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The Toxic Effect of Bisphenol A on Male Rat Testicles and The Possible Protective Value of Propolis and Vitamin E

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Abstract:

This study aimed to examine the impact of propolis (Pro) on various physiological parameters in male rats experiencing induced testicular dysfunction by Bisphenol A. Five groups of ten male rats apiece were created by random selection from the rats. Group 1 the rats, which performed as the group under negative control, received normal food and water. In Group 2, rats were subjected to intraperitoneal administration of 0.2 mL of corn oil, utilized as the vehicle for bisphenol A (BPA), thereby serving as the vehicle control group. For Group 3, rats were administered in corn oil, BPA dissolved at a dosage of 50 mg/kg body weight administered three times a week for three weeks using an intraperitoneal injection. In Group 4rats received a dose of 250 mg/kg body weight of propolis (protected) orally via a gavage needle. Subsequently, intraperitoneal injections are administered to them from BPA delivered three times weekly for three weeks, the dosage is 50 mg per kilogram of body weight dissolved in corn oil. In Group 5, rats received protection with vitamin E orally, at a dose of 100 mg/kg body weight, using a gavage needle. The intraperitoneal injection of BPA, dissolved in corn oil at a dose of 50 mg/kg body weight, was then given three times a week for three weeks. After three weeks, animals were sacrificed, and testes and blood were gathered for additional analysis. Superoxide Dismutase (SOD), Sperm Viability, General motility, Progressive motility, and Morphology, were all significantly reduced in the BPA group compared to the control groups, according to the results. While malondialdehyde (MDA) increased in the BPA group compared to the control groups. In conclusion, propolis enhanced physiological parameters adversely affected by BPA, suggesting its potential as a protective agent against BPA-induced testicular dysfunction.

Keywords: Antioxidant, Bisphenol A, Propolis and Vitamin E.

Introduction:

Bisphenol A (BPA) is produced more widely than any other chemical in the world. Food and drink containers may release BPA [1] Humans readily absorb BPA after consumption and break it down into its

metabolites [2]. There is mounting evidence that Then, BPA can be freely distributed throughout the body. It is forbidden for children's products to contain BPA as a result of the chemicals found in children's urine. BPA at low doses is still there in items that come into contact with food, like coating agents in cans [3].

One of the most often utilized artificial compounds in the world is BPA which has estrogenic and antiandrogenic properties that impact the reproductive systems as an endocrine disruptor. Research indicates that BPA is part of the process of creating several endocrine problems, like hormone-dependent cancers, precocious puberty, infertility, and reproductive dysfunction [4]. Alternatively, using Cyperus esculentus hydroethanolic extract on Testicular failure brought due to lead acetate in Wistar rats [5]. Alternatively, by exposure to malathion [6], cadmium chloride [7], and by extracting the seeds of Portulaca oleracea, this study would do the induction of testicular dysfunction by exposure to bisphenol A [8].

Bees use tree sap, bee saliva, and beeswax to make propolis, a naturally formed resinous material. 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 [9] The propolis Consists of different bioactive substances, such as flavonoids, phenolic acids, and other polyphenols through the neutralization of reactive oxygen species (ROS) and other free radicals, these substances cooperate to counteract the negative consequences of oxidative stress [10].

Propolis was investigated for its potential to enhance sperm quality in male Wistar rats with sexual impairment induced by paroxetine [11]. Moreover, there were notable enhancements in sperm motility and the count was recorded. [12]. Vitamin E has been studied for its potential protective effects against testicular toxicity, particularly in the context of oxidative stress [13,14].

The present study aimed to investigate the toxic effects of BPA on the sperm characteristics, MDA and SOD, as well as, the protective effect of propolis, if any, against BPA-induced reproductive dysfunction in male rats and comparison with vitamin E.

Material and methods:

Animal Ethical Approval:

The University of Kufa's Faculty of Veterinary Medicine ethics committee accepted this work, which complies with the UK.VET.2023.27152. A Guide for the Use and Care of Animals in Research.

Animals:

In this experiment, fifty adult male albino rats in good health each weighing between 200 and 250 grams, were employed for this study and had been acquired from the University of Kufa's Faculty of Veterinary Medicine's animal housing. In hygienic conditions, rats were kept in four-per-cage steel wire cages at the University of Kufa's Physiology Animal House [15-16-17].

