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Research Paper

Bisphenol A: Cause of Male Infertility

Zoology

KEYWORDS : bisphenol A, seminiferous tubules, spermatogenesis, epididymis, testosterone

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ABSTRACT

The aim of this study was to evaluate the effect of bisphenol A (BPA), a contaminant of resin-based dental composites and sealants, on the fertility of male adult wistar albino rats. Adult male albino rats of strain weighing approx.150-200gm. were divided into four groups of 10 each. BPA (5, 50 and 100 µg /100gm/bw) was administered orally daily to the rats in the test groups and olive oil to the control group for 90 days. Male fertility was assessed periodically by mating each rat with two untreated females. There were significant reductions in the absolute weights of the testes and disrupted spermatogenesis was observed at 50 and 100µg dose level of BPA whereas at 5 µg dose level did not show any such significant effect.

Epididymal epithelium degeneration was observed at 5, 50 and100µg dose level. Cauda sperms were found to be reduced in the 100µg dose treated animals. Reduction in the serum testosterone was also observed in treated animals. Study concludes that bpa affects reproduction with impaired fertility in male rats.

Introduction

In recent years, scientists have increasingly reported evidences that certain pollutants in the environment plays important roles in contributing causes of fertility problems. A wide range of compounds, including components from pesticides to plastics, from detergents to cosmetics registered as pollutant. Bisphenol A (2, 2-bis (4-hydroxyphenyl) propane) (BPA), is the chemical found in polycarbonate plastics, leaches from plastic containers during normal usage, and detectable amounts can be found in many commercial food products and dental sealant (Goodson et al., 2004; Mountfort et al., 1997). It is reported that 10-20 µg of BPA in canned food is leached from the lacquer lining (Brontons et al., 1995). In the saliva of patients who had been treated with a dental sealant, 20-30 µg of BPA/mL was detected (Olea et al., 1996). The current reference dose [50µg/kg/day (U.S. EPA 1993)] determined by the U.S. Environmental Protection Agency (EPA) is 1/1000 of the level that exerts the lowest adverse effect (LOAEL; 50 mg/kg/day).

It is chronically ingested by humans, 95% of adults and children testes have detectable total urinary BPA (Matsumot et al., 2003; Calafat et al., 2008). BPA has also been measured in maternal serum and ovarian follicular fluid, as well as in fetal plasma and amniotic fluid, indicating passage across the placenta (Schonfelder et al., 2002; Ikezuki et al., 2002). BPA has both estrogenic and anti-androgenic effect (Akingbemi et al., 2004; Lee et al., 2003; Wetherill et al., 2007). Toxicological studies (Richter et al., 2007) have pointed out that rodents exposed to BPA during the prenatal period show a large variety of adverse reproductive outcomes, including decreased epididymal weight and daily sperm production, increased sloughing from seminiferous epithelium (Salian et al., 2009a, 2009b; vom Saal et al., 1998) and increased prostate weight (Nagel et al., 1997). Postnatal exposure disrupt blood testis barrier by this germ cell cannot develop into mature sperm and also increases the activation of caspase-3 which cause germ cell apoptosis (Li et al., 2009). Hence, there is a significant risk of BPA exposure during critical development period that are particularly sensitive to changes in estrogenic environment (Vandenberg et al., 2009). During adult exposure changes in sperm morphology like abnormalities in acrosomal cap, vesicle and deformed nuclei were found in Wistar and Swiss rat at 20µg/kg/day(Chitra, Latchoumycandane, & Mathur, 2003). Recent studies showed that the level of testosterone decrease in rats when exposed to different dose level of BPA (Tohei et al., 2001, Mendiola et al., 2010, Wisniewski et al., 2015). Reviewing to all these studies the present study was planned to determine whether exposure of BPA to different dose level affects testicular spermatogenesis or cause any histological changes in reproductive organs, if so, to identify the mechanisms associated with observed effect.

Material and Method

Adult male Wistar albino rats (*Rattus norvegicus*), 3 months old, weighing 150-200 grams, were used in present investigation. The animals were maintained in the Departmental Experimental Facility with light and dark (12h: 12h) schedule in individual polypropylene cage (size 43×27×15cm). Animals were fed with rat pellet diet and water ad libitam. The animals were maintained under perfect veterinary supervision and accordance to the guidelines of CPCSEA (CPCSEA, 2010).

Test Chemical

Bisphenol A (2, 2-bis (4-hydroxyphenyl) propane) (<99% pure) was purchased from sigma Aldrich. This compound was diluted in olive oil to obtain final concentration of the 5, 50 and 100µg/100gm body weight of the animals respectively.

Experimental Design

Animals were divided into four groups with ten in each and olive oil was used as vehicle.

