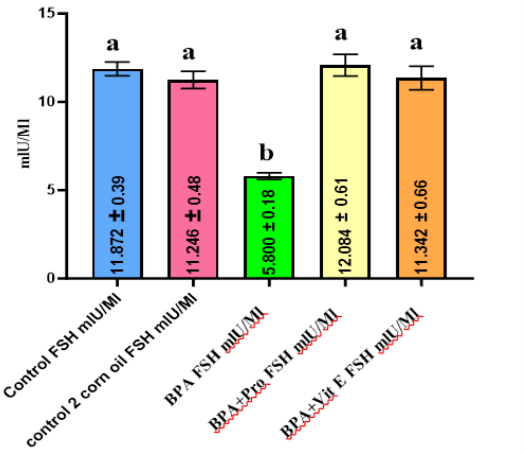

Figure 1. Effect of Pro and Vit E on testicular serum FSH levels in adult male rats with testicular dysfunction

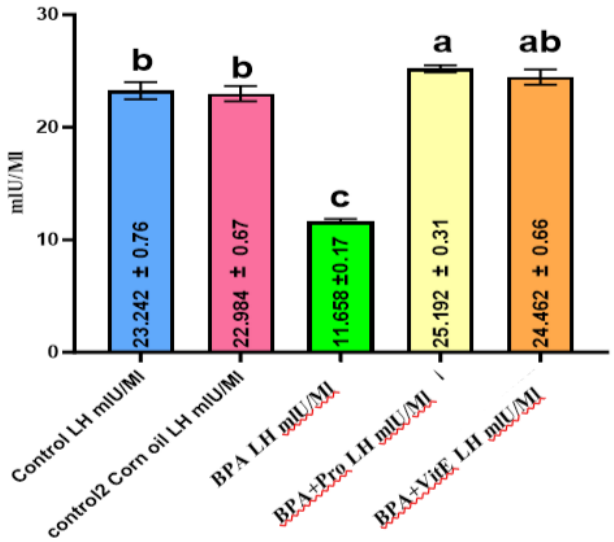

Figure 2. Effect of Pro and Vit E on testicular serum LH levels in adult male rats with testicular dysfunction

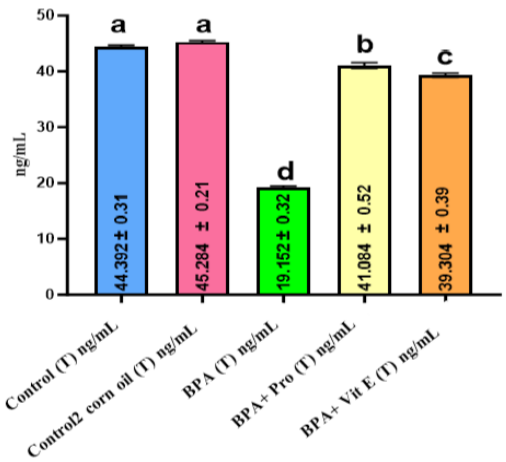

Figure 3. Effect of Pro and Vit E on testicular serum t levels in adult male rats with testicular dysfunction

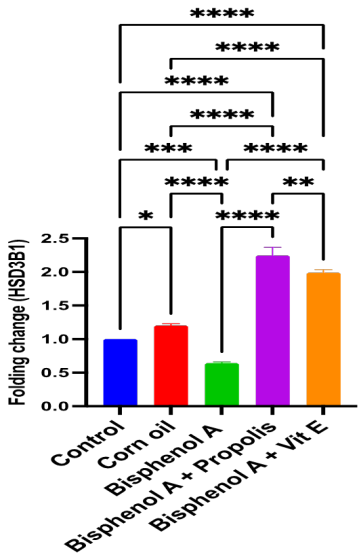

Figure 4. Effects of Pro and Vit E administration on the expression of *3β-HSD1* mRNA in the testis of male rats with testicular dysfunction