Experimental Design:

The male rats in the experiment were placed into five groups at random, each holding ten male rats, in the manner described below:

Group 1 was utilized as the negative control for the experimental subjects they were provided with food and drink to function as the vehicle control group, Group 2 underwent intraperitoneal (IP) delivery of 0.2 mL of corn oil, the BPA vehicle. In group 3, rats received for three weeks, BPA was Soluble in corn oil and injected intraperitoneally three times a week at a level of 50 mg/kg body weight [18]. Group 4, Propolis was given orally to male rats at a dose of 250 mg/kg body weight to protect them, delivered with a gavage needle. [19] This was followed by the intraperitoneal injection of BPA in corn oil for three weeks, at a dosage of 50 mg/kg body weight, three times a week. Group 5, rats received protection with vitamin orally, at a dose of 100 mg/kg body weight, using a gavage needle. The intraperitoneal injection of BPA, dissolved in corn oil at a dose of 50 mg/kg body weight, was then given three times a week for three weeks. [20]. after three weeks, every animal was sacrificed, and testes and blood were gathered for additional analysis.

Studied parameters

Assessment of Testicular Oxidative Stress and Antioxidant Systems (MDA and SOD)

The Competitive-ELISA principle is employed in conjunction with this ELISA kit, which includes a micro-ELISA plate pre-coated with MDA. The equipment used was sourced from the China catalogue (Elabscience, E-EL-0060). (SOD) assay kit (FineTest Cat.No: ER1950) was used to assess the amount of SOD in the testis.

Sperm Motility Analysis

Spermatozoa were obtained from the epididymal tubules by microscissoring the left caudal epididymis into small fragments, which were subsequently placed in normal saline. Following an incubation period at 37°C, the epididymal semen suspension was assessed for both general and progressive motility [21]. This procedure was carried out at the artificial insemination and frozen semen straw production unit within the Faculty of Veterinary Medicine at the University of Kufa.

Sperm Viability and Morphology Analysis

The study utilized an eosin-nigrosin stain (EN) method following Baiee et al.'s (2018) [22]. In brief, 20 μL of EN was mixed with a 10 μL suspension of epididymal semen, applied to a slide, and dried at 45°C. Sperm viability was assessed under a ×400 a phase-contrast microscope, distinguishing between dead (pink-stained) and living (unstained) sperm. Morphology was examined at ×1000 magnification, with normal head, mid-piece, and tail considered.

Statistical analysis:

After doing an analysis of variance on the data, an examination of the significant differences at $P \leq 0.05$ using an ANOVA one-way was conducted using the statistical program's the least significant difference (LSD) to assess the differences among means using computerized SPSS version 20. ANOVA revealed a significant ($P<0.05$) difference and GraphPad Prism 8.

Results:

Male Adult Rats with Dysfunctional Testicles and Propolis and Vitamin E's Effect on Testicular Tissue (MDA and SOD).

MDA

Figure (1) demonstrates that in the Bisphenol A Group's tissue MDA concentration, the observed difference was statistically significant ($P \leq 0.05$) greater than in groups under control. The treated groups (BPA plus Pro, BPA plus Vit E) revealed that the tissue MDA concentration was significantly ($P \leq 0.05$) less than the Bisphenol A Group, but nonsignificant the treated group BPA plus Pro compared to BPA plus Vit E and control groups, also non a significant the treated group BPA plus Vit E and control group (corn oil) finally, Tissue MDA levels are significantly ($P \leq 0.05$) lower in the treatment groups BPA plus Vit E than in the control group (normal saline).

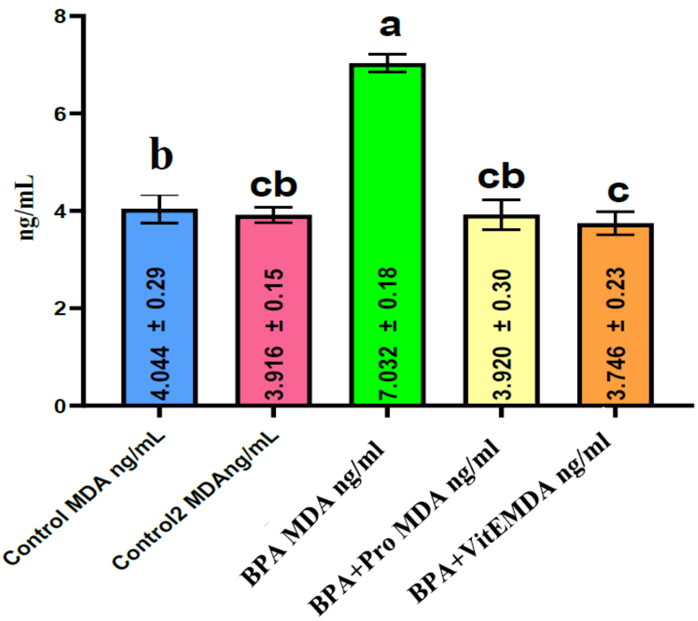

Figure 1: Impact of Propolis and Vit E on Testicular Tissue MDA in Testicular dysfunction Adult Male Rats (Mean ± SE) (n=10). LSD=0.715

SOD

Figure (2) demonstrates that the tissue SOD concentration Significantly ($P \leq 0.05$) less than the control groups was the effect Bisphenol A Group. The BPA plus Pro and BPA plus Vit E treated groups exhibited a noteworthy ($P \leq 0.05$) rise in tissue SOD concentration as compared to the Bisphenol A group. The group that received treatment also showed a significant ($P \leq 0.05$) raise in tissue SOD concentration. BPA plus Pro compared to the BPA plus Vit E group and control groups, finally non a significant between BPA plus Vit E group and control groups, also a significant between the control groups with them.