Group I: Control (vehicle treated)
Group II: Oral administration of 5µg BPA/100 g/bw
Group III: Oral administration of 50µg BPA/100 g/bw
Group IV: Oral administration of 100µg BPA/100 g/bw
Doses were given for consecutive 90 days. On 91th day of experiment, animals were sacrificed by overdose of anesthetic ether.

Body and reproductive organ weight:

The body weights of all animals were measured fortnightly. The weights of all reproductive organs of all animals will be obtained at the time of scarification schedule of each group.

Fertility Test:

The treated adult males were mated with the normal adult females. Each cage contains one male and two female. Mating was confirmed by visual appearances and presence of spermatozoa in the vaginal smear. Following mating pregnant animals will be allowed to complete the term and pregnancy.

Histopathology:

A portion of reproductive organs testis, epididymis, were fixed in 4% paraformaldehyde, dehydrated in ethanol, cleared in Xylene and embedded in paraffin wax. Five micron thick sections were stained with haematoxylin and eosin for light microscopic observation.

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Hormone analyses:
Circulatory levels of testosterone in all treated group of rats exposed to 5µg BPA/100 g/bw, 50µg BPA/100 g/bw and 100µg BPA/100 g/bw and control group of rats which were vehicle treated assayed by ELISA kit.

Statistical analysis:
The mean values will be compared using respective standard error of mean followed by statistical comparison between control and test groups. The significance of differences among means was evaluated using one-way ANOVA. Differences at $P < 0.05$ were considered statistically significant.

Results

Body and Reproductive organ weight
Weights of all animals were within the normal range. No significant alteration was observed in the body weight of treated animals at different doses of BPA as compared to control. Reductions in reproductive organ weight were observed. No significant alteration was observed in group I i.e. control animals. Non significant decline in the weight of testis and epididymis was observed in Group II and III whereas Group IV animals treated with 100µg BPA/100 g/bw showed a significant alteration. (Table 1) (Figure 1 a and 1b)

Table 1.Body and Organs Weight of Control and BPA treated male Rats

Treatment (For 90 days)	Initial Body weight (gms.)	Final Body weight (gms.)	Reproductive organs weight (mg/100 gm.) body weight.		
			Testis	Cauda epididymus	Testosterone level(ng/ml)
Group I Control (Vehicle Treated)	120± 1.66	160 ± 1.66	1498± 33.02	.398± .01	5.3±0.3
Group II 5µg/100g.b.wt/day	113.33±5.77	137.5±5.77	1280±43.26	.361±.02	4.93±0.4
Group III 50µg/100g.b.wt/day	115±5.77	162.5±49.24	1139 ±50.69	.335 ±.03	3.18±0.3
Group IV 100µg/100g.b.wt./day	142.5±5.77	165±5.77	1050±71.62*	.251 ±.02*	2.42±.8*

(Mean ± SEM) P<0.05	Group II, III & IV Compared with Group I

*= significant

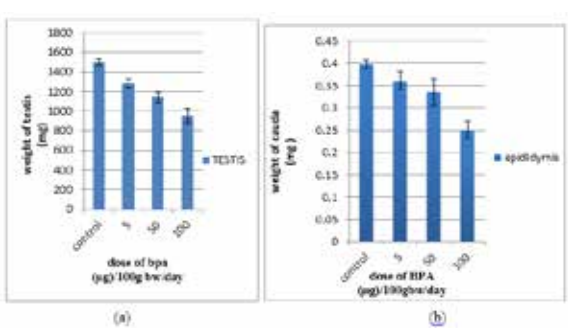


Figure: 1 weight of testis and cauda epididymis in BPA treated groups

Fertility test
Fertility test was performed every 15 days and we found that the treated rats mated normally to the females and they began to mount within 30 min and ejaculate after insertions. The females delivered normal pups. The litter numbers were 5 to 6.

Influence of BPA on spermatogenesis
Histologically group II, III and IV animals showed altered spermatogenesis as observed by reduction in the number of round spermatids, elongated spermatids and no spermatids as compared to control, in the three treated groups respectively.(fig:2)

Effect on epididymis
Histologically group II, III and IV animals showed degenerative changes as observed by altered epithelium and almost nil sperms in the treated animals of group IV.(fig:3)

Effect on testosterone level
The levels of serum testosterone were lower in the BPA treated rats than those in the control rats in all the three groups of treated animals compared to control .(fig: 4) These results indicate that the reduction in testosterone levels by BPA arrests the process of spermiation.

