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Almoeiz Y. Hammad *

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The Impact of Bisphenol A on Gonadal Hormones and Histological Structure of Wistar rats

Almoeiz Y. Hammad [1]*, Shama I. Y. Adam [2] and Warda S. Abdelgadir [3]

[1]Department of Biochemistry – Faculty of Medicine, Omdurman Ahlia University, Omdurman, Sudan.

[2]Department of Biochemistry and Molecular Biology, Faculty of Science and Technology, Al NeelainUniversity, P.O. Box 12702, Khartoum, Sudan.

[3]National Food Research Centre, Ministry of Agriculture and Natural Resources, P.O. Box 213, Khartoum North, Sudan.

***Correspondence Author:** Almoeiz Y. Hammad, Department of Biochemistry – Faculty of Medicine, Omdurman Ahlia University, Omdurman, Sudan.

Received Date: September 10, 2024 | Accepted Date: September 24, 2024 | Published Date: October 08, 2024

Citation: Almoeiz Y. Hammad, Shama I. Y. Adam and Warda S. Abdelgadir, (2024), The Impact of Bisphenol A on Gonadal Hormones and Histological Structure of Wistar rats, *Clinical Trials and Case Studies*, 3(5); **DOI:**10.31579/2835-835X/082

Abstract:

Introduction: Bisphenol A (BPA), a chemical found in plastics and consumer goods, significantly impacts fertility in female rats, disrupting oocyte maturation, ovulation, and sperm quality, leading to impaired implantation and embryonic development. Further research is needed to develop strategies to mitigate BPA's reproductive toxicity.

Materials and methods: The study involved 40 Wistar rats from the University of Khartoum, Sudan, who were given Extra Pure Bisphenol A powder for four weeks. After anesthesia and slaughter, their blood was analyzed for sexual hormones and lesions.

Results: The study investigated the impact of BPA on rats' reproductive hormones, showing significant changes in both control and treated groups, including reduced FSH, LH, testosterone, estrogen, progesterone, and Prolactin levels, and abnormal ovaries.

Conclusion: BPA exposure reduced reproductive hormones in rats, with elevated Prolactin levels. Males experienced seminiferous epithelium degeneration, sperm reduction, and abnormal antral, cystic, and atretic follicles.

Keywords: bisphenol a; estrogen; progesterone; prolactin; reproductive system; testosterone; wistar rats

1.Introduction

Bisphenol A (BPA), recognized as an endocrine-disrupting chemical, is notably prevalent in the environment and has found extensive application in the manufacturing of plastics, epoxy resins, and various consumer goods[1]. Owing to its omnipresent nature and potential repercussions on health, apprehensions about BPA exposure and its implications for the well-being of both humans and animals have been on the rise. A multitude of research endeavors have delved into the possible reproductive hazards associated with BPA, particularly emphasizing its effects on fertility [2]. One critical area impacted by BPA exposure is oocyte maturation and ovulation in female rats [3]. Research has shown that BPA can disturb the maturation of oocytes and the process of ovulation, resulting in a significant decrease in ovulation rates and ultimately reducing fertility levels among the exposed subjects. Similarly, in male rats, BPA has been linked to a decline in sperm quality and function [4]. Specifically, exposure to BPA is associated with impaired sperm quality, reduced sperm motility, and a decrease in sperm count [5-6].

Moreover, the effects of BPA extend to implantation and embryonic development. Studies have indicated that BPA exposure can hinder successful implantation and proper embryonic development, potentially leading to increased instances of miscarriage and the occurrence of fetal abnormalities in affected individuals [7]. In addition to these direct impacts on fertility, BPA-induced hormonal deregulation further exacerbates reproductive challenges. By disrupting the production and regulation of crucial reproductive hormones such as estrogen, testosterone, and follicle-stimulating hormone (FSH), BPA perpetuates the adverse effects on overall reproductive function -- underscoring the urgency of understanding and mitigating the consequences of BPA exposure on reproductive health[8].

