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Evaluation of Propolis and Vitamin E Roles on Pituitary-Gonad Axis and Gene Expression of Testosterone Hormone of Testicular Toxicity Male Rats

Propolis and Vitamin E in Male Rat Testicular Toxicity

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Abstract

BACKGROUND The study took place at the animal facility of the University of Kufa College of Veterinary Medicine. The primary objective was to examine the impact of propolis (Pro) and Vitamin E on the reproductive hormones (follicle-stimulating hormone FSH, Luteinizing hormone LH and Testosterone T), histopathological changes, and gene expression of 3β-HSD1mRNA in male rats with induced testicular dysfunction by Bisphenol A(BPA).

OBJECTIVE The primary objective of the current study was to examine the toxic effects of BPA.on the reproductive organs and roles propolis on the regulation of the Pituitary- Gonad Axis and Gene Expression of adult male rats, and comparison with vitamin E. which focused on evaluating, male reproductive hormones (Follicle Stimulating Hormone FSH, Luteinizing Hormone LH and Testosterone T) histopathological changes, and gene expression of 3β-HSD1mRNA.

MATERIAL AND 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 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 intraperitoneal injection three days a week for a duration of three weeks. In Group 4, rats were protected with Propolis at a dosage of 250 mg/kg body weight orally, administered through a gavage needle. This was followed by the intraperitoneal injection of BPA at 50 mg/kg body weight dissolved in corn oil, conducted three days a week over a three-week period. For Group 5, rats received protection with vitamin E at a dosage of 100 mg/kg body weight orally, administered through a gavage needle. This was followed by the intraperitoneal

injection of BPA at 50 mg/kg body weight dissolved in corn oil, administered three days a week over a three-week period.

RESULTS the result showed the Bisphenol A was significant adverse effects male reproductive hormones (FSH, LH and T) histopathological changes, and gene expression of 3β-HSD1 mRNA. In contrast, the propolis and vitamin E groups showed was positive influence on all parameters.

Conclusion: Bisphenol A exposure induced histopathological changes and male reproductive hormones (FSH, LH, and T) of male rats and gene expression of 3β-HSD1 mRNA of (Testosterone). Propolis and Vitamin E were positively influenced by histopathological changes and male reproductive hormones (FSH, LH, and T) induced by Bisphenol A or restored their normal architecture.

Keywords: Bisphenol A, Propolis, Spermatogenesis, Vitamin E and 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. Here are some potential causes and aspects of testicular dysfunction in male rats (Hormonal Imbalances, Genetic Factors,

Nutritional Deficiencies, and Toxic Exposures) (Usende *et al.,* 2022; Sartorius & Handelsman, 2023; Agarwal *et al.,* 2020).

Bisphenol-A 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 in rubber manufacturing (Liu *et al.,* 2021).

Bisphenol-A 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 Bisphenol A 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 Types 1 (3β-HSD1) is responsible for the formation testosterone an enzyme involved in steroidogenesis, the process by which steroid hormones are synthesized. In particular, 3β-HSD1 plays a role in the formation of testosterone, which is an important male sex hormone (Gao *et al.,* 2021) The synthesis of testosterone 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). 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 testosterone (Elzenaty et al., 2022) Finally, androstenedione is converted into testosterone, the primary male sex hormone. This conversion can occur in various tissues, including the testes and the adrenal glands.

The genes associated with testosterone formation in male rats include:

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 dehydroepiandrosterone (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 testosterone) (Jayaraman et al., 2020; Khalid, 2024).

Hsd17b (17β-Hydroxysteroid Dehydrogenase): The Hsd17b gene family encodes enzymes that convert androstenedione to testosterone. 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 testosterone to dihydrotestosterone (DHT), which is a more potent androgen. The specific isoforms involved include Srd5a1 and Srd5a2 (Corti et al., 2022) In this study select 3β-Hydroxysteroid Dehydrogenase (3β Hsd1) for gene expression.

Propolis is a natural resinous substance crafted 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 propolis (Bae et al., 2022).

Propolis 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 testosterone levels, accompanied by improvements in both sperm count and motility. (Polat et al., 2019) Propolis 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 propolis altered plasma levels of reproductive hormones. Propolis increases the plasma level of FSH, LH, and Testosterone (Polat et al., 2019).

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

Vitamin 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. Vitamin E is known for its antioxidant properties, which means it helps 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 vitamin E in mitigating testicular toxicity, and the results have shown some promising effects:

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

*Sperm Quality: Some research suggests that vitamin 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 vitamin E (Sabetian et al., 2021).

Material and methods:

Animal 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 (UK.VET.2023.27152.).