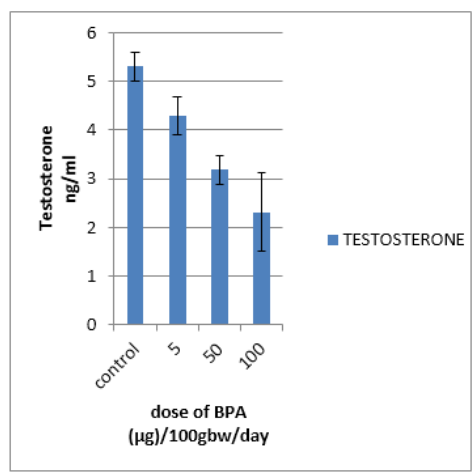
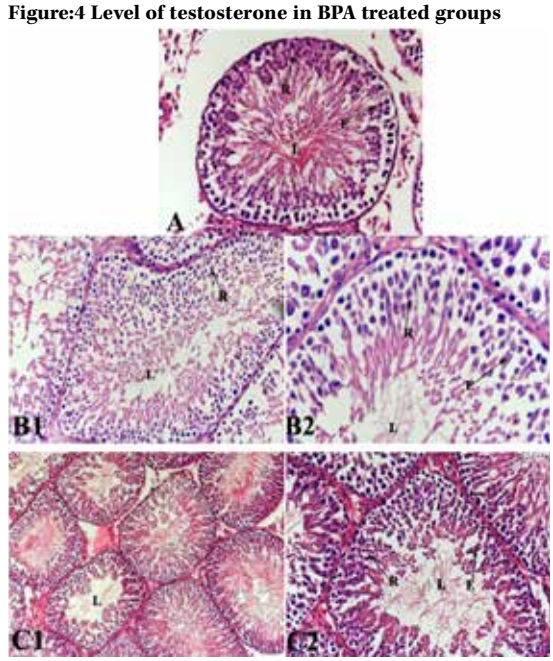


Figure:4 Level of testosterone in BPA treated groups



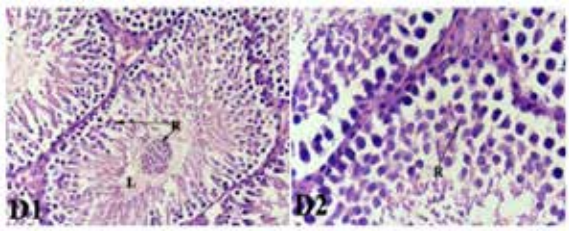


Figure 2: Photomicrograph showing histological changes in testis of rats after 90 days of bisphenol A treatment. A: Control; B1-epididymis (R) denotes round spermatid, (E) denotes elongated spermatid and (L) denotes Lumen of seminiferous tubule

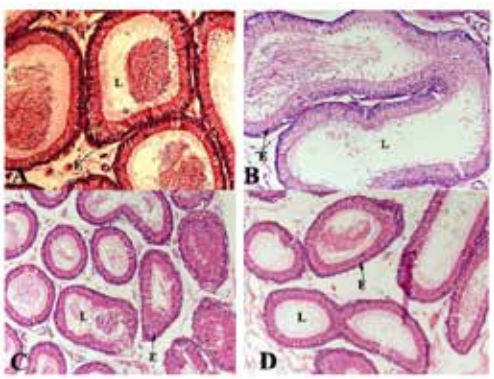


Figure 3a: Photomicrograph showing histological changes in caput epididymis of rats after 90 days of bisphenol A treatment . A: Control; B (-) D: 5µg/100g/day, 50 µg/100g/day and 100 µg/100g/day, respectively. (E) denotes epididymal epithelium, (L) denotes lumen of the epididymis

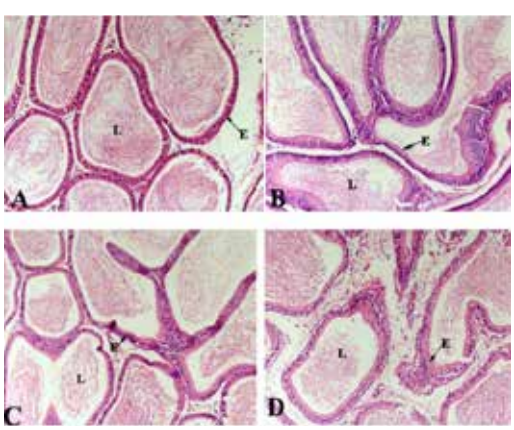


Figure 3b: Photomicrograph showing histological changes in corpus epididymis of rats after 90 days of bisphenol A treatment. A: Control; B (-) D: 5µg/100g/day, 50 µg/100g/day and 100 µg/100g/day, respectively. (E) denotes epididymis

