

Teratogenic Effects of Bisphenol A Exposure During Maternal Pregnancy and Lactation on Developing Rat Pups Testes

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ABSTRACT

Objective: To study the teratogenic effects of Bisphenol A exposure during maternal pregnancy and lactation on the testicular histology of developing rat pups.

Design of Study: Laboratory-based experimental study.

Place and Duration of Study: Department of Anatomy, Islamic International Medical College Rawalpindi in collaboration with the National Institute of Health (NIH) Islamabad Pakistan from Sep 2018 to Sep 2019.

Methodology: Eight weeks old, ten pregnant female rats were divided into groups, each comprising five pregnant rats. Pups were born by spontaneous vaginal delivery. Control group A consisted of 15 male rat pups delivered from 5 pregnant rats, fed on a standard diet during pregnancy and lactation till the 21st day. Experimental group B consisted of 15 male rat pups delivered from 5 pregnant female rats who were administered subcutaneous Bisphenol A at a dose of during their pregnancy and lactation till the 21st day.

Results: Significant deterioration of quantitative parameters in testes of the experimental group was seen. The seminiferous tubule diameter in group B was 81.99 ± 10.88 compared to group A which was 111.59 ± 8.36 . The germinal epithelial height in group B was 10.47 ± 2.34 compared to 30.41 ± 2.43 in group A. The number of Leydig cells in group B was 17 ± 3.45 compared to 47 ± 4.44 in group A. The number of rounded spermatids in group B was 10 ± 3.74 compared to 38 ± 4.02 in group A.

Conclusion: Bisphenol A has teratogenic effects on testicular histology of developing rats when exposed to mothers during pregnancy and lactation. This may deteriorate male fertility by adverse effects on spermatogenesis.

Keywords: Bisphenol A, Developing rat testes, Lactation, Pregnancy, Teratogenic effects.

How to Cite This Article: Khurshid T, Ali S, Ali H, Bano M, Cheema WH, Hussain N. Teratogenic Effects of Bisphenol A Exposure During Maternal Pregnancy and Lactation on Developing Rat Pups Testes. *Pak Armed Forces Med J* 2022; 72(3): 986-989. DOI: <https://doi.org/10.51253/pafmj.v72i3.6451>

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INTRODUCTION

BPA is an organic compound and a well-known endocrine-disrupting chemical. It is widely used to manufacture epoxy resins and polycarbonate plastic in food cans, dental sealants and thermal receipt papers. In US and Canada, national surveys have revealed that BPA has been detected in urine samples (0.2 or >0.4 ng of total BPA/mL) of more than 90% of the general population.¹ In addition, a study has shown plastic lined pipes leached more BPA than the ones with newer technology. The concentration of BPA found was 0.25 $\mu\text{g/L}$ and 10 ng/L , respectively. In addition, 99% of BPA exposures in preschool children come from the diet with an oral exposure from 52 to 74 ng/kg/day, whereas the estimated inhalational exposure was from 0.24 – 0.41 ng/kg/day.² In humans, BPA has been detected in maternal and fetal plasma, amniotic fluid, placental tissue at birth, and milk of lactating mothers.³ Pregnant females are particularly at risk

from BPA exposure since it is a known teratogen as well.⁴

BPA causes the production of reactive oxygen species such as inducible nitric oxide synthase, superoxide, hydrogen peroxide and hydroxyl radicals. It decreases the expression of the androgen receptor (AR) gene in the testis of male rats significantly.⁵ BPA acts as a selective estrogen receptor modulator at the estrogen receptor. BPA has decreased testosterone levels in mice and altered their sexual response.⁶ It decreases testicular and epididymal weight, testicular and epididymal spermatozoa counts and plasma levels of testosterone. This oxidative damage is also an important reason for morphological deformities of sperms.⁷

The testis is thus a very important organ in males, which is affected during development by BPA and later can lead to infertility. The adverse effects of BPA on testicular histogenesis are a significant concern and need investigation, especially in our country. To the best of the researcher's knowledge, in Pakistan, where a substantial amount of BPA is leached into water bodies, a study was needed to be carried out in

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Received: 12 Mar 2021; revision received: 13 Aug 2021; accepted: 20 Aug 2021

pregnant and lactating rats to see the teratogenic effects of BPA on testes of developing rats.

METHODOLOGY

This laboratory-based experimental study was carried out at the animal house of the National Institute of Health, Islamabad (NIH) Pakistan, from September 2018 to September 2019. Ethics Review Committee approved the study (Ref. No. Riphah/IIMC /IRC/21/35). The study comprised ten female and five male rats.