Animals and Housing The present study was conducted at the College of Veterinary Medicine-University of Kufa, during the period extended from 1/8/2023 to 6/3/2024. The study comprises two experiments a total of seventy adult male rats 12 weeks old, weighing (200-250g) was used. rats were kept for an adaptation period of three weeks at the animal house of the College of Veterinary Medicine / University of Kufa. The animals were housed in cages,4 rats in each cage, under optimum conditions (12/12 light, dark cycle, 22 ± 2 C˚) Animal had ad-libitum to fed 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 randomly divided into different cages, with each cage containing six animals at the Animal Housed in the faculty of the College of Veterinary Medicine, University of Kufa. 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.

In Group 2, rats were administered 0.2 mL of corn oil (the vehicle for BPA) through the 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 intraperitoneal injection three days a week for three weeks (Othman et al., 2014).

in Group 4, rats received protection with Propolis at a dosage of 250 mg/kg body weight orally, administered through a gavage needle. This was followed by the intraperitoneal 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)

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

Animal Preparation

The animals were anesthetized by using Ketamine (90 mg/kg body weight) and Xylazine (40 mg/kg body weight). Post anesthesia, the bilateral testes for histological and homogenized gene expression and blood were collected, and the animals were subsequently euthanized.

Blood Sample Collection

Ten rats from each group were sacrificed when the therapy was completed. Blood samples were taken from the heart's inferior vena cava of each sacrificed rat by sterile syringe in a plain tube without anticoagulant and serum was extracted and stored after 15 minutes of centrifugation at 3000 rpm in micro-Eppendorf tubes at -20C^{o} to conduct laboratory analysis for biochemical tests and take the testis for pathohistological

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 a duration of forty-eight 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 degrees Celsius for two hours The tissues were sliced to a thickness of 5 micrometers using a microtome. The subsequent step included dewaxing and staining with Eosin and Harris Hematoxylin (E&H). 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 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 ddH2O 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).

Table (1) Primers Used in this Study

Organism	Target gene	Primer name	5'-3'	PCR Product	Reference	Accession number
Rattus rattus	**Hsd3b1**	F	CCCTGCTCTACTGGCTTGC	189 bp	Ji et al., 2021	XM_032897634.1
		R	TCTGCTTGGCTTCCTCCC			
Rattus rattus	**GAPDH**	F	ATGACTCTACCCACGGCAAG	89 bp	Kunst et al., 2012	NM_017008

		R	CTGGAAGATGGTGATGGGTT			

Statistical analysis:

The data underwent an analysis of variance, and the significant differences at P≤0.05 were assessed by ANOVA, one-way by utilizing the statistical software's sigma statistical

Results

This present investigation aimed to determine the positive effect of propolis (Pro) and vitamin E on animals in which we created a testicular dysfunction by BPA to shed light on its potential impact on the complex processes that control male reproductive health and evaluation of a range of assessments, including, the male reproductive hormones (FSH, LH, and T), gene expression analysis of 3β-HSD1 mRNA and histopathological examination.

The impact of Propolis and Vitamin E on serum pituitary-gonadal-axis hormones in adult male rats with testicular dysfunction.

FSH

In Figure (1), a significant (P≤0.05) reduction in serum FSH concentration is observable in the Bisphenol A Group compared to the control group. Conversely, the protected groups (BPA plus Pro, BPA plus Vit E) exhibit a substantial (P≤0.05) rise in serum FSH concentration compared to

the Bisphenol A Group, though not significantly different (P>0.05) from the control groups. Importantly, there is no significant difference between the (BPA plus Pro and BPA plus Vit E) groups themselves.

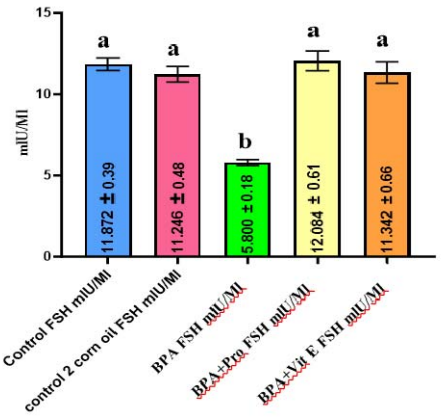

Figure (1) Effect of Propolis and Vit E on Testicular serum FSH in Testicular dysfunction Adult Male Rats

LH

Figure (2) indicates a significant (P≤0.05) reduction in serum LH concentration in the Bisphenol A Group compared to the control groups. While, the protected groups (BPA plus Pro, BPA plus Vit E) demonstrated a significant (P≤0.05) increase in serum LH concentration compared to the Bisphenol A Group, While, The protected group BPA plus Vit E showed a significant compared to the control groups, but a significant (P≤0.05) increase in serum LH concentration in protected

group BPA plus Pro compared to the control groups. Finally, no significant between (BPA plus Pro, and BPA plus Vit E) .