The current research aimed to study the impact of exposure to small doses of BPA to Wistar rats of both sexes on the gonodal hormones and histological structure

2. Materials and methods

The present study was carried out in the Department of Biochemistry and Molecular Biology, Faculty of Science and Technology, El Neelain, University, Sudan, after getting approval from Scientific Research Ethical Committee. Forty Wistar rats were obtained from the Faculty of Pharmacy University of Khartoum, reared within the premises of the animal house under 12 hours' photoperiod with standard feed and drinking water provided *adlibitum* before the commencement of experimental feeding. Room temperature was maintained at 25±2 0C at adequate house ventilation.

Then the animals were randomly allotted into four groups 1, 2, 3, and 4 each of ten rats. Group 1 was designated as the male control group, group 2 was designed as the male bisphenol-treated group. Group 3 is the female control group, whereas Group 4 is the female bisphenol A- treated group . Extra Pure (97%) Bisphenol A powder (Sangon, China) was thoroughly dissolved in distilled water and rats of group 2 (male rats) and 4 (female rats) received this test chemical by oral gavage dose at 25µg /kg body weight/day for four weeks period.

Figure 1: Wister rats

2.1 Data collection

2.1.1 Serum analysis

After the end of the experimental period, rats of the control and treatment groups were anaesthetized with diethyl ether and humanely slaughtered. Blood was collected at slaughter in clean sterile vials and sera were separated thereafter to be analyzed for the sexual hormones, Follice-Stimulating Hormone (FSH), Leutinizing Hormone (LH), Testosterone, Progesterone, Estrogen and Prolactin according to Aviva Systems Biology [9].

2.1.2 Histopathological methods

Necropsy was conducted to identify gross lesions and specimens of tests and ovaries were immediately being collected immediately after slaughter of rats, fixed in 10% neutral buffered formalin and embedded in paraffin wax, sectioned at 5m and stained with Haematoxylin and Eosin (H & E) [10].

2.2. Statistical analysis

Mean values of Testosterone, Prostaglandin, Progesterone, Estrogen and Prolactin concentration, were compared using student's t-test [11].

3. Results:

3.1. Clinical observations

The control Group 1 remained clinically normal throughout the experimental period. On the fourth day of the experiment, rats of group 2 showed nervous signs (aggressiveness and defensive behavior) and dosing resistance.

3.2. Serum sex hormones concentration

Changes in concentrations of serum reproductive hormones are presented in Table 1. In male and female groups exposed to 25 µl/kg/day BPA, all the measured hormones were altered. Serum concentrations of FSH were reduced by 36.9% and 69.5% in BPA treated male and female, respectively and those of LH were reduced by 48.1% and 43.4%, respectively. Serum concentrations of testosterone were significantly reduced (P<0.05-0.01) in the two treatment groups. No estrogen was detected in both male control and treated groups, while the concentration in the female rats was significantly decreased (P<0.05) in BPA treated group. Significant decrease (P<0.05) in progesterone concentration was observed in female treated rats, but that of the male treatment group did not change. Significantly (P<0.05-0.01) higher concentration of Prolactin was recorded in both male and female treated rats than the controls.

3.3. Histopathological findings

Testis and ovaries of the male and female control groups remain normal throughout the experimental period. Significant histopathological alterations were observed in the treated group. Marked reduction of the thickness of the seminiferous tubules, along with an increased degeneration of the seminiferous epithelium was visualized. Associated with these alterations, a significant reduction in the number of sperms in the lumen of the seminiferous tubule was found (Fig. 2). Ovaries of treated female rats given BPA at 25µg /kg body weight/day showed lack of normal appearing antral follicles, enlarge cystic follicles and showing a number of atretic follicles (Fig. 3).