Inclusion Criteria: Albino Sprague Dawley rats which were eight weeks old and kept in stainless steel cages under the standard temperature of $22 \pm 0.5^{\circ}\text{C}$ at a 12-hour light-dark cycle with 50% humidity, were included in the study.

Exclusion Criteria: Mice with any obvious injury and disease were excluded from the study.

Two female rats were caged with one adult male ad libidum for 7-days to acclimatize and provide food and water. Rats were mated under standard conditions. The female rats with vaginal plugs were considered pregnant at day 0. The pregnant females were then divided into two groups. Each group consisted of 5 pregnant rats. Control group A was fed a standard diet, and experimental group B was administered BPA (Table-I). The pups were born by spontaneous vaginal delivery. Twenty-one days old male pups were included in the study. Pups with obvious deformities or pups more than 21 days of age were not included. Newborn male pups were then divided into two groups (Table-I). The sample size was 30 male rat pups.

After the completion of 42 days of this study, male rat pups (21 days old) were dissected after euthanasia. The testes were fixed in Bouin’s fixative. Eosin and Hematoxylin staining was done. The examination was done at X10 and X40 power of the light microscope. Microscopic parametric data included seminiferous tubule diameter, epithelial height, number of Leydig cells and round spermatids. Parameters were measured by Image J software. Independent samples t-test was applied to assess the data. SPSS version 21 was used for data analysis. The *p*-value of ≤ 0.05 was considered significant.

RESULTS

The microscopic quantitative parameters of testes were assessed in 15 male rat pups of groups A and B. The seminiferous tubule diameter in group B was 81.99 ± 10.88 compared to group A which was 111.59 ± 8.36 (Figure-1 and Figure-2).

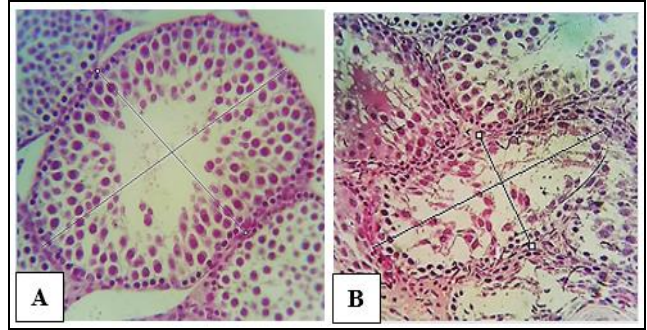


Figure-1A, 1B): Diameter of seminiferous tubules in testes of albino rat in control group A and experimental group B (H&E, X40).

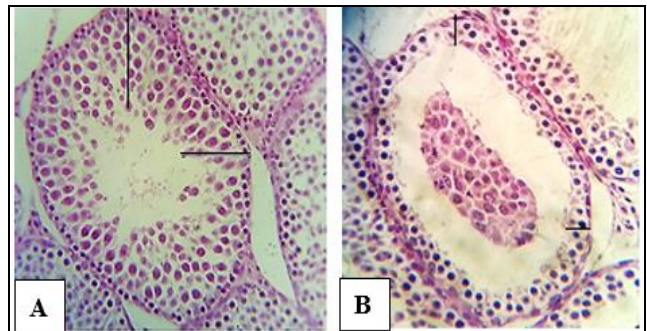


Figure-2A, 2B): Germinal epithelial height in testes of albino rat in control group A and experimental group B (H&E, X40).

The germinal epithelial height in group B was 10.47 ± 2.34 compared to 30.41 ± 2.43 in group A. The number of Leydig cells in group B was 17 ± 3.45 compared to 47 ± 4.44 in group A. The number of rounded spermatids in group B was 10 ± 3.74 compared to 38 ± 4.02 in group A (Figure-3).

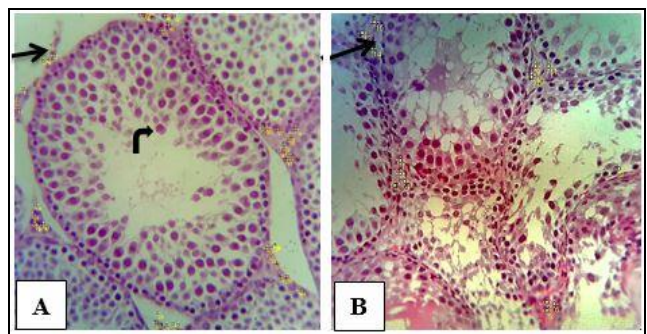


Figure-3A, 3B): Number of Leydig cells (Straight Arrow) and Number of rounded spermatids (Curved Arrow) in testes of al-bino rat in control group A and experimental group B (H & E, X 40).