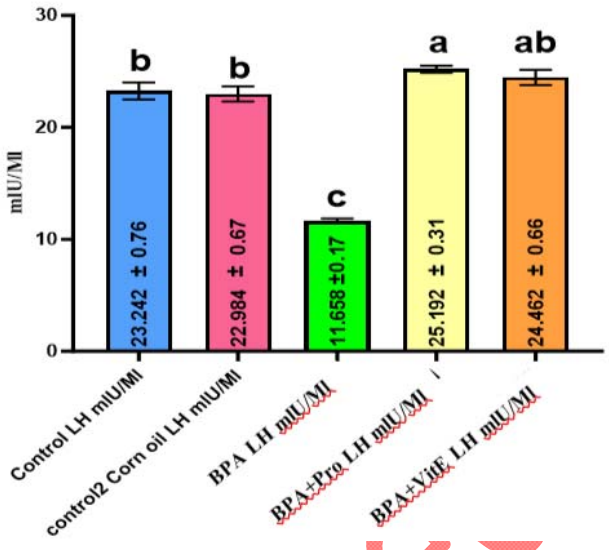

Figure (2) Effect of Propolis and Vit E on Testicular serum LH in Testicular dysfunction Adult Male Rat

Testosterone (T)

Figure (3) shows A significant (P≤0.05) decline in serum testosterone (T) concentration noted in the Bisphenol A Group compared to the control groups. In contrast, the protected groups (BPA plus Pro, BPA plus Vit E) exhibit a significant (P≤0.05) increase in serum T concentration compared to the Bisphenol A Group. However, there is also a significant (P≤0.05) decrease in serum T concentration in the protected groups, particularly in (mention specific protected group).

group BPA plus Pro compared to the control groups, also a significant (P≤0.05) decrease in serum T concentration in protected group BPA plus Vit E compared to the protected group BPA plus Pro and control groups. Finally, no significant between the control groups.

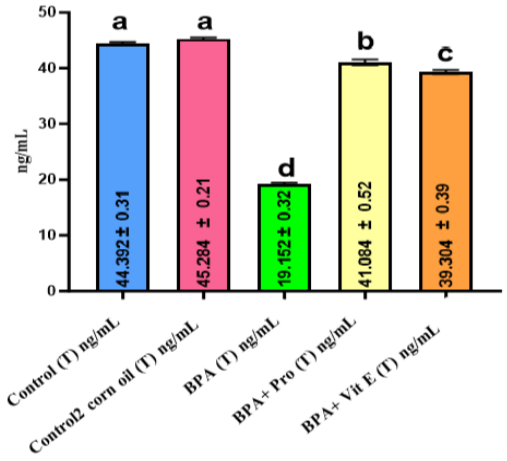

Figure (3). Effect of Propolis and Vit E on Testicular serum T in Testicular dysfunction Adult Male Rats.

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 ($p<0.0001$) decreases in the Bisphenol A group compared to the control groups. Conversely, the 3β-HSD1 mRNA expression significantly ($p<0.0001$) increases in all protective groups (BPA + Pro and BPA + Vit E) compared to both the BPA group and the control groups.

Table (2): Effect of propolis and Vitamin E on Expression of 3β-HSD1 mRNA in testicular dysfunction Adult Male Rats.

Multiple comparisons test	Significant	Summary	Adjusted P Value
Control vs. Corn oil	Yes	*	0.0132
Control vs. Bisphenol A	Yes	***	0.0002
Control vs. Bisphenol A + Propolis	Yes	****	<0.0001
Control vs. Bisphenol A + Vit E	Yes	****	<0.0001
Corn oil vs. Bisphenol A	Yes	****	<0.0001
Corn oil vs. Bisphenol A + Propolis	Yes	****	<0.0001
Corn oil vs. Bisphenol A + Vit E	Yes	****	<0.0001
Bisphenol A vs. Bisphenol A + Propolis	Yes	****	<0.0001
Bisphenol A vs. Bisphenol A + Vit E	Yes	****	<0.0001
Bisphenol A + Propolis vs. Bisphenol A + Vit E	Yes	**	0.0027

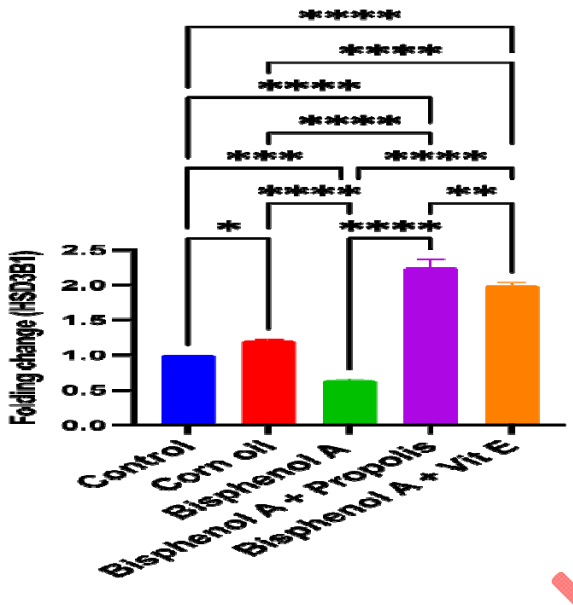

Figure (4) Effects of propolis and Vitamin E administration on the Expression of 3β-HSD1mRNA in the testis of male rats with testicular dysfunction.