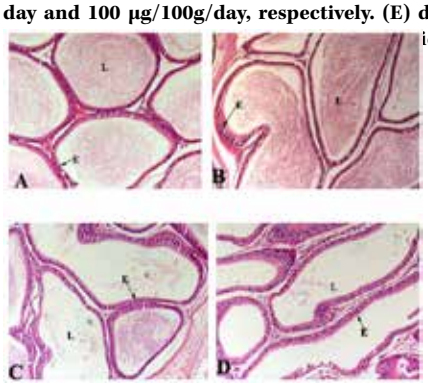


Figure 3c: Photomicrograph showing histological changes in cauda epididymis of rats after 90 days of bisphenol A treatment. A: Control; B (-) D: 5µg/100g/day, 50µg/100g/day and 100µg/100g/day, respectively. (E) denotes epididymal epithelium, (L) denotes lumen of the epididymis

Discussion

The testes are most vulnerable organ to measure the fertility effect. Changes in homeostasis of hormonal level and disrupted spermatogenesis process show the toxicity of the compound on fertility. In our study decrease in serum testosterone level was observed in rats exposed to BPA which indicates that spermatogenesis also get disrupted. Histological examination of different regions of testes shows the slowing down of spermiation process and epididymal epithelium show degenerative changes. Lesser amount of sperms in cauda epididymis indicates that sperm count also gets reduced Consistent with our results, Tohei et al., 2001 and Mendiola et al., 2010 reported that exposure of adult rats to BPA decreased the plasma concentration of testosterone. Wisniewski et al., 2015 also reported that exposure of BPA at 5mg and 50mg/kg/day dose level for 60 days decreased the plasma level of testosterone in adult male rats which effect the sperm concentration.

The doses in our study are selected with human applicability in mind. Studies suggest that an adult is exposed to low concentrations of BPA, no more than 0.4–1.5 µg BPA/kg bw/day (Lakind JS, et al., 2008).The environmentally human-relevant dose of BPA in rodent experiments is considered as 20 µg/kg bw/day(Susiarjo M, et al., 2007). Although the dose of BPA (5, 50 and 100µg/100 bw/day) that induced significant reproductive impairment in our study cannot be considered truly environmentally relevant, it can be considered low. Based on the available data, a conference sponsored by the National Institute of Environmental Health Sciences in 2007 predicted that internal BPA exposure (plasma or serum concentrations) in humans is 435 µg/day (500µg/kg/day) (Vandenberg LN, et al., 2007). Moreover, the actual exposure level may be much higher than the current accepted level in some countries or areas (Huang YQ et al., 2012). The effects of bpa have been documented in various previous studies using short term exposure and by injecting directly. The documented action of BPA includes orally ingested 25 and 100 µg kg^{-1} BPA for 28 days showed a significant reduction in testicular sperm counts and in the efficiency of sperm production(Al-Hiyasat,2002). Reduced sperm count and epididymal epithelium degeneration in Wistar rat at 20µg/kg / day dose level in 60 days exposure study was observed. (Chitra, Latchoumycandane, & Mathur, 2003). Similarly, our result also shows these effects on 5 µg, 50 µg and 100µg/100gm bw/day dose level in 90days exposure. In another study oral administration of low dose of BPA (2µg/kg) for consecutive 14 days in adult rats also impairs spermatogenesis (Pengpeng Jin,et al.,2013) but in our study at 5µg dose this effect is less. At 50µg and100µg dose level BPA affect spermatogenesis process more. In rodent testes, the multiple types of germ cells are arranged in characteristic cellular associations that succeed each other in a given area of the seminiferous tubule, which are known as the stages of spermatogenesis. Their succession in time has been defined as the cycle of the seminiferous epithelium. These cycle of spermatogenesis are essential for continuous sperm production, which is dependent upon numerous factors, both intrinsic (sertoli cell and germ cell) and extrinsic (androgen and retinoic acid) as well as species specific (Rex A, Hess et al., 2008).

Interestingly, in our study, the BPA treated groups at 5 µg dose level lesser no. of round spermatid were observed where as at 50 µg dose level the whole lumen of seminiferrous tubule filled with cell debris and round spermatid showed a higher frequency of stage VII and a lower frequency of stage VIII compared with control animals, suggesting a possible delay in the progression

of spermiation, which takes place at stage VIII. These results are consistent with previous studies showing that environmental toxin-induced lower daily sperm production and this sperm count reduction is highly associated with the disruption of spermatogenesis. The sex hormones important for controlling the process of spermatogenesis were also altered after BPA treatment but some studies also reported that at 2, 20 and 200µg/kg/d orally administrated dose given for 60 days, any significant decrement in testosterone level in adult wistar rats not observed (C Liu, W Duan et al., 2013). During the treatment of different doses of BPA the body weight of rat did not effected but the weight of testes and cauda epididymis got decreased which was also reported in earlier studies by short term exposure treatment.

In conclusion, results from present study demonstrate that BPA has an adverse effect on testicular spermatogenesis at different dose level when exposed orally for long duration of 90 days. BPA also suppressed the level of testosterone hormone which is secreted by leydig cells and also degenerate the epididymal epihtilium.

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