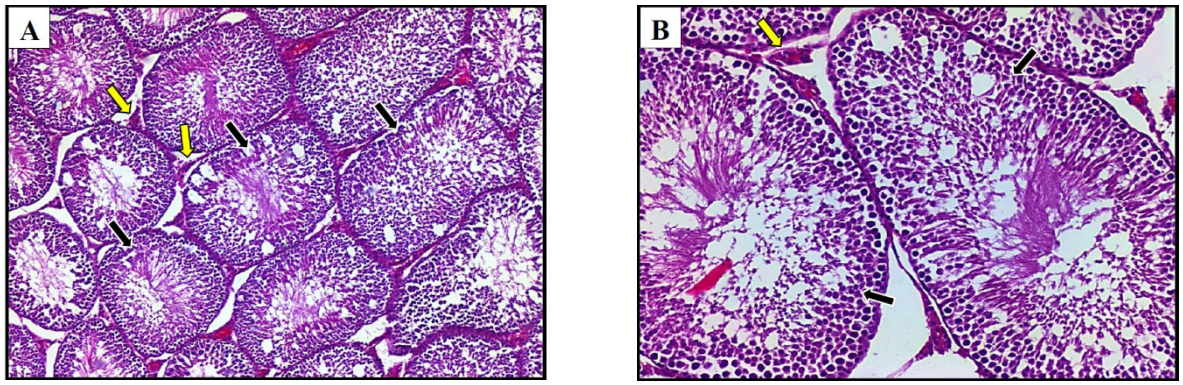

Figure 5. Photomicrographs of the testis from the control negative group

A & B) Normal histology of testicular architecture.

Note the seminiferous tubules (black arrow) and Leydig cells (yellow arrow) observed in the intra-seminiferous tubule spaces. H&E. A: ×40 and B: ×100.

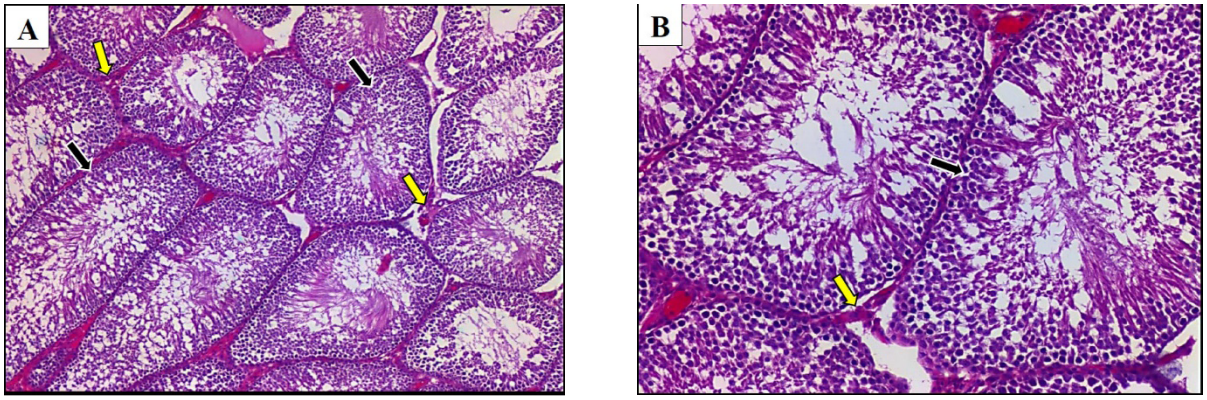

Figure 6. Photomicrographs of the testis from the control negative group

A & B) Normal histology of testicular architecture. Note the seminiferous tubules (black arrow) and Leydig cells (yellow arrow) observed in the intra-seminiferous tubule spaces. H&E. A: ×40 and B: ×100.

