Original Article

Changes of Testicular Histogenesis during Prenatal and Early Postnatal Life in Bisphenol A Exposed Rats by Ascorbic Acid

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[1,2] *Analysis/Interpretation/Discussion*
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Abstract

Objectives: In this study, we aimed to investigate the changes in testicular histogenesis in bisphenol-a exposed rats by ascorbic acid during prenatal and early postnatal life.

Materials and Methods: Eight weeks old 15 pregnant rats were divided into 3 groups, each containing 5 rats. Pups were delivered by spontaneous vaginal delivery. Group A had 15 male rat pups, from 5 pregnant female rats, which were fed on a standard diet during pregnancy and lactation till day 21. Group B had 15 male rat pups from 5 pregnant female rats which were given 250 µg/kg/day of Bisphenol A subcutaneously during pregnancy and lactation till day 21. Group C had 15 male rat pups from 5 pregnant female rats which were given 250 µg/kg/day of Bisphenol A subcutaneously and 150 mg/kg/day of ascorbic acid orally during pregnancy and lactation till day 21.

Results: In group B, 86.6% of rats had irregular seminiferous tubules. This irregularity was reduced to 26.6% in group C. The basement membranes of tubules were irregular along with a detachment of germinal epithelium in 80% of rats in group B which reduced to 26.6% in group C. The cell debris was present in 80% of tubular lumina in group B rats with only 13.3% in group C. These parameters were improved with ascorbic acid in group C with a significant p-value.

Conclusion: Biphenol A adversely affects the histogenesis of testes by causing oxidative stress when given during pregnancy and lactation and ascorbic acid improves BPA exposure to developing testes and may preserve spermatogenesis and male fertility.

Keywords: Bisphenol A, ascorbic acid, testicular histogenesis, rat, prenatal, early postnatal.

Introduction

Bisphenol A (BPA, 2,2-bis(4-hydroxyphenyl)) is an endocrine-disrupting chemical (EDC) that induces oxidative damage. It is a key component of polycarbonate plastics and resins.[1] More than 6 billion pounds of BPA are produced annually and more than 100 tons are released into the environment on yearly basis by industries worldwide.[2] The maximum amount of BPA i.e; up to 17 mg/L was reported in landfill leachates and effluents of pulp mills however in the sediments of the river and marine up to 191mg/kg of dry weight was reported.[3] In Pakistan, Phenol,2,4-bis(1,1-dimethylethyl) has been detected in the river Indus.[4] BPA concentration has been detected in freshwater fish in Pakistan with DNA damage in their lung, liver, and brain tissues.[5] A cross-sectional study in Pakistan has shown that dietary exposure to BPA led to the development of diabetes mellitis with a detectable amount of BPA in 75% of the study participants.[6]

BPA-induced lipid peroxidation has resulted in the mal-development of fetal Leydig cells and consequent spermatogenic arrest.[7] In a rat study, it was reported that anogenital distance which is an index of testicular testosterone production was markedly reduced in male rat pups after exposure of BPA to the pregnant mothers at a dose of 250 µg/kg/day BPA showing that there are detrimental effects of BPA on testes of rat embryo.[8]

The need for antioxidants to combat BPA-induced oxidative stress is the need of the hour. Ascorbic acid has been reported to scavenge reactive oxygen species by rapid transfer of electrons to impede lipid peroxidation.[9,10] Ascorbic acid inhibits disruption of the blood testes barrier induced by oxidative stress.[11] Animal studies have shown that ascorbic acid played a protective role in the male rat offspring against reproductive damage caused by certain drugs.[11,12] Ascorbic acid supplementation prenatally among smokers has been associated with a reduction in preterm birth and placental abruption.13 Maternal deficiency of vitamin C during pregnancy results in fetal and placental growth retardation(14). The present study was thus conducted to counter the histological damage done by Bisphenol A in developing rat pup testes with the administration of ascorbic acid during the prenatal and early postnatal period with the aim to improve future male fertility.