Rat Sex	BPA Dose 25 µg/kg/day	FSH mIU/mL	LH mIU/mL	Testosterone ng/mL	Estrogen pg/mL	Progesterone ng/mL	Prolactin ng/mL
Male	**Control M**	5.85±0.16	4.10±0.05	7.06 ±0.08	ND	1.52±0.23	4.17±0.07
	Treated M	2.16±0.02*	1.97±0.16*	2.13 ±0.04**	ND	2.15±0.10^{NS}	7.26±0.12*
Female	**Control F**	8.24±0.16	7.49±0.05	1.64±0.12	10.51±0.57	5.61±0.40	2.30±0.12

	Treated F	5.73±0.13*	3.25±0.02**	0.65±0.14 *	7.10±0.12*	2.4±0.15*	8.54±0.29**

Values are means ±SE, NS = not significant, *Denotes mean values significant at (P<0.05), **Significant= (P<0.01),
M= Male, F= Female

Table 1: Effects of BPA at 25 µg/kg/day on male and female reproductive hormones

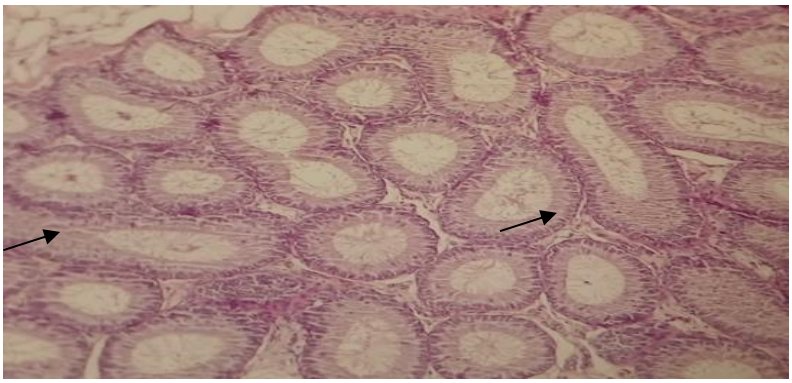

Figure 2: Reduction of the thickness and an increase in the degeneration of the seminiferous epithelium as well as a reduction in the number of sperms in the lumen of the seminiferous tubule. H&E x100

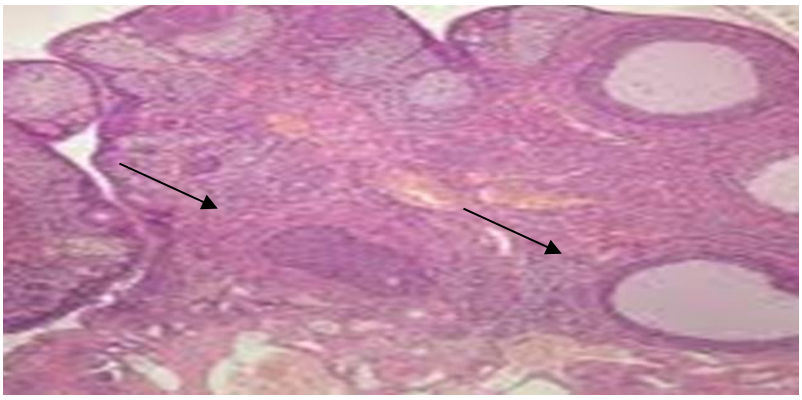

Figure 3: Lack of normal appearing antral follicles, enlarge cystic follicles and showing a number of atretic follicles in a female rat receiving oral BPA at 25 µg/kg H& E x100.

