Research Article

A Study on Developmental Toxicity of Bisphenol A (BPA) in Maternal Exposed Wistar Rats

Seema Srivastava*, Nupoor Dhagga
Reproductive Physiology laboratory, Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India.
***Corresponding author's E-mail:** drseemaa07@gmail.com

Received: 09-05-2017; **Revised:** 16-07-2017; **Accepted:** 30-07-2017.

ABSTRACT

The potential teratogenic effects of environmental estrogenic disruptors have become a great concern in recent years. Humans are routinely exposed to bisphenol A (BPA), an estrogenic compound that leaches from CDs, dental sealants, plastic bottles and beverage containers. Observing its harmful effects on male as well as in female reproductive system, the present study was designed to evaluate embryo toxic and teratogenic effects of BPA exposure during the maintenance of pregnancy and embryo fetal development in wistar rats. Adult females were administered by gavage at the dose of 5 and 50 mg/kg b.wt./week BPA orally using olive oil as a vehicle for 3 months. During the treatment period, treated females were allowed to mate depending on the possible outcomes (non pregnant and pregnant). The halves of the experimental animals (pregnant) were sacrificed on 20th day of gestation and the other half were allowed to complete their full term and deliver their pups. No significant changes were seen in number of implantations and resorptions, corpus lutea, weight of litters and litter size in cesarean. No teratogenic effects were observed in fetus of full term females. Maternal body weight had no significant changes. The study infers no adverse signs of either maternal toxicity or developmental toxicity in BPA administered rats.

Keywords: Teratogen, BPA, cesarean, pregnant, resorption.

INTRODUCTION

Bisphenol A (BPA) is a highly produced synthetic chemical used in the manufacture of polycarbonate plastics and epoxy resins. These materials can be found in reusable water bottles, food cans and containers, compact discs, flooring, and paints.[1]The primary mode of exposure for adults is through ingestion of foods in contact with BPA containing materials. Pregnant women who regularly consume canned vegetables and food, work as cashiers, or are exposed to tobacco smoke have higher urinary BPA concentrations than pregnant women without these characteristics.[2] BPA with weak estrogenic properties[3,4] and appears to interfere with typical development of embryos, affecting skeletal anomalies.[5] Animal studies have demonstrated detrimental effects of BPA on reproductive function[6], on maternal behavior[7], on increased body weight in females[8,9], and on sex-typical behaviors.[10] Human studies have linked environmental BPA exposure with reduced testosterone[11] and infertility[12,13] in adult men, as well as fewer oocytes and serum estradiol levels in women undergoing in-vitro fertilization.[14]

Exposure of the developing fetus to BPA is of particular concern as the compound readily crosses the placental barrier of pregnant rat and women and accumulates both in the placenta and in the fetus and potentially impacting the developing fetus.[15-17] The present study aimed to shed more light on the BPA exposure on embryonic development in pregnant rats and their effects.

MATERIALS AND METHODS

Test Material

Bisphenol A [2, 2-bis (4-hydroxyphenyl propane)] (purity 99.5%, CAS no. 80-05-7) was purchased from Sigma Aldrich.

Animals

Adult female Wistar rats, 5-6 months old, weighing 180-220 grams, were used in the investigation. The animals bred in our laboratory and maintained in the Departmental Experimental Facility with light and dark (12h:12h) schedule in individual cage. The temperature in animal house during study period was maintained at 23±2 ^{0}C and relative humidity was ranged between 32 and 70%. Animals were fed with rat pellet diet (Ashirwad Industries Limited, Chandigarh) and free access to safe drinking water *ad libitum* in glass bottles. The animals were maintained under perfect veterinary supervision and accordance to the CPCSEA guidelines.[18]

Experimental Design

Animals were allocated into three groups, containing ten animals each. Parallel control animals were used for termination of each phase. During treatment all animals were allowed to mate and get pregnant depending on no pregnancy, cesarean and full-term of pregnancy and considered as day 0 of pregnancy.

Group I: Control (vehicle treated)

Group II: Oral administration of 5 mg BPA/kg b.wt. /week

Group III: Oral administration of 50 mg BPA/kg b.wt. /week

Doses and Autopsy Schedule

During treatment, doses were administered to all females orally. All pregnant females received dose during gestation also and half of the cesarean females were subjected to necropsy on 20th day of gestation and other half females were subjected to deliver their pups.

Body Weight

The body weight of all animals measured twice; initially (before starting the experiment) and final body weight obtained at the time of euthanization at the termination of schedule or fatality.

Maternal-Fetal Investigations

Numbers of implantation, resorptions, corpus lutea, and weight of litters and size of litters were recorded.

Teratological Examination

F1 progenies (full term fetus) of the test groups were examined for skeletal morphology. The offspring was eviscerated and skinned following external and visceral examination, later, fixed in 95% alcohol and double stained with Alician blue and Alizarin red. Subsequently, macerated in 0.5% KOH and processed in graded series of glycerol.[19,20]

Statistical Analysis

The mean values were compared using respective standard deviations followed by statistical comparison between control and test groups for evaluation of significant changes in values by Student's t-test. P<0.05 was considered as significant.