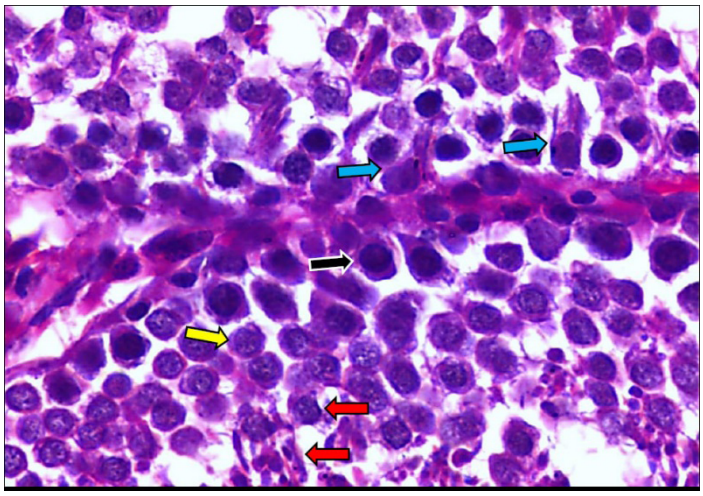

Figure 7. Photomicrograph of the testis from the control negative group. Normal histology of seminiferous tubules. Note the spermatogenic cells: spermatogonia (black arrow), spermatocytes (yellow arrow), round or elongated spermatids (red arrow), and Sertoli cells (blue arrow). H&E. ×400.

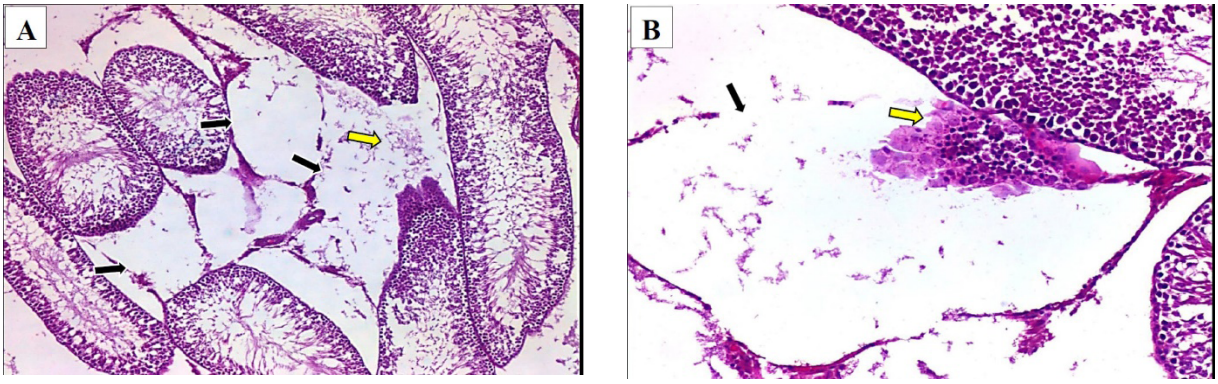

Figure 8. Photomicrographs of the testis from the control positive group

A & B) Complete necrosis of spermatogenic cells in the seminiferous tubules led to the complete absence of affected seminiferous tubules, forming spaces (black arrow) in the testicular parenchyma.

Note that debris from necrotic cells (yellow arrow) was observed in the spaces of the affected seminiferous tubules. H&E. A: ×40 and B: ×100.

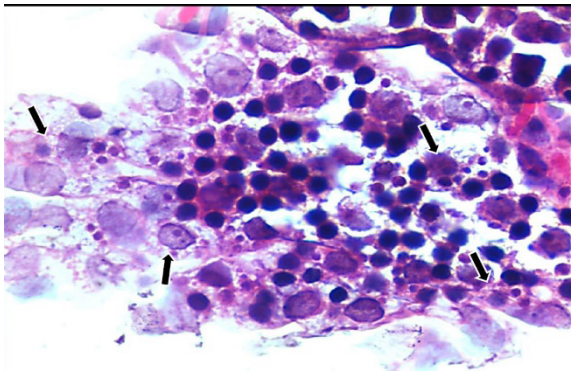

Figure 9. Photomicrograph of the testis from the control positive group

Notes: Necrotic cells (black arrow) were observed in the spaces of the affected seminiferous tubules. H&E, ×400.