4. Discussion

The results of the present study have indicated that the serum hormonal levels of FSH, LH, Testosterone, Estrogen and Progesterone of female Wistar rats were significantly decreased with a significant increase in prolactin in the test group when BPA was orally administered at a dose of 25 µg/kg/day. A study by [12] revealed that non- pregnant female Wistar rats exposed to different oral doses of BPA (5, 50, 300, 600 and 800 mg/kg body weight /week) exhibited significant decreased levels of LH, FSH, E2, PROG and PRL with altering the mating activity, reducing the reproductive capacity and leading to decrease female fertility and weight of reproductive organs (ovary and uterus). [13] Study findings indicated that Adult female Sprague Dawley rats orally exposed to 330 mg/kg BW of BPA for 10 and 12 weeks revealed reduction in estrogen and progesterone concentration [14] found that adult female rats exposed to 0.1 mg/kg BW of BPA for 90 days showed decreased estradiol concentration. This result might be attributed to the effect of BPA that causes down regulation of P450 aromatase mRNA expression in granulose cells [15] that play an important role in estrogen biosynthesis [16] or it might be due to the reduction in steroidogenic acute regulatory protein [17] , which is a critical steroidogenic protein responsible for the transfer of cholesterol from the outer to the inner mitochondrial membrane during androgen biosynthesis [18] . On the 67other hand, bisphenol A disrupts estrogen hormone, and exposure to BPA resulted in increased atresia of the ovarian follicles [19] Delclos *et al*. [20] observed no significant treatment-related effects on serum hormone levels in the low BPA dose range (2.5–2700 µg/kg/day) in females, but serum estradiol was significantly increased by 67% and 113%, respectively in the 100,000 and 300,000 µgBPA/kg/day exposures. Progesterone was significantly decreased by 51% in the highest BPA dose (300,000 µg BPA/kg /day). The highest dose of BPA had significantly elevated prolactin levels, although they did not differ significantly from the control females. No significant differences in FSH and LH levels were observed in 100,000 and 300,000 µg BPA/kg /day dose groups. In contrast,[21] observed a significant positive association between increased urine BPA concentration and higher prolactin and progesterone levels in a study done from BPA exposed and unexposed factories in China on 106 exposed and 250 unexposed female workers. In addition, a positive association between urine BPA and estrogen was observed among exposed workers with borderline significance, while a statistically significant inverse association between urine BPA and FSH was observed among unexposed group. These authors reported that BPA exposure was found to be linked to higher prolactin level among adult females. However, this finding is consistent with the results of the present study and other reported findings from *in vitro* and *in vivo* studies. *In vitro* studies showed that except for estrogen receptors alpha, beta, gamma, BPA can also bind to membrane estrogen receptor (mER), and thesemembrane bound receptors are capable of non-genomic steroid actions [22]. GH3/B6 pituitary cells, which express mER, respond to low level BPA exposure by producing a calcium flux which leads to PRL

