ORIGINAL ARTICLE

# Bisphenol A: A Testicular Teratogen in Developing Rats During Prenatal and Early Postnatal Life

Tooba Khurshid, Rehana Rana, Shabana Ali, Huma Beenish, Ali Ahmed, Syeda Sara Bano

**ABSTRACT**

**Objective:** To study the teratogenic effects of Bisphenol A on the testicular histogenesis of developing rat pups during prenatal and early postnatal life.

**Study Design**: Laboratory-Based Randomized Control Trial.

**Place and Duration of Study:** The present study was carried out at Anatomy Department of Islamic International Medical College Rawalpindi in association with National Institute of Health (NIH) Islamabad from September 2018 to September 2019.

**Materials and Methods:** Eight weeks old 10 pregnant female rats were divided into 2 groups each containing 5 pregnant female rats. Pups were delivered by spontaneous vaginal delivery. Control group A was composed of 15 male rat pups from 5 pregnant female rats fed on the standard diet during pregnancy and lactation till day 21. Experimental group B was composed of 15 male rat pups from 5 pregnant female rats which were given 250µg/kg/day Bisphenol A subcutaneously during pregnancy and lactation till day 21.

**Results:** Deterioration of histological parameters was significantly seen in group B. Tubule differentiation index(TDI) in group B was 69.22%; significantly reduced as compared to control group A. The modified Johnsen's criteria in group B was score 3 (in 33.3% rats ) and score 1 (in 26.6% rats) as compared to score 7 (in 100% rats) in control group A. 66.6% rats in group B showed severe (grade 4) and 26.6% rats showed moderate (grade 3) epithelial degeneration as compared to control group A. 46.6% rats in group B showed severe (grade 4) and 40% had moderate (grade3) congestion as compared to control group A.46.6% rats in group B showed severe (grade 4) vacuolization and 33.3% had moderate (grade3) vacuolization as compared to control group.

**Conclusion:** The teratogenic effect of BPA on developing rat testes is evident when given during prenatal and early postnatal life. The teratogenicity of BPA may adversely affect spermatogenesis and male fertility.

**Key Words:** *Bisphenol A, Developing Rats, Early Postnatal Testicular Teratogen, Prenatal.*

## Introduction

Bisphenol A was manufactured first by A. P. Dianin in 1891 and investigated in 1930s during the hunt for synthetic estrogens and is thus an important endocrine disrupting chemical.[1,2] BPA is a xenoestrogen which mimics estrogens and is able to bind estrogen receptors.[3] Edward Charles Dodd first identified the estrogenic characteristics of BPA when he was in search of an estrogen.[4] A study on BPA reported that humans are exposed to 10µg/day of bisphenol A.[5] The European Food Safety Authority (EFSA) has determined the temporary tolerable daily intake of BPA which is 4µg/kg/day.[6] According to the data published by EFSA in 2015; BPA exposure for men and women is 1.01 to 1.06µg/kg/day.[7]

Bisphenol A is a ubiquitous endocrine disrupting chemicals (EDC) which is used in synthesis of epoxy resins and polycarbonate plastics, food cans, water pipes, receipt papers, baby feeding bottles and currency notes.[3] Reproductive system is one of the main systems targeted by this endocrine disruptor.[8] Exposure to such chemicals in humans is a probable cause of male infertility.[9] Developing testis is fundamental component of male reproductive system which is susceptible to teratogens. BPA has deteriorating effects in pregnancy when exposed during critical period of embryonic testicular development.[10] Animal studies revealed that BPA has a potent teratogenic potential.[11] In rats during prenatal testicular development (gestational days:

*Department of Anatomy*
*Islamic International Medical College*
*Riphah International University, Islamabad*
*Correspondence:*
*Dr. Tooba Khurshid*
*Senior lecturer*
*Department of Anatomy*
*Islamic International Medical College*
*Riphah International University, Islamabad*
*E-mail: khursheedtooba@gmail.com*

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13.5-16.5), there is migration of primordial germ cells towards genital ridge along with sertoli cells differentiation, formation of seminiferous cords and leydig cells differentiation. Postnatal testicular development includes neonatal period (3–7 post natal days), early infantile period (8–14 days), late infantile period (15–20 days), juvenile period (21–32 days) and peri-pubertal period (32–55 days).[12] Oxidative stress induced by BPA during the testes development is the key factor in aetiology of male infertility.[11] Exposure of pre- implantation embryos to BPA impairs testes development and disrupts testosterone synthesis.[6] A study of BPA exposed rats revealed abnormal spermatids, interstitial congestion, scanty cellular components and empty tubular lumina with significant decrease in Leydig cell count, germinal epithelium height and tubular diameter.[5]