Histopathological examination testes

Normal histology testicular architectures. Note Seminiferous tubules (black arrow) and Leydig cells (yellow arrow) that were observed in intra-seminiferous tubule spaces as shown in Figures (5,6,7,8,9). While, the testes in male rats treated with Bisphenol A, experiencing testicular toxicity, exhibit histopathological changes including Complete necrosis of spermatogenesis cells of seminiferous tubules led to the complete absence of affected seminiferous tubules, forming spaces in testicular parenchyma. Note the debris of necrotic cells was observed in spaces of affected seminiferous tubules. in Figures (10,11,12). Another

histopathological section of testis in Testicular toxicity treated with Bisphenol A male rats showed Necrosis of spermatogenesis cells of seminiferous tubules that involved all seminiferous tubules, however, individuals of spermatogonia was observed in these tubules. Note one of the seminiferous tubules showed severe necrosis that involved all spermatogenesis. However, Sertoli cells (red arrow) did not show any necrosis in affected seminiferous tubules. Fig. (13,14,15).

In contrast, male rats treated with Propolis exhibited no significant occupied lesions in the testis. (SOL) normal spermatogenesis in some seminiferous tubules as shown in Fig. (18,19,20). Moreover, the testis in male rats treated with vitamin E showed Necrosis of spermatogenesis cells of seminiferous tubules that involved less than 50% seminiferous tubules, where necrotic cells debris was aggregated in the center of seminiferous tubules lumen and Necrosis of spermatogenesis cells of seminiferous tubules led to minimized the spermatogenesis cells population as shown in Fig. (16,17).

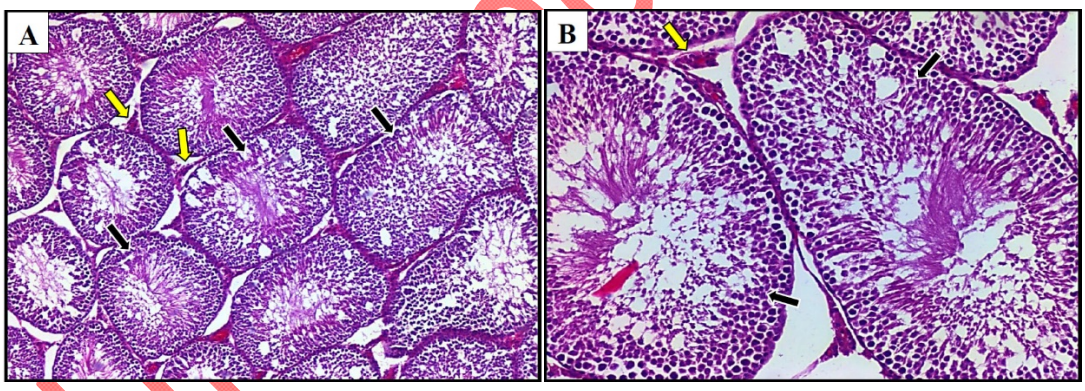

Figure (5,6): Photomicrograph of testis of control negative 1 group rat. A&B/ Normal histology testicular architectures. Note Seminiferous tubules (black arrow) and leydig cells (yellow arrow) that observed in intra-seminiferous tubules spaces. **H&E. A: 40x and B: 100x.**