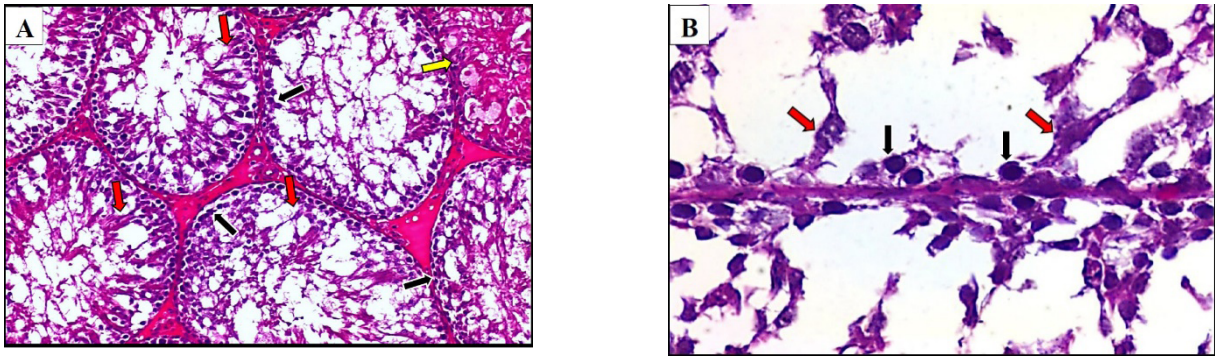

Figure 10. Photomicrographs of the testis from the control positive group

A & B) Necrosis of spermatogenic cells in the seminiferous tubules involved all seminiferous tubules; however, some spermatogonia (black arrow) were observed in these tubules.

Note: One of the seminiferous tubules showed severe necrosis (yellow arrow) that involved all spermatogenic cells. Sertoli cells (red arrow) did not show any necrosis in the affected seminiferous tubules. H&E. A: ×100 and B: ×400.

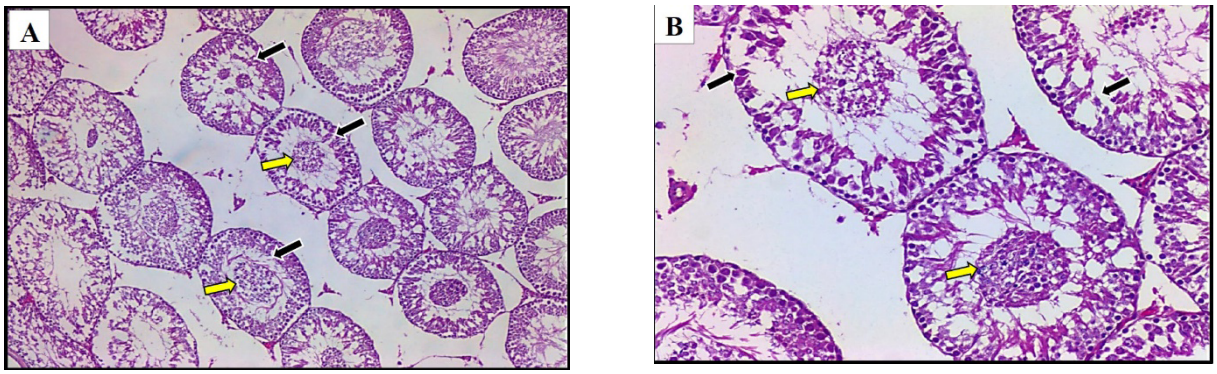

Figure 11. Photomicrographs of the testis from the Vit E-protected group

A & B) Necrosis of spermatogenic cells (black arrow) in the seminiferous tubules involved less than 50% of the seminiferous tubules, where necrotic cell debris (yellow arrow) was aggregated in the center of the seminiferous tubule lumen. H&E. A: ×40 and B: ×100.

stems from its effects at the level of the hypothalamus-pituitary, where it inhibits luteinizing hormone-releasing hormone (LH-RH) and LH secretions (Gharravi et al., 2006). Conversely, these findings contradict the results reported by other researchers (Goncalves et al., 2010; Mosallanejad et al., 2021), who observed an elevation