Numerous phenol derivatives were detected in water bodies of river Indus in Pakistan. All samples were adulterated with Phenol,2,4-bis(1,1-dimethylethyl).[13] To the best of researcher's knowledge, in Pakistan where significant quantity of BPA is leached into water, such a study was needed to be conducted in pregnant rats to see teratogenic role of BPA during prenatal and early postnatal life. With exposure to BPA, this study could highlight the probable reason of increasing male infertility in Pakistan by identifying the problem in progression of spermatogenesis in rat pups with the help of seminiferous tubule differentiation and histological scoring.

## Materials and Methods

The experimental study was carried out by randomized control trial in animal house of National institute of health, Islamabad (NIH) from September 2018 to September 2019 after approval of Ethics Review Committee. Randomization was by simple random sampling. Eight weeks old 10 female and 5 male albino Sprague Dawley rats were kept under standard temperature at 22 ± 0.5˚C in stainless steel cages in 12-hour light dark cycle with 50% humidity. Adult female rats were caged with adult male rats (2 females/ male). They were given food and water ad libidum for 7-days to acclimatize. They mated under normal conditions in cage. Female rats with presence of vaginal plugs were considered at day 0 of pregnancy. Pregnant rats were divided into 2 groups. Each group comprised of 5 pregnant rats. One group was fed on normal diet and other group was given BPA (Table I). Pups were delivered by spontaneous vaginal delivery at time of birth. Male rat pups which were 21 days old were included in the study. Male pups with obvious pathology or male pups older than 21 days old were rejected. Delivered male pups were divided into two groups as in Table I. This study constituted a sample size of 30 male rat pups.

**Table I: Grouping of Animals (N=30)**

| Control Group (Group A), n=15 | Experimental Group (Group B), n=15 |
|---|---|
| 15 male rat pups from 5 pregnant female rats. Their mothers were kept on standard diet during pregnancy and lactation till day 21. | 15 male rat pups from 5 pregnant female rats. They were given standard diet orally. BPA 250µg/kg/day was given to their mothers via subcutaneous route during pregnancy and lactation till day 21. |

After 42 days of experiment, 21 days old male rat pups were euthanized and dissected. Testes were fixed in Bouin's fixative, stained with Eosin and Hematoxylin and examined under X10 and X40 power of light microscope and parametric data was collected by the principal researcher. Microscopic parameters included tubule differentiation index (% of tubules with more than 3 layers of differentiated germinal epithelial cells) and assessment of spermatogenesis with modified Johnsen's crirteria from 1-7 with 1 being the lowest (absence of germinal epithelium and cells) and 7 being the highest (presence of rounded spermatids). In between 7 and 1 spermatogonia, spermatocytes and sertoli cells were seen[14]. These two parameters were assessed by independent samples T test. The parameters of epithelial degeneration, congestion and vacuolization were classified according to International Harmonization of Toxicologic Pathology Nomenclature.[15] These three parameters were assessed by Chi square test. The data was analysed by SPSS version 21. A *p-value* of less than 0.05 was considered significant.

## Results

Tubule differentiation index (TDI) and modified Johnsen's criteria were reduced in group B significantly as compared to control group A (Figure 1, Table II). Severe and moderate grades of epithelial

degeneration, interstitial congestion and vacuolization were seen in rats of group B as compared to control group A. (Figure 2, Table III, Figure3, Table III, Figure 4, Table III respectively).