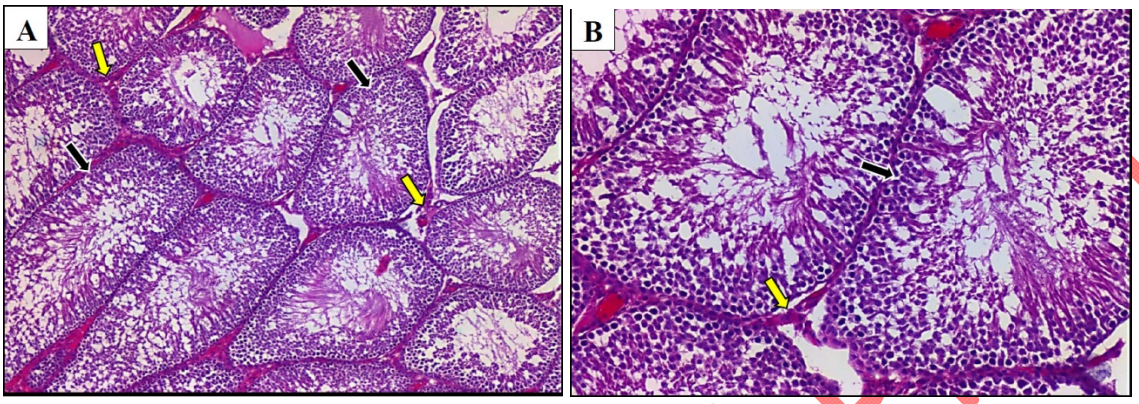

Figure (7,8): Photomicrograph of testis of control negative 2 group rat. A&B/ Normal histology testicular architectures. Note Seminiferous tubules (black arrow) and Leydig cells (yellow arrow) that observed in intra-seminiferous tubules spaces. **H&E. A: 40x and B: 100x**

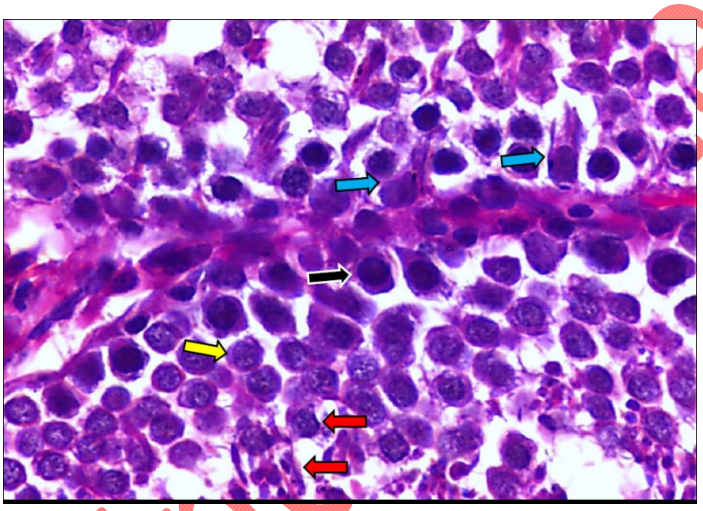

Figure (9): Photomicrograph of testis of control negative 2 group rat. Normal histology of seminiferous tubules. Note spermatogenesis cells: spermatogonia (black arrow)spermatocytes (yellow arrow), round or elongated spermatid (red arrow) and Sertoli cells (blue arrow). **H&E. 400x.**

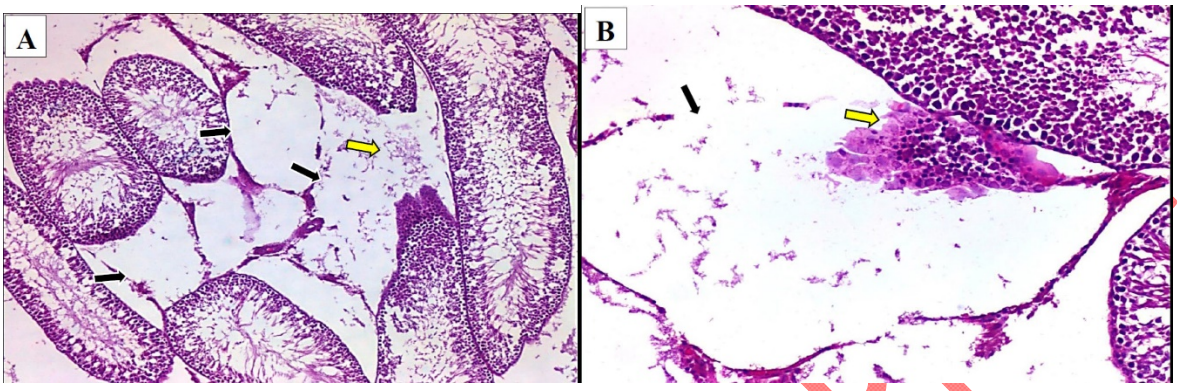

Figure (10,11): Photomicrograph of testis of control positive group rat. A&B/ Complete necrosis of spermatogenesis cells of seminiferous tubules led to complete absence of affected seminiferous tubules, forming spaces (black arrow) in testicular parenchyma. Note the debris of necrotic cells (yellow arrow) was observed in spaces of affected seminiferous tubules. **H&E. A: 40x and B: 100x.**