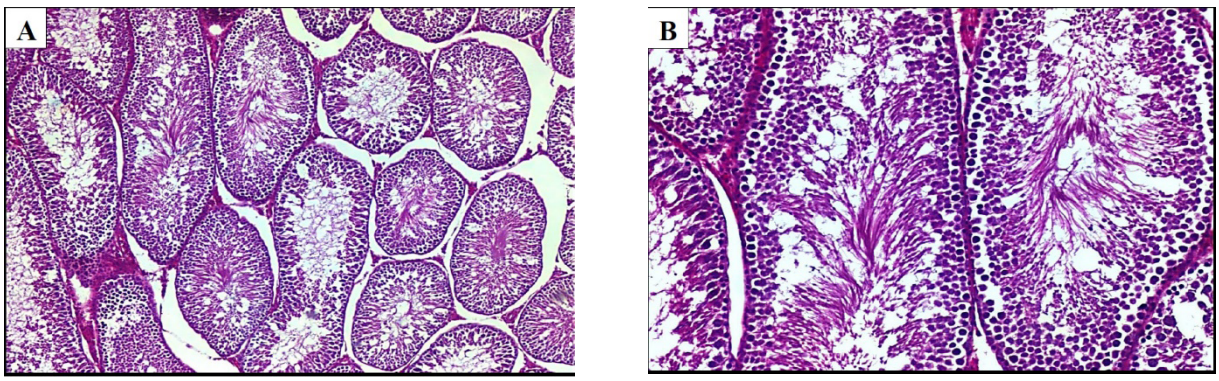

Figure 12. Photomicrograph of the testis from the pro-protected group

A & B: Normal histology of testicular architecture. H&E. A: ×40 and B: ×100.

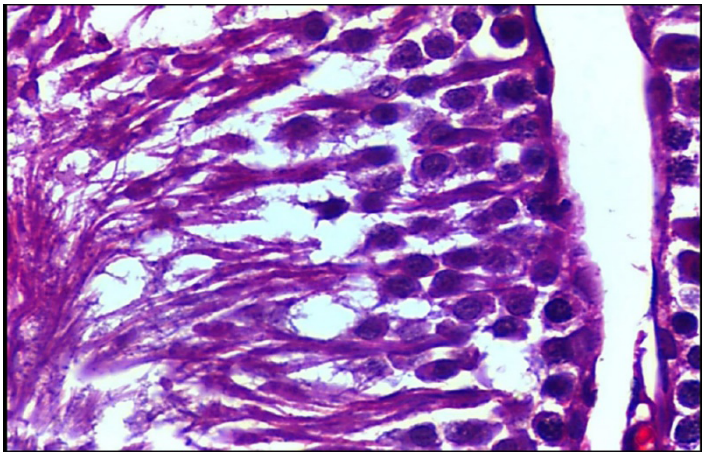

Figure 13. Photomicrograph of the testis from a Pro-protected group

A & B) Normal histology of testicular architecture. H&E. ×400.

in serum LH levels after subcutaneous administration of 0.3 mg BPA/kg body weight/day to male rats for two weeks. We propose that the discrepancy in LH levels may be attributed to variations in the administered doses.

T plays a pivotal role in initiating and sustaining spermatogenesis, including the differentiation of male genital organs and the development of secondary sexual characteristics. Any factor affecting the viability of Leydig cells or disrupting testicular steroidogenesis has the potential to compromise the endocrine regulation of spermatogenesis, ultimately impairing fertility. BPA is recognized as an endocrine-disrupting chemical owing to its ability to interfere with hormonal systems. Within the testis, BPA can bind to estrogen receptors (ERs) and androgen receptors (ARs), resulting in altered hormonal signaling. This disruption can impact T production, a critical factor for maintaining testicular function and supporting spermatogenesis (Walker et al., 2021).

The decline in T levels is linked to low concentrations of BPA, negatively impacting the genes that encode steroidogenic enzymes, such as StAR, P450scc, 3β-HSD, Cyp17a1, Cyp19a1, and 17β-HSD in Leydig cells (Xu et al., 2020).