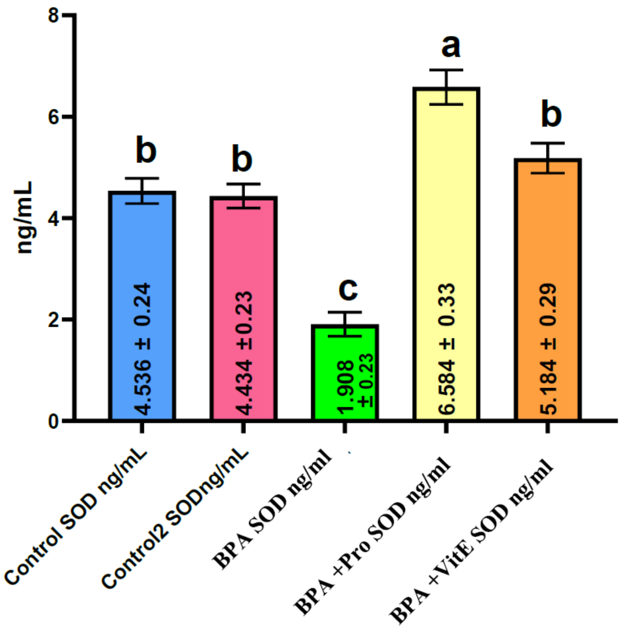

Figure 2: Impact of Vitamin E and Propolis on SOD on Testicular Tissue in Testicular Dysfunction Adult Male Rats. (Mean ± SE) (n=10). LSD=0.809.

Propolis and vitamin E's impact on epididymal sperm analysis in adult male rats with testicular dysfunction.

Table (1) compares the Bisphenol A Group to the Control Group and demonstrates a significant (P≤0.05) decline in the general sperm motility percentage, progressive motility percentage, morphologically normal sperm percentage, and viability of sperm percentage. When compared to the Bisphenol A Group, the treated groups (BPA plus Pro, BPA plus Vit E) exhibit a significant (P≤0.05) rise in the percentage progressive motility percentage, morphologically normal sperms percentage, and viability of sperm percentage, but not a significant (P>0.05) rise in comparison to the groups under control. and last, there wasn't any discernible difference between them (BPA plus Pro, BPA plus Vit E).

Table 1: shows how propolis and vitamin E affect the analysis of epididymal sperm in adult male rats with testicular dysfunction. Rats that are male. (Mean +/- SE) n = 10. Significant differences at the (P<0.05) level are indicated by values written in small letters**.**

Parameters / Groups	Motility		Morphology (%)	Viability (%)
	General Motility (%)	Progressive Motility (%)		
Control (N. S)	84.60±1.43a	80.00±0.70a	84.40±1.43a	86.60±0.97b
Control 2 (Corn oil)	82.80 ±1.65a	79.60±0.74a	84.60±1.63a	91.20±0.73a
BPA	42.20±0.58 b	44.00±0.54b	70.00±0.70b	76.00±1.14c
BPA+Propolis	83.40±1.24 a	80.80±1.28a	84.00±1.87a	90.60±0.67a
BPA+Vit E	82.80±1.65 a	78.60±0.91a	83.00±1.54a	87.60±0.67b
LSD	4.053	2.585	4.399	2.544

Discussion:

This study set out to determine whether male rat reproductive organ Oxidative stress is brought on by bisphenol A (BPA) exposure [23,24]. and whether administering propolis after BPA exposure can lessen the harmful effects of BPA. Propolis, rich in flavonoids and phenolic compounds, is acknowledged for its antioxidant qualities and the ability to scavenge free radicals, [25,26]. This antioxidant capacity, especially linked to propolis's flavonoids' capacity to scavenge free radicals [27], may contribute to its ability to alleviate the oxidative stress toxicity induced by BPA [28,29]. Notably, these flavonoids are acknowledged to have antioxidant advantages surpassing those of vitamins C and E, as highlighted by [30].

Propolis demonstrated the ability to counteract the induced reduction via enhancing antioxidant enzymes in the testis, and antioxidant defence mechanisms as observed in studies by [31,32]. Considering that a reduction in antioxidant defence mechanisms has a relationship to reduced semen quality, as highlighted by [33]. it can be inferred that the favourable antioxidant effects of propolis may contribute to improving semen quality in the presence of induced reproductive toxicity [34].