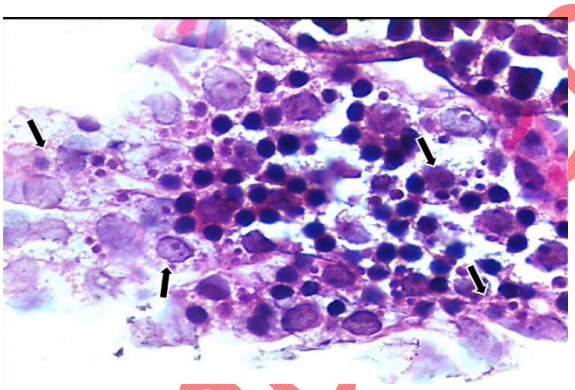

Figure (12): Photomicrograph of testis of control positive group rat. the necrotic cells (black arrow) were observed in spaces of affected seminiferous tubules. **H&E. 400x.**

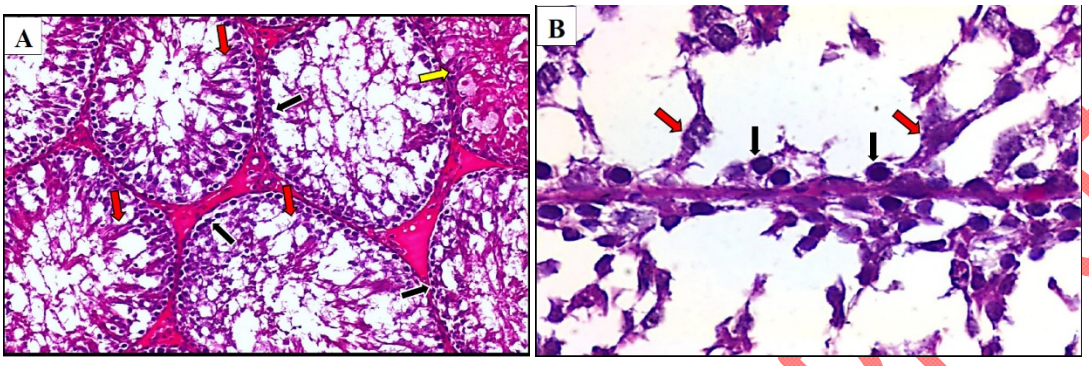

Figure (13,14): Photomicrograph of testis of control positive group rat. A&B/ Necrosis of spermatogenesis cells of seminiferous tubules that involved all seminiferous tubules, however individuals of spermatogonia (black arrow) was observed in these tubules. Note one of seminiferous tubules showed severe necrosis (yellow arrow) that involved all spermatogenesis. However, Sertoli cells (red arrow) did not show any necrosis in affected seminiferous tubules. **H&E. A: 100x and B: 400x.**

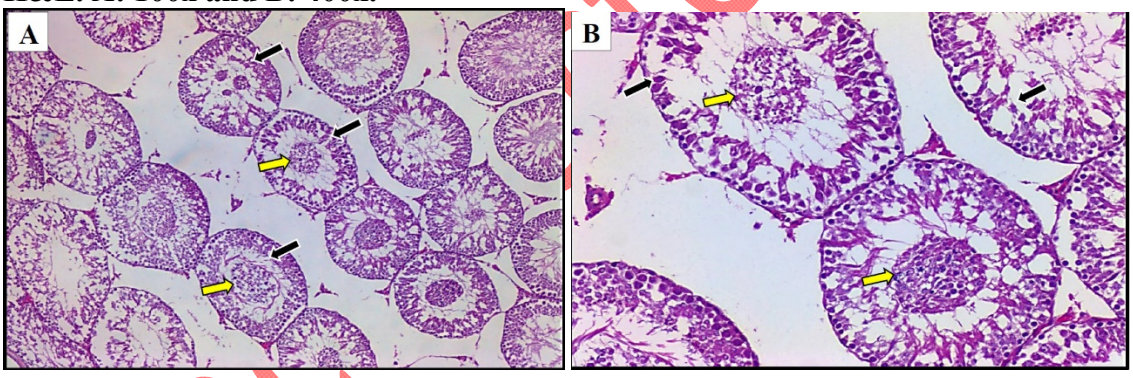

Figure (15,16): Photomicrograph of testis of vitamin E protected group rat. A&B/ Necrosis of spermatogenesis cells (black arrow) of seminiferous tubules that involved less than 50% seminiferous tubules, where necrotic cells debris (yellow arrow) were aggregated in center of seminiferous tubules lumen. **H&E. A: 40x and B: 100x.**

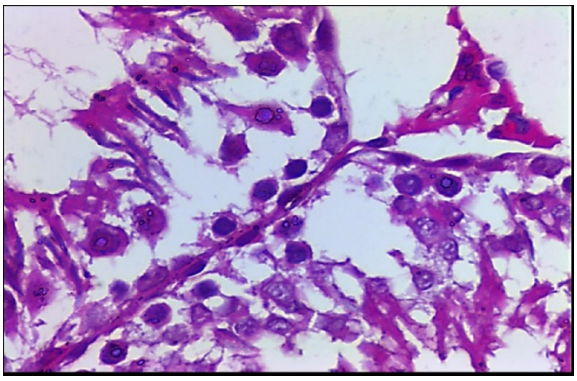

Figure (17): Photomicrograph of testis of vitamin E protected group rat. A&B/ Necrosis of spermatogenesis cells (black arrow) of seminiferous tubules led to minimized the spermatogenesis cells population. **H&E. 400x**