Materials and Methods

The study was conducted with a mutual collaboration of the National Institute of Health (NIH) and the Anatomy Department of Islamic International Medical College after the approval of the Ethics Review Committee. It was a randomized control trial. The duration of the study was 12 months. Eight weeks old 15 female and eight weeks old 5 male Albino Sprague Dawley rats (220-310 gm) were kept in stainless steel cages at a standard temperature of 22 ± 0.5˚C in clean stainless steel cages under 12 hours light and dark cycle with 50% humidity in the animal house of NIH. Adult female rats were caged with adult male rats (3 females/male). They were given food and water ad libidum for 7-days to acclimatize. The animals mated under normal conditions in the cage. Female rats with the presence of vaginal plugs were considered at day 0 of pregnancy. Pregnant rats were divided into 3 groups one control group and two experimental groups. Each group comprised 5 pregnant rats. Pups were delivered by spontaneous vaginal delivery. Male pups were breastfed by the mother till day 21 postpartum. 15 male rat pups were randomly selected from each of the 3 groups. This constituted a sample size of 45 male rat pups divided into three groups.

Chemicals

A crystalline form of 97% pure Bisphenol A imported from Sigma Aldrich USA was used with MAFCAN scientific traders courtesy (Lot # MKBS4674V, Pcode1002028684). A crystalline form of Ascorbic acid was purchased from Nobel drug store (25 grams per bottle).

Treatment of animal groups

Group A comprised 15 male rat pups from 5 pregnant female rats. These pregnant females were kept on a standard diet orally throughout pregnancy and during lactation till day 21. Group B comprised 15 male rat pups from 5 pregnant female rats. These pregnant females were given a standard diet orally. Bisphenol A 250 µg/kg/day was given to these mothers via subcutaneous injection (powder form of BPA dissolved in almond oil to make 0.5ml subcutaneous injectable solution) throughout pregnancy and during lactation till day 21. Group C comprised 15 male rat pups from 5 pregnant female rats. These pregnant females were given a standard diet orally. Bisphenol A 250 µg/kg/day was given to these mothers via subcutaneous injection and ascorbic acid 150 mg/kg/day was given to these mothers via the oral route after dissolution in water throughout pregnancy

and during lactation till day 21. The testes were removed after dissection.

Histological methods

The testes were fixed in Bouin's fixative and fixed tissues were processed. Hematoxylin & Eosin (H&E) and Periodic Acid Schiff (PAS) were used for staining the processed tissue. Nikon S4300 (16.02 megapixels) was used to take images from Olympus light microscope. All four quadrants of each slide of each rat were observed for the parameters under the 40X power of a microscope.

Data analysis

Data were analyzed in SPSS version 22. The Chi square test was applied for this qualitative study. The parameters were seminiferous tubule regularity, basement membrane regularity, detachment of germinal epithelium, and presence of cell debris in the tubular lumen. A *p*-value of less than 0.05 was considered significant.

Results

Seminiferous tubule regularity

In control group A, the appearance of seminiferous tubules was regular in 100% of rats with spherical in shape and contours while in experimental group B, 86.60% of rats had irregular tubules with loss of normal arrangement and distorted shape as compared to control group. 13.30% of rats in group B had regular seminiferous tubules with normal shapes and contours. However, in experimental group C, 73.3% of rats had regular and 26.6% of rats had irregular tubules (Figure 1, Table 2). The difference in seminiferous tubule regularity was significant among all groups (*p-value*: 0.000).

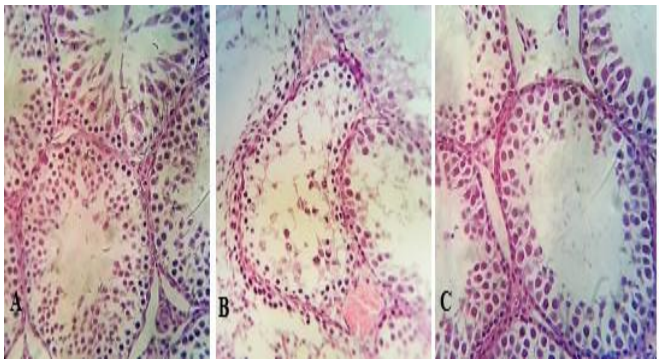

Figure 1: Seminiferous tubules regularity in testes of albino rat (A: control group A, B: experimental group B, C: experimental group C) (H&E, X40)

Table 1: Group-wise distribution of seminiferous tubules regularity among control and experimental groups in testes of albino rats (N=45)

Seminiferous tubules regularity				
Groups	**Regular (N, %)**	**Irregular (N, %)**	**Total**	***p-value***
A(control)	15 (100%)	0 (0%)	15	0.000*
B(BPA)	2 (13.3%)	13 (86.60%)	15	
C(BPA+Vit.C)	11 (73.30%)	4 (26.60%)	15	

* *P-value* ≤ *0.05*

Basement membrane regularity

In control group A, the basement membrane was regular in 100% of rats while in experimental group B, 80% of rats had irregular basement membrane and it was puckered, thinned out, or humped and 20% had no cell debris in the tubular lumen. However, in experimental group C, 26.6% of rats had irregular basement membranes and 73.3% of rats had regular basement membranes of the seminiferous tubules (Figure 2, Table 2). The difference in basement regularity was significant among all groups (*p-value*: 0.000).