release [23]. BPA can also induce prolactin gene expression and cell proliferation in both primary anterior pituitaries cells and GH3 cells [24]. In an animal study, injecting approximately 15 mg/(kg/day) of BPA into neonatal Fisher 344 rat pups resulted in an increase in serum prolactin levels [25]. Similarly, treatment of ovary-ectomized Wistar rats with BPA doses of 11–250 mg/kg per day induced hyperprolactinemia [26]. The same result was found when perinatal administration of BPA (0.05 mg/kg/day, 20 mg/kg/day) was used, the exposed animals of F1 females reached adulthood and became pregnant, and it induced alterations in serum progesterone and estrogen 68hormonal levels[27] The exposure to 500 μg/kg/day BPA in rats leads to an ovulation and infertility[28] Moreover, BPA may target GnRH neurons and as a result cause the decrease in GnRH mRNA expression[29] . The uterus responds to the changing hormone levels produced by the brain as well as the ovaries. This process is initiated in the hypothalamus through the production and release of GnRH, which leads to FSH and LH release from the anterior pituitary gland. As a result, oocyte development takes place in the ovaries, and estradiol is produced from the growing ovarian follicle. BPA can affect the hypothalamic system. The hypothalamic-pituitary ovarian axis controls the ability of the mammalian female to ovulate and to prepare the reproductive organs to support potential pregnancy. BPA exposure resulted in the decrease of the reproductive capacity and delay or elimination of puberty [31]. BPA affects ovarian steroidogenesis by modulating the expression of key steroidogenic enzymes. For example, BPA decreases aromatase (CYP19A1) expression and E2 production in human granulosa cells [32]. In mice, BPA inhibits P4, testosterone (T) and E2 synthesis by decreasing the expression of steroidogenic acute regulatory protein (Star), 3β-hydroxysteroid dehydrogenase (Hsd3b1) and 17α- hydroxylase (Cyp17a1) [33] . In rats, however, BPA increases P and T synthesis, as well as the expression of Star, cholesterol side-chain cleavage enzyme (Cyp11a1) and Cyp17a1, but decreases E2 synthesis and Cyp19a1 expression (Zhou *et al*., 2008). In pigs, BPA increases basal and FSH-induced P4 synthesis, whereas it decreases FSH-induced E2 synthesis [34] Histopathological changes in rat ovaries are consistent with Adult female rats exposed to BPA had atretic follicles, which were characterized by the initial elimination of granulosa cells proximal to the antrum, pyknotic nuclei, and remnants of mitochondrial and plasma membranes [35]. The presence of atretic follicles may be attributed to decreased estradiol concentration as reported by [36]. The present study has shown that orally administered BPA at 25 µg/kg/day caused significant decrease in the reproductive hormones, FSH, LH, Testosterone, with an elevated level of prolactin. The toxic effect of BPA on the male reproductive functions is 69 well defined in animal models and demonstrated by physiological changes throughout foetal, pubertal and adult life of male rats [37-39]. An *in vivo* study showed that when low doses of BPA were given to rats via oral administration an impairment of spermatogenesis caused by the reduction of reproductive hormones serum level (FSH, LH, GnRH) and stopping germ cells meiosis process, thus activating the apoptosis pathway in germ cells. BPA administration reduces testosterone biosynthesis and secretion, thus inhibiting the activity of GnRH neurons, and lowering the expression of steroidogenic enzymes. Consequently, a decline of testosterone levels and a reduction in spermatozoa concentration was seen [40]. BPA effect on testosterone level has been tested on prepubertal rats after subcutaneously administered doses of 0, 20, 100, and 200 mg/kg/day, for six weeks. This study has demonstrated the decrease in testosterone levels only after higher doses (100 and 200 mg/kg/day) [41]. Other studies have shown that BPA inhibits steroidogenesis in the rat testis and reduces testosterone secretion, thus inhibiting the activity of GnRH neurons, and lowering the expression of steroidogenic enzymes [42-43] . Spermatogenesis is dependent on a well-orchestrated hormonal environment. Leydig cells stimulated by LH provide the local production of testosterone, and Sertoli cells stimulated by FSH provide the local production of estradiol. In addition, Sertoli cells maintain the spermatogonial stem cells responsible for the continuity of spermatogenesis [44] . In the present study, BPA exposure caused an imbalance in these hormones, which may have contributed to defects in spermatogenesis and sperm maturation. Using prepubertal rats as an experimental model,[45] also reported a dose-dependent reduction in testosterone and LH serum concentrations (20, 100 and 200mg BPA/kg/day). The reduction in the LH serum concentration may be directly responsible for the reduction in testosterone production by Leydig cells in BPA-treated animals. In the seminiferous tubules, testosterone is carried by androgen binding protein (ABP) through the testis toward the epididymis. Testosterone is converted to dihydrotestosterone (DHT) by 5-alpha reductase enzyme [46-47]. The androgenic 70activity of DHT is two-fold higher than testosterone, and the epididymis is highly dependent on androgens to complete its transport and storage of spermatozoa prior to ejaculation [46-47]. Therefore, the reduction in testosterone observed in the BPA-treated animals can affect these processes and may be at least partly responsible for the alterations observed in the spermatozoa of these rats.

5. Conclusion:

In a comprehensive study conducted on both male and female rats, a distinct decrease in reproductive hormone levels was prominently noted when exposed to Bisphenol A (BPA) at a dosage of 25 µg/kg per day. Interestingly, despite this suppression in hormonal activity, one notable exception was found in the case of Prolactin, which exhibited an unusual increase compared to the baseline levels. Furthermore, the repercussions of BPA exposure seemed to manifest differently in males and females. Male rats displayed concerning signs of seminiferous epithelium degeneration, which led to a significant decline in sperm production. Conversely, female rats exhibited notable abnormalities in their ovarian structure, characterized by the absence of healthy antral follicles, the enlargement of cystic follicles, and the presence of atretic follicles. These findings shed light on the detrimental effects of BPA on the reproductive systems of both male and female rats, emphasizing the urgent need for further investigations into the impact of this chemical on overall reproductive health.

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