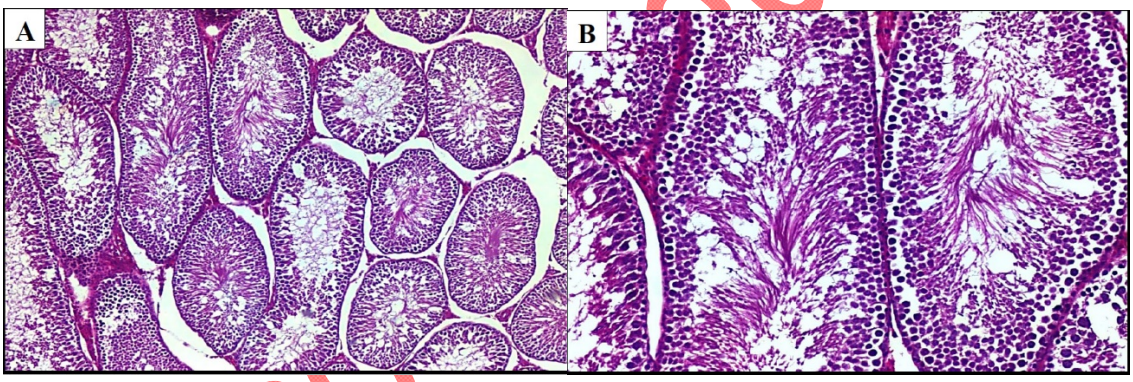

Figure (18,19): Photomicrograph of testis of propolis protected group rat. A&B/ Normal histology testicular architectures. Note. **H&E. A: 40x and B: 100x.**

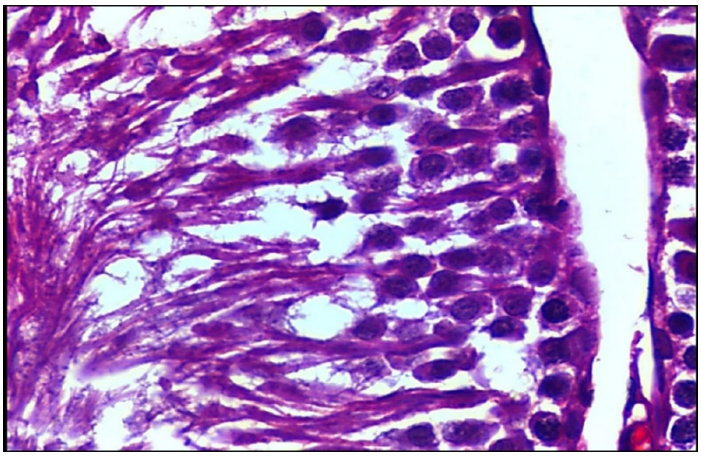

Figure (20): Photomicrograph of testis of propolis protected group rat. A&B/ Normal histology testicular architectures. **H&E. 400x.**

Discussion

Effect of Propolis on FSH, LH, and T in Testicular Dysfunction Male Rats Induced by Bisphenol A. The administration of BPA for 21 days resulted in a decrease in the concentrations of FSH, LH, and T hormones in the Bisphenol A group when compared to the control groups. These results are in agreement with (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). Indeed, Sertoli cells play a crucial role in influencing 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 a deficiency of supportive functions, this condition has the potential to lead to the loss of spermatogenic cells. It has been observed that Sertoli cells function as targets for pituitary-derived FSH and testosterone. 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 testosterone levels. It's noteworthy that testosterone secretion takes place in Leydig cells within the testicular interstitium, responding to the stimulation of LH. (Urriola-Muñoz et al., 2014) Hence, the absence of LH stimulation in the BPA-protected groups could provide a rationale for the diminished presence of Leydig cells, interstitial tissue atrophy, and the subsequent decrease in testosterone production. The observed decrease in serum LH levels depicted in Figure (2) aligns with the findings reported by (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 Luteinizing Hormone (LH) stimulation of steroidogenesis, 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. Hyperprolactinemia has been demonstrated to induce reproductive dysfunction, as confirmed by. (Edinoff et al., 2021) This dysfunction is not mediated through a direct action on the testis but rather stems from its effects at the level of the hypothalamus-pituitary, where it inhibits luteinizing hormone releasing hormone (LH-RH) and LH secretions confirmed by (Gharravi et

al., 2006). Conversely, these findings contradict the results reported by (Goncalves et al., 2010; Mosallanejad et al., 2021), who observed an elevation in serum LH levels after subcutaneous administration of male rats with 0.3 mg BPA/kg b.wt/day for 2 weeks. We propose that the discrepancy in LH levels may be attributed to variations in the administered doses.