In this study, Vitamin E (Vit E) significantly every one of the parameters listed in the Vit E + BPA group compared to the BPA group. Antioxidant therapy, including Vit E, has been shown to enhance sperm parameters [35] and testicular tissue as well [36]. against cytotoxic harm and may be useful in lowering the toxicity of BPA [37, 38]. By increasing the activity of antioxidant enzymes and decreasing lipid peroxidation (LPO), vitamin E mitigates the harmful effects of BPA on testicular tissue, sperm parameters, testosterone, and MDA levels. [38],[39]. Conversely, in rats, vitamin E can raise serum testosterone levels and decrease LPO [40]. Vit E's primary job is to prevent phospholipid membrane peroxidation and shield cell membranes from damage by acting as an antioxidant [39]. Tocopherol is found inside the cell membrane due to its lipophilic qualities [41]. Additionally, free radicals can have one electron removed from it by tocopherols-OH by transferring their hydrogen atom to it before the free radical comes into contact with the cell membrane. Consequently, vitamin E can inhibit lipid peroxidation and stabilize membranes [36]. In addition, the testicular mitochondria's non-enzymatic defensive mechanism involves vitamin E. This vitamin can stop the development of apoptosis in spermatogenic, Sertoli, and Leydig cells [42] and lowering oxidative damage in the testis [38]. This is crucial to both their count and the viability of the sperm [41]. Furthermore, vitamin E lowers the levels of oxidative stress and restores glutathione to normal levels by intracellular free radical scavenging [43]. By lowering peroxidation and oxidative stress, maintaining cell membranes, and adjusting testosterone levels, vitamin E may enhance spermatogenesis in rats given BPA. This study demonstrates how propolis can help reduce testicular damage caused by Bisphenol A (BPA) in rats. Our study's initial section revealed that sperm quality was negatively impacted by a daily dose of 50 mg/kg of BPA for three weeks. BPA exposure in this study resulted in a significant reduction in various reproductive function parameters, including abnormal sperm morphology, sperm viability, and sperm motility. These findings align with similar reports in the literature, as indicated by [44-45-46]. which revealed a significant drop in testosterone levels. Furthermore, a drop in testosterone concentrations may compromise sperm production and testicular function. [47] found that rats given sub-acute oral BPA treatment experienced changes in their serum testosterone levels as well as morphological abnormalities in their prostate glands. According to our investigation, BPA reduced testosterone levels and induced testicular tissue necrosis by impairing sperm quality.

In rats treated with BPA, propolis treatment improved sperm motility and stopped its decline (Table 2). in line with another research [48,49]. According to the current study, propolis can increase male rat fertility. Propolis might be useful in this situation as an adjuvant treatment. Rats that received propolis and BPA at the same time were shielded from LPO-induced testicular injury. Rats receiving propolis extract had higher epididymal sperm counts than the control group, according to a prior study [49]. It has been demonstrated that giving rats propolis increases the sperm's motility and ability to fertilize when they pass through the epididymis. This implies that changes in enzyme activity when oxidative phosphorylation occurs, which is necessary for the production of ATP, the energy source that propels sperm forward, could affect the motility of sperm [50,48].

Propolis has potent antioxidant qualities, which could be the cause of this [51]. The administration of Vit E, a potent antioxidant, showed a beneficial effect on sperm characteristics by enhancing sperm motility, and viability and improving the morphology of sperm.

It has been demonstrated that vitamin E reacts with free radicals by generating the alpha-tocopherol radical. Once generated, this radical can neutralize other free radicals [52]. Conversely, as testosterone is necessary for the spermatogenesis process, a lower sperm count may be linked to a lower secretion of testosterone [53]. Research has demonstrated that BPA injection results in the death of Leydig cells and subsequently lower testosterone levels.

In the current investigation, the BPA-induced unfavourable changes in the sperm parameters were significantly improved in the rats receiving BPA when Vit E was concurrently administered [54]. Vit E's capacity to control ROS overproduction and so regulate oxidative stress is probably what gives it protection against BPA-induced reproductive damage [55,56]. It has been demonstrated that vitamin E possesses anti-inflammatory and antioxidant properties, and enhances sperm parameters and embryo development [57], which is consistent with our findings.

The way that vitamin E modulates the oxidant status protects sperm shape and concentration from Cadmium (Cd) damage [58]. This may also be explained by a prior study that showed how important vitamin E is for shielding spermatozoon DNA and chromatin components from harm and for raising serum levels of the hormone LH [59].

Conclusion:

Propolis may prevent testicular dysfunction caused by bisphenol A by maintaining sperm characteristics and returning body weight to normal due to its antioxidant properties.

Recommendations:

Study of the other herbal or drugs, that affect testicular dysfunction, after induction by bisphenol A.

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Conflict of Interest:

There is no conflict of interest.

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