On the other hand, plasma FSH, LH, and T levels were significantly higher in the Pro plus BPA and vit E plus BPA groups compared to the BPA group, which is similar to the results obtained by El-Naggar et al. (2015), who found that Pro enhances the HPG axis. Shalaby and Saleh (2011) found that testicular toxicity caused a significant decline in the activity of testicular 17-ketosteroid reductase (17-KSR) compared to the control group. The significant reduction in the activity of this enzyme in rats expe-

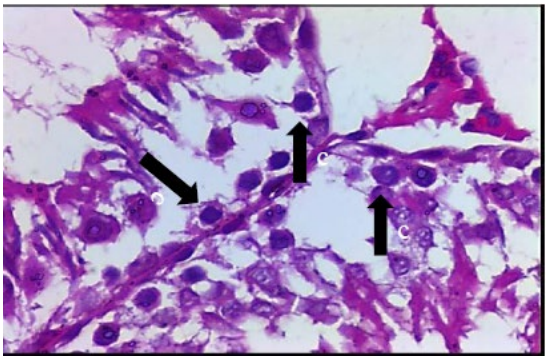

Figure 14. Photomicrograph of the testis from a Vit E-protected group

A & B) Necrosis of spermatogenic cells (black arrow) in the seminiferous tubules led to a reduced population of spermatogenic cells. H&E. ×400

riencing testicular toxicity corresponds to the decreased levels of T in that group. Conversely, the administration of Pro led to an elevation in the activity of this enzyme, subsequently resulting in increased T levels in the Pro-treated group (El-Naggar et al., 2015; Makiabadi, 2022).

Similar effects on 17-KSR and T in rats due to oxidative toxicity induced by cadmium have been reported in previous studies (Salama & El-Bahr, 2007). Specifically, rats protected with Pro exhibited an increase in testicular protein, enhancing the activity of the 17-KSR enzyme, and consequently, elevating blood T and LH concentrations. This stands in contrast to the toxic effect, which resulted in low T and LH plasma levels.

The decrease in T concentrations within the BPA group may be attributed to reduced LH levels, as LH action is mediated by the intracellular secondary messenger cAMP. This messenger enhances the conversion of acetate to squalene, the precursor for cholesterol synthesis, and promotes the conversion of cholesterol to pregnenolone, a crucial step in T formation.

The diminished release and synthesis of T could also result from a decline in the activity of testicular 17-KSR, responsible for converting androstenedione to T (Al-Zaiyadi et al., 2019; Hamzah et al., 2025). Pro appears to alleviate the toxicity induced by BPA, as indicated by the increased activity of 17-KSR and the concentration of T.

Vit E, as an antioxidant, has been studied for its potential to protect testicular cells from oxidative damage. Some research suggests that Vit E supplementation may have a positive impact on T levels in rats by preventing oxidative stress in the testes. T synthesis in Leydig cells can be influenced by oxidative stress, and Vit E's antioxidant properties may help maintain T production (Wu et al., 2010).

As detailed in the present study, the administration of Vit E led to a substantial increase in serum LH and T concentrations. Additionally, there was a noteworthy modulation of the elevated FSH levels induced by BPA administration. These observations align with a previous study (Abdel-Wahab et al., 2021), reporting a significant increase in the expression of FSH and LH hormones in the pituitary gland following Vit E supplementation. The heightened levels of gonadotropins are expected to stimulate Leydig cells, promoting the production of the T hormone, thus corroborating the results obtained in the current study.

Effect of Pro and Vit E on the expression of 3β-HSD1 mRNA in adult male rats with testicular dysfunction

BPA has the potential to interfere with the endocrine system, including the synthesis and function of steroid hormones. While research on the specific effects of BPA on 3β-HSD1 is not as extensive as that on some other endocrine-related pathways, there is evidence suggesting potential impacts. Some studies have suggested that BPA may inhibit the activity of 3β-HSD1. This inhibition could disrupt the normal conversion of precursor steroids to more potent and active forms, affecting the synthesis of androgens and estrogens (Yang et al., 2019). BPA exposure has been associated with alterations in steroid hormone levels. This includes changes in the concentrations of androgens and estrogens, which are substrates and products of 3β-HSD1 activity. Research at the cellular and molecular levels has indicated that BPA can affect gene expression, signal transduction pathways, and the activity of enzymes involved in steroidogenesis, potentially including 3β-HSD1.