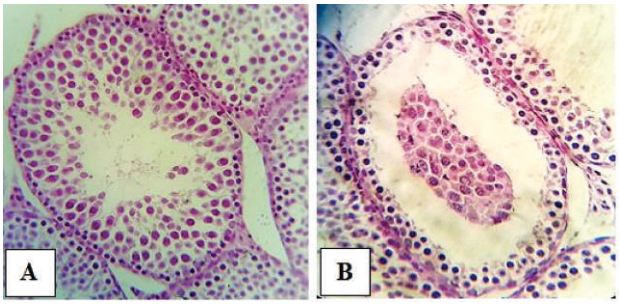


**Fig 1: Figure 19 TDI and Assessment of Spermatogenesis with Modified Johnsen's Criteria in Testes of Albino Rat Showing Score 7 in Control Group A and Score 3 in Experimental Group B. (A: Control Group A, B: Experimental Group B) (H&E, X40)**

**Table II: Group Wise Distribution of Tubule Differentiation Index (ANOVA) and Assessment of Spermatogenesis with Modified Johnsen's Criteria (Chi square test) of Seminiferous Tubules of Testes among the Control and the Experimental Groups in Albino Rats (N=30).**

| Tubule differentiation index (%) | | | | Assessment of spermatogenesis with modified Johnsen's criteria (N) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Groups | Mean | S.D | SEM | Groups | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| A | 100 | 0 | 0 | A | 0 | 0 | 0 | 0 | 0 | 0 | 15 |
| B | 69.22 | 3.97 | 1.03 | B | 4 | 0 | 5 | 2 | 1 | 2 | 1 |
| *p value* | 0.002 | | | 0.000* | | | | | | | |

*p-value ≤ 0.05*

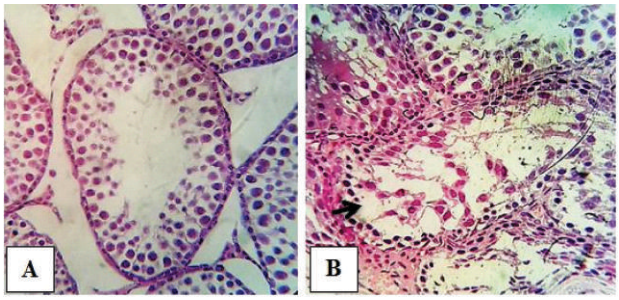


**Fig 2: Germinal Epithelial Degeneration (Severe) in Testes of Albino Rat (Arrow) Shown in Experimental Group B. Negligible Degeneration is Seen in Control Group A (A: Control Group A, B: Experimental Group B) (H&E, X40)**

## Discussion

Infertility is defined as failure to conceive after unprotected and frequent sexual intercourse for more than a year which affects 15% couples

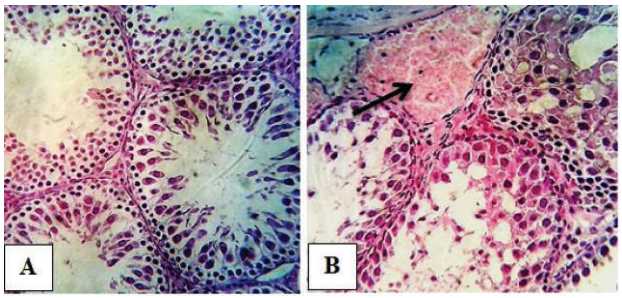


**Fig 3: Interstitial Congestion (Severe) in Testes of Albino Rat (Arrow) Shown in Experimental Group B. Negligible Congestion is Seen in Control Group A (A: Control Group A, B: Experimental Group B) (H&E, X40)**

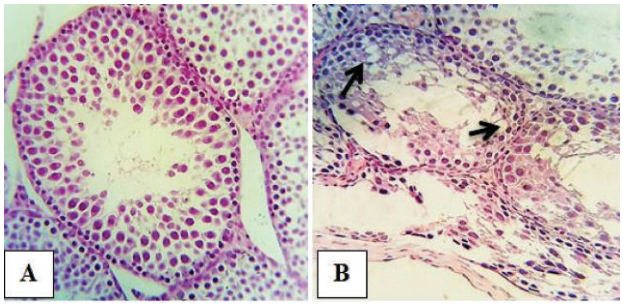


**Fig 4: Vacuolization of Germinal Epithelium (Severe) in Testes of Albino Rat (Arrows) Shown in Experimental Group B. Negligible Vacuolization is Seen in Control Group A (A: Control Group A, B: Experimental Group B) (H&E, X40)**