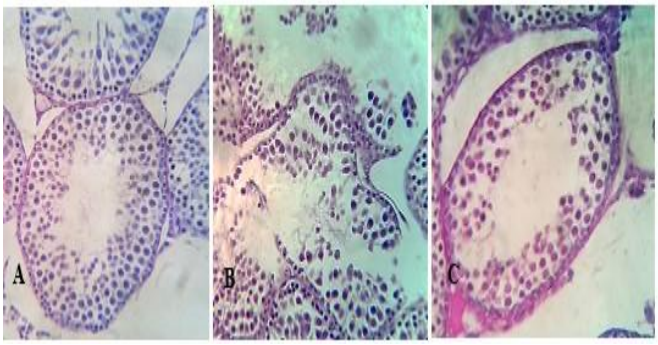

Figure 2: Basement membrane regularity in testes of albino rat (A: control group A, B: experimental group B, C: experimental group C) (PAS, X40)

Table 2: Group-wise distribution of basement membrane regularity among control and experimental groups in testes of albino rats (N=45)

Basement membrane regularity				
Groups	**Regular (N, %)**	**Irregular (N, %)**	**Total**	***p-value***
A(control)	15 (100%)	0 (0%)	15	0.000*
B(BPA)	3 (20%)	12(80%)	15	
C(BPA+Vit. C)	11 (73.3%)	4 (26.6%)	15	

Detachment of germinal epithelium

In control group A, the germinal epithelium was intact in 100% of rats while in experimental group B, 80% of rats had detached germinal epithelium and 20% had intact germinal epithelium. However, in experimental group C, 73.3% of rats had intact germinal epithelium and 26.6% of rats had detached germinal epithelium (Figure 3, Table 3). The difference in detachment of germinal epithelium was significant among all groups (*p-value*: 0.000).

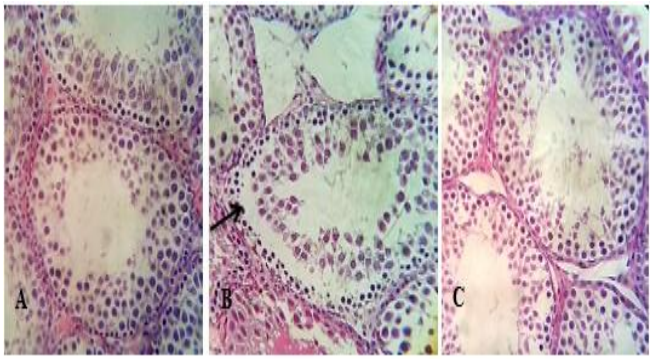

Figure 3: Detachment of germinal epithelium in testes of albino rat (A: control group A, B: experimental group B, C: experimental group C) (H&E, X40)

Table 3: Group-wise distribution of detachment of germinal epithelium among control and experimental groups in testes of albino rats (N=45)

Detachment of germinal epithelium				
Groups	**Intact (N, %)**	**Detached (N, %)**	**Total**	***p-value***
A(control)	15 (100%)	0 (0%)	15	0.000*
B(BPA)	3 (20%)	12(80%)	15	
C(BPA+Vit.C)	11 (73.30%)	4 (26.60%)	15	

Cell debris in the tubular lumen

In control group A, the cell debris was absent in 100% of rats while in experimental group B, 80% of rats had cell debris present in the tubular lumen and 20% had no cell debris in the tubular lumen. However, in experimental group C, 13.3% of rats had cell debris present and 86.6% of rats had no cell debris in the tubular lumen (Figure 4, Table 4). The difference in cell debris in the tubular lumen was significant among all groups (*p-value*: 0.000).