Some studies suggest that Pro and Vit E may have anti-inflammatory effects and could potentially influence certain enzymatic activities, but more research is needed to understand its specific impact on 3β-HSD1. Pro and Vit E enhance the concentration of LH. Dai et al. and Monageng et al. found that LH may have a direct or indirect effect on 3β-HSD1. The androgens produced in response to LH stimulation serve as substrates for enzymes, like 3β-HSD1, which further convert them into more potent androgens or estrogens, depending on the tissue and local enzymatic activity (Dai et al., 2017; Monageng et al., 2023). In this study, Pro and Vit E improved the antioxidant system (SOD and GSH); on the other hand, they decreased oxidative stress (MDA), which might enhance the enzymes responsible for androgen formation, such as 3β-HSD1.

When focusing on the histopathological examination of testicular tissue, the testes of rats treated with BPA exhibited complete necrosis of spermatogenic cells in the seminiferous tubules, leading to the complete absence of affected seminiferous tubules and the formation of spaces in the testicular parenchyma. Notably, debris from necrotic cells was observed in the spaces of the affected seminiferous tubules. The aforementioned observations align with earlier findings (Olukole et al., 2018; Zahra et al., 2020; Rashad et al., 2021), reporting spermatogenic cell vacuolization, sloughing, and reduction, alongside testicular atrophy. This corresponds to a substantial loss of spermatogenesis in the majority of seminiferous tu-

bules. Additionally, observations included interstitial bleeding, as well as vacuolated, degenerated, and poorly formed Leydig cells.

BPA is suggested to potentially inhibit the growth of Sertoli cells by concurrently inducing ROS production, loss of mitochondrial membrane potential, apoptosis, autophagy, and necrosis. These effects offer insights into the underlying mechanisms of BPA toxicity in male reproduction (Zhang et al., 2017). Furthermore, T levels decrease in response to low BPA concentrations, impairing the genes encoding steroidogenic enzymes, such as StAR, P450scc, 3β-HSD, Cyp17a1, Cyp19a1, and 17β-HSD in Leydig cells (Xu et al., 2020).

Several antioxidant substances have been studied for their potential protective effects against BPA-induced oxidative stress, such as Asparagus officinalis extract, cinnamon treatment, N-acetylcysteine, and boron (Acaroz et al., 2019; Hussain et al., 2024).

The administration of Pro and Vit E antioxidants demonstrated protective effects against BPA-induced testicular dysfunction. These antioxidants caused improvements in reproductive function parameters, as assessed by sperm count, sperm motility, and serum levels of FSH, LH, and T.

The enhanced effect of Pro and Vit E on testicular tissue is attributed to the regulation of the hypothalamus-pituitary-gonadal (H-P-G) axis, as well as the observed increase in antioxidant systems.

Conclusion

BPA exposure induced histopathological changes and affected male reproductive hormones (FSH, LH, and T) in male rats, along with gene expression of *3β-HSD1* mRNA of (T). Pro and Vit E positively influenced the histopathological changes and male reproductive hormones (FSH, LH, and T) induced by BPA or restored their normal architecture.

Author Contributions Both authors, Ali Maan Mudhaffer and Fouad Ziedan Hamzah, designed and performed the experiments to collect and analyze the data. They also prepared the tissue slices, examined them, and photographed the results using an optical microscope camera. Additionally, they wrote the manuscript and agreed to its publication.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the Faculty of Veterinary Medicine at the University of Kufa, Kufa, Iraq (Code.: UK.VET.2023.27152), and conforms to the guide for the care and use of laboratory animals.

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interception of the results and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

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