**Table III: Group wise Distribution of Tubular Degeneration, Interstitial Congestion and Germinal Epithelial Vacuolization (Chi Square Test) In Testes of Albino Rats among the Control and the Experimental Groups**

| Parameters | Tubular Degeneration (N, %) | | Congestion (N, %) | | Vacuolization (N, %) | |
|---|---|---|---|---|---|---|
| Study Groups | A | B | A | B | A | B |
| Grades | | | | | | |
| Negligible (Grade 0) | 15(100%) | 0 (0%) | 15(100%) | 0 (0%) | 12 (80%) | 0 (0%) |
| Minimal (Grade1) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (6.6%) | 3 (20%) | 1 (6.6%) |
| Mild (Grade2) | 0 (0%) | 1 (6.6%) | 0 (0%) | 1 (6.6%) | 0 (0%) | 2 (13.3%) |
| Moderate (Grade3) | 0 (0%) | 4 (26.6%) | 0 (0%) | 6 (40%) | 0 (0%) | 5 (33.3%) |
| Severe (Grade 4) | 0 (0%) | 10 (66.6%) | 0 (0%) | 7 (46.6%) | 0 (0%) | 7 (46.6%) |
| *p*-value | 0.000* | | 0.000* | | 0.000* | |

*p-value ≤ 0.05*

worldwide. Almost half of these cases are attributed to male partner. A study by Majid et al (2019) depicted that oxidative stress due to EDCs is one of major mechanisms involved in causing male infertility.[16] Programming of masculinization occurs during the gestation days 15–18 in laboratory albino

rats and in humans between weeks 8–14 of gestation.[17] Animal studies have shown that pregnant females are at risk from BPA exposure since BPA is a known teratogen as well.[18,19] In humans, BPA has been detected in maternal and fetal plasma, amniotic fluid, placental tissue and in milk of lactating mothers.[20]

In present study, BPA was administered during pregnancy and 21 days after delivery during lactation. Testicular histogenesis was assessed by quantitative and qualitative parameters. BPA adversely affected its development owing to its teratogenicity. TDI and modified Johnsen's criteria have been evaluated in this study both prenatally and in early postnatal period to see teratogenic potential of BPA and its role in development of subsequent male infertility.

Differentiation of spermatogenic epithelium is indicated by tubule differentiation index. In present study, TDI in group B was significantly reduced as compared to control group which was due to disruption of germinal epithelial cells lipid membranes which occurred by generation of ROS by BPA. A study on diabetic rat has shown reduction in TDI in testes showing tubular damage.[21]

Modified Johnsen's criteria manifests progression of stages of spermatogenesis. The reduced score in this study with BPA indicated spermatogenic arrest due to Leydig cells disruption which caused testosterone levels to fall. Since testosterone is principally implicated in progression of spermatogenesis so the score fell. Along with tubular degeneration there was loss of cells of spermatogenic lineage. This has been supported by a study in which BPA with dose of 100mg/kg/day was used.[22]

In a study, BPA has induced mitochondria-mediated apoptosis due to activation of caspases in cytosol of hepatic cells.[23] In present study group B, significant degeneration of germinal epithelium was seen. Loss of normal cellular array and architecture of spermatogenic lineage was clearly evident in group B. As a result, sloughing of germinal epithelium from basement membranes occurred due to increased movement of smooth muscle cells as a result of ongoing inflammation by the released ROS. This result is in line with a study in which BPA-induced oxidative damage is seen in kidney, testis and liver of rats with degenerative changes.[24,25]

In present study, testicular interstitium showed significant congestion in BPA group. This was due to ongoing inflammation by free radicals which led to recruitment of inflammatory mediators in testicular blood vessels. This caused increased blood flow and hyperemia by focal dilation of blood vessels evident as congestion.[26] This result has been supported by various studies on rat testes when exposed to BPA.[27]

Vacuolization is due to disturbance of fluid balance in spermatogenic and Sertoli cells. Uncharged lipophilic bases and phospholipids enter cells by diffusion which become positively charged in organelles. Accumulation of charged form of weak bases increases intraorganellar osmotic pressure which attracts water from extracellular compartment and thus vacuoles appear.[28] In present study, severe vacuolization of spermatogenic cells and Sertoli cells was seen in group B rats as compared to control group. This result is in accordance with other studies.[29]

Being a rodent based developmental study it has offered constraints in inducing pregnancy in all the rats at the same time. Lactation was an important event during the current study in which mothers and newborn pups showed individual variations.

This study recommends future investigation into the imperative male infertility markers like semen analysis, sperm morphology and immunohistochemistry.

## Conclusion

The present study indicates that BPA has detrimental effects on histology of developing testes owing to its teratogenic potential. Thus, spermatogenesis and male fertility may be adversely affected by BPA especially when exposed to pregnant and nursing mothers.

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