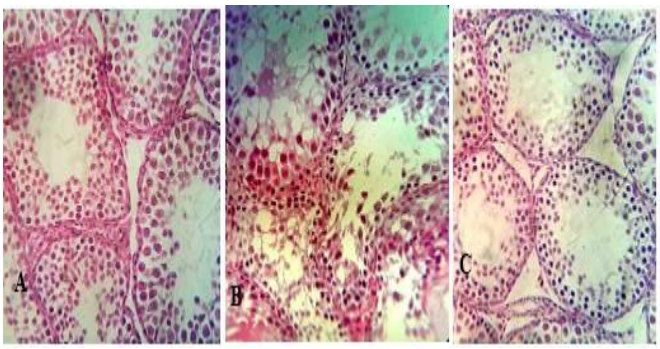

Figure 4: Cell debris in the tubular lumen in testes of albino rat (A: control group A, B: experimental group B, C: experimental group C) (H&E, X40)

Table 4: Group-wise distribution of cell debris in tubular lumen among control and experimental groups in testes of albino rats (N=45)

Cell debris in the tubular lumen				
Groups	**Absent (N, %)**	**Present (N, %)**	**Total**	***p-value***
A(control)	15 (100%)	0 (0%)	15	0.000*
B(BPA)	3 (20%)	12(80%)	15	
C(BPA+Vit.C)	13 (86.6%)	2 (13.3%)	15	

Discussion

Infertility is the failure to conceive after unprotected and frequent sexual intercourse for more than a year with 72.4 million couples affected worldwide. About half of such cases are ascribed to the male partner. 90% of these cases are due to reduced sperm count and quality. One of the causes linked to male infertility includes exposure to endocrine disruptors such as BPA.[7] The DNA base modification by reactive oxygen species has been shown to induce the nuclear factor kappa-light-chain-enhancer of activated B cells.[15]

The present study highlighted the antioxidant role of ascorbic acid in the amelioration of testicular damage done in rat pups after BPA exposure. The histologic appearance of seminiferous tubules regarding their regularity, shape, contours, and arrangement was studied. The loss of tubular regularity in group B was due to distortion of the cellular array with BPA. This result has been supported by M. Tolba et al conducted a study in 2019 on adult male rats using 25 mg/kg/day BPA for 30 days and showed that BPA caused irregularity and loss of circular shape of tubules in testes.[16] In another study by Shuang Ma et al similar result was seen in which BPA was given to

pregnant mice at 50 mg/kg, 500 mg/kg, and 2500 mg/kg/day during the gestation period till 8 weeks of age.[17] In the current study appearance of tubules in group C was regular mostly. A study conducted by Sadeghzadeh et al in Iran (2018) showed similar results by using ascorbic acid in male rats against dexamethasone.[18]

Owing to the lipid peroxidation by BPA, the tubular basement membrane was severely affected which led to irregular contours of this membrane. It was puckered, humped up, and thinned out as well. The tubular basement membrane was highly irregular in most of the rats of group B. This result has been supported by previous studies.[3,19] In the present study, the regularity of the basement membrane was returned by the use of ascorbic in group C in a majority of the rats. The fact depicted by this result confirmed that ascorbic acid has an assertive influence on maintaining the integrity of the tubular basement membrane in developing testes.

Germinal epithelium of seminiferous tubules was detached from the basement membrane in 80% of the rats in group B as compared to the control group (in which epithelium was intact). The detachment was due to the disruption of the basement membrane and loss of tight junctions and intercellular junctions in the testes due to the damage to the Sertoli cell membranes after exposure to BPA. This result is supported by a study conducted in China by Shuang Ma (2017) in which BPA was administered to pregnant mice at 50 mg/kg, 500 mg/kg, and 2500 mg/kg/day BPA at 8 weeks of age.[3] As compared to group B, 73% of the rats in the group in group C showed intact basement membranes.

Detachment of germinal epithelium caused sloughing off of spermatogenic cells which later on led to the presence of cell debris in the tubular lumen. The sloughed-off cells after apoptosis were maximally seen in the seminiferous rats in the BPA group. This result has been supported by a study conducted by Abdel-Maksoud et al in 2019.[20] The shedding of epithelial cells into the tubular lumina has been observed in the testes of rats affected by metabolic syndrome.[21] In the present study, in group C however; most of the rats had normal tubular architecture with clear lumina since the basement membranes and germinal epithelia were intact without abnormal loss of cells. Thus, ascorbic acid preserved the cells of germinal epithelium from abnormal shedding of germinal epithelial cells into the lumen.

Conclusion

The present study showed that the antioxidant potential of ascorbic acid has improved the histological parameters of testicular damage in rat litter after BPA exposure during prenatal and early postnatal life. Therefore, spermatogenesis and male fertility may be improved at the early stages of life by the use of ascorbic acid in pregnant and nursing mothers exposed to BPA. An important endorsement of this study is to add a group of vitamin C-enriched natural foods to the rodent diet along with the chemical form of ascorbic acid among the groups and compare their histological effects.

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