These parameters were significantly reduced with a *p*-value of <0.001 in group B as compared to group A with a *p*-value of <0.001 (Table-II).

Table-I: Study groups (n=30).

Control Group (A), n=15	Experimental Group (B), n=15
15 male rat pups from 5 pregnant female rats which were fed on standard diet during pregnancy and lactation till 21st day.	15 male rat pups from 5 pregnant female rats which were fed on standard diet. 250 µg/kg/day subcutaneous BPA was administered to these mothers during pregnancy and lactation till 21st day.

Table-II Group wise distribution (independent samples t test) of the mean seminiferous tubule diameter (µm), Germinal epithelial height (µm), the mean Number of Leydig cells (count) and Rounded spermatids of testes among control and experimental groups of albino rats (n=30).

Parameters (Mean ± SD)	Study Groups		p-value
	Group A, (n=15)	Group B, (n=15)	
Seminiferous Tubule Diameter (µm)	111.59 ± 8.36	81.99 ± 10.88	<0.001
Germinal Epithelial Height (µm)	30.41 ± 2.43	10.47 ± 2.34	<0.001
Number of Leydig Cells (count)	47 ± 4.44	17 ± 3.45	<0.001
Number of Rounded Spermatids (count)	38 ± 4.02	10 ± 3.74	<0.001

DISCUSSION

Bisphenol A is a universal endocrine-disrupting chemical which induces the development of free radicals and thus disrupts the histology and function of the male reproductive system. BPA is manufactured for almost five to six million pounds per annum, with serious repercussions on male fertility. The association of decreased sperm count, abnormal sperm morphology, and sperm DNA damage with higher BPA concentration has been linked to reduced semen quality.⁸ Testicular development is thus adversely affected due to BPA exposure. In addition, programming of masculinization occurs during the gestation days 15–18 in laboratory albino rats and humans between weeks 8–14 of gestation.⁹

In the present study, BPA was administered during pregnancy and a month afterwards during lactation. Quantitative parameters assessed testicular histogenesis. BPA adversely affected testicular development owing to its teratogenicity. For quantitative parametric analysis of developing testes, seminiferous tubule diameter and epithelial height, Leydig cells number and rounded spermatids number were assessed. The mean diameter of seminiferous tubules in experimental group B was significantly reduced compared to group A, which was supported by a study conducted by John *et al*, (2019).¹⁰ The decrease in diameter caused by BPA is due to degeneration of germinal epithelial cells and reduction of testosterone due to oxidative stress induced by BPA on the tissue.¹¹

The germinal epithelial height was significantly reduced in group B as compared to group A. A study conducted in Turkey by Gules *et al*, (2019) supported a similar decrease in seminiferous epithelial height induced by BPA in which a 50 mg/kg/day dose was administered to adult rats for 8 weeks.¹² This decrease in epithelial height was due to epithelial degradation

by ROS produced by BPA, which caused cellular apoptosis of spermatogenic lineage leading to the spermatogenic arrest.

Interstitial cells of Leydig are the most important source of testosterone. In a study, BPA has been identified to suppress its production by inhibiting enzymes used in steroidogenesis, such as CYP11A1 and 3β-hydroxysteroid dehydrogenase (3β-HSD).¹³ In the current study, BPA led to the reduction in the number of Leydig cells in group B. This result has been supported by down-regulation of the expression of insulin-like peptide 3 (INSL3) produced by the Leydig cells due to BPA led to cryptorchism which was reported in a study by Lv *et al*, (2018).¹⁴

The number of rounded spermatids in the spermatogenic lineage represents a stage developed in neonate rat pups. The number was significantly reduced in BPA-exposed rats of group B as compared to group A. A similar result is being supported by a studies conducted in 2019 in China in which newborn male mice pups were subcutaneously injected with 5mg/kg/day BPA for 35 days and showed a significant increase in the apoptotic index of rounded spermatids.^{15,16}

Being a rat-based developmental study, there were many limitations like timely pregnancy induction in all rats. In addition, during lactation, mothers and newborn pups showed individual variations.^{17,18}

This study recommends future investigation of antioxidants against BPA-exposed testes in pregnant and nursing mothers to ameliorate the detrimental effects of BPA on the histology of developing testes.

CONCLUSION

Bisphenol A has teratogenic effects on testicular histology of developing rats when exposed to mothers during pregnancy and lactation. This may deteriorate the subsequent male fertility by adverse effects on spermatogenesis.

Conflict of Interest:

Author's Contribution

TK: Crossponding author, SA: Concept and review of manuscript, HA: Helping in carry out Lab, based animal experiment in the animal house of NIH, Islamabad, MB; NH: Formatting of manuscript, HWC: Use of image and data interpretation,

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