Testosterone 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 testosterone production, a critical factor for maintaining testicular function and supporting spermatogenesis (Walker et al., 2021).

The decline in testosterone 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 testosterone levels were significantly high in Pro plus BPA and vit E plus BPA groups compared to BPA group is similar to the results obtained by (El-Naggar et al., 2015), Which found the propolis lead to enhance the HPG axis. Shalaby and Saleh 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 experiencing testicular toxicity corresponds to the decreased levels of testosterone in that group. Conversely, the administration of propolis led to an elevation in the activity of this enzyme, subsequently resulting in increased testosterone levels in the propolis-treated group (El-Naggar *et al.,* 2015; Makiabadi, 2022) Similar effects on 17-KSR and testosterone in rats due to oxidative toxicity induced by cadmium have been reported in previous studies (Salama & El-Bahr, 2007). Specifically, rats protected with propolis exhibited an increase in testicular protein, enhancing the activity of the 17-KSR enzyme, and consequently, elevating blood testosterone and LH concentrations. This stands in contrast to the toxic effect, which resulted in low testosterone and LH plasma levels. The decrease in testosterone 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 testosterone formation. The diminished release and synthesis of testosterone could also result from a decline in the activity of testicular 17-KSR, responsible for converting androstenedione to testosterone (Huang et al., 2016) Propolis appears to alleviate the toxicity induced by BPA, as indicated by the increased activity of 17-KSR and the concentration of testosterone.

Vitamin E, as an antioxidant, has been studied for its potential to protect testicular cells from oxidative damage. Some research suggests that vitamin E supplementation may have a positive impact on testosterone levels in rats by preventing oxidative stress in the testes. Testosterone synthesis in Leydig cells can be influenced by oxidative stress, and vitamin E's antioxidant properties may help maintain testosterone production (Wu et al., 2010).

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

Effect of Propolis and Vit E on Expression of 3β-Hydroxysteroid Dehydrogenase Types 1 (3β-HSD1) mRNA in Testicular Dysfunction Adult Male Rats. 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-Hydroxysteroid Dehydrogenase Type 1 (3β-HSD1) is not as extensive as 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 propolis and vitamin 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.propolis and vitamin E enhance the concentration of LH, Dai, and Monageng found the LH may be effected direct or indirect on 3β-HSD1, The androgens produced in response to LH stimulation serve as substrates for enzymes like 3β-HSD1 to 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 the propolis and vitamin E improved the antioxidant system (SOD and GSH) in other hand decreases oxidative stress (MDA) might enhance the enzymes responsible for androgen formation such as 3β-HSD1.

When focusing on the histopathological examination of testicular tissue, Rats testes treated with Bisphenol A, Complete necrosis of spermatogenesis cells of seminiferous tubules led to complete absence of affected seminiferous tubules, forming spaces in testicular parenchyma. Note the debris of necrotic cells was observed in spaces of affected seminiferous tubules. The aforementioned observations align with the findings reported in studies by (Olukole et al., 2018; Zahra et al., 2020; Rashad et al., 2021) These findings reported spermatogenic cell vacuolization, sloughing, and reduction, alongside testicular atrophy. This aligns with a substantial loss of spermatogenesis in the majority of seminiferous tubules. Additionally, observations included interstitial bleeding, vacuolated, degenerated, and poorly formed Leydig cells.

Bisphenol A 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, testosterone 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 Propolis and vitamin E antioxidants has demonstrated protective effects against BPA-induced testicular dysfunction. These antioxidants have shown improvements in reproductive function parameters, as assessed by sperm count, sperm motility, and serum levels of FSH, LH, and testosterone.

It is possible that the enhanced effect of Propolis and vitamin The impact of vitamin E on testicular tissue is attributed to the regulation of the H-P-G axis. Additionally, the observed increase in antioxidant systems.

Conclusion

Bisphenol A exposure induced histopathological changes and male reproductive hormones (FSH, LH and T) of male rats and gene expression of 3β-HSD1 mRNA of (Testosterone). Propolis and Vitamin E were positively influenced on histopathological changes and male reproductive hormones (FSH, LH, and T) induced by Bisphenol A or restored their normal architecture.

Author Contributions Both authors Ali Maan mudhaffer and Fouad Ziedan Hamzah designed and performed the experiment to collect the data and analyze also prepared the tissue slices and

examined to be photographed using an optical microscope camera, wrote the manuscript and agreed to the published of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

Ethics statement

Ethical approval was granted through the local committee of the animal care and use at the College of Veterinary Medicine within the University of Kufa (Number 27152/PG) before starting this study.

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