RESULTS

Pregnancy Status

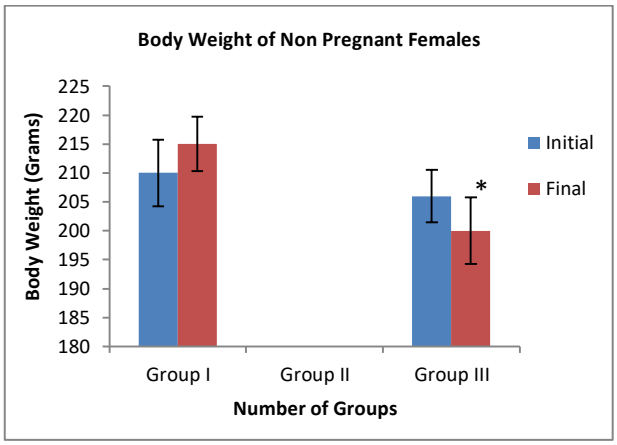
The animals of group I (control) and group II (5 mg/kg b.wt./week) got pregnant but group III animals (50 mg/kg b.wt./week) did not get pregnant at all.

Body Weight

A non significant difference in body weights was observed of cesarean females in group II (5 mg/kg b.wt./week)(Table 1) and a significant changes in body weight was noted in group III (50 mg/kg b.wt./weeky) of non pregnant in comparison to group I (control) females (Graph 1).

Maternal-Fetal Investigations

No significant changes were recorded of cesarean females of group II (5 mg/kg b.wt./week) including number of implantation, resorptions, corpus lutea, weight of litters and and size of litters as compared with group I (control) (Table 1).



Graph 1: Body weight (gram) following control and treated group (5 mg/kg b.wt./week) in Wistar rats of non pregnant. Note remarkable alteration in final body weight. Each column and vertical bar denotes mean±SD. Asterisk denotes significance at p<0.05.

Table 1: Effect of bisphenolA dose on maternal rats and their offsprings

Parameters	Group I	Group II
Initial body weight (grams)	215±4.08	213±5.87
Final body weight (grams)	258±5.89	256±4.62
Number of implantations	7.7±0.48	7.5±0.52
Number of resorptions	0	0
Number of corpus lutea	7.7±0.48	7.5±0.52
Weight of litters	3.48±0.035	3.47±0.037
Litter size	7.7±0.48	7.5±0.52

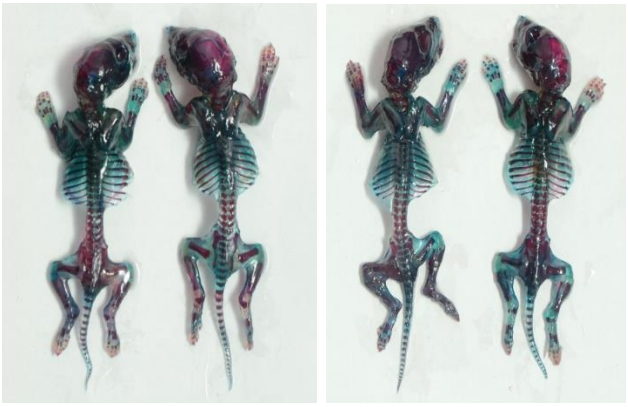
Values expressed as mean ± standard deviation.

Teratological Examination

Examining the skeletal bones and cartilage, no abnormalities were seen in group II (5 mg/kg b.wt./week) BPA dosed animals in full term pregnancy as compared with group I (control) (Table 2; Figure 1A-B).

Table 2: Teratogenic effects of BPA on skeletal system of rat

Ossification	Group I	Group II
Skull	Normal	Normal
Ribs	Normal	Normal
Vertebral Centra	Normal	Normal
Fore Limbs	Normal	Normal
Hind Limbs	Normal	Normal



(A) (B)

Figure 1: Skeletal morphology of F_1 progeny in (A) group I and (B) group II. Note normal ossification and chondrification in all F_1 progenies.

DISCUSSION

Several studies report subtle effects of BPA exposure in rodents at different doses. In the scientific reviewed literature of Gal and Flaws (2016), the potential effects of BPA on fertility outcomes (ability to become pregnant, number of off springs) and reproductive related process (implantation) was published which indicates that BPA may be associated with infertility, may alter reproductive capacity and implantation.[21]

In the present study, pregnancy was found at 5 mg/kg b.wt of dose of BPA but at 50 mg/kg b.wt BPA dose, no pregnancy was observed. In support Monje et al (2009) also reported significantly lower levels of perceptive behavior in neonatal rats at 20 and 0.05 mg/kg/day exposure of BPA.[22]Kim et al. (2001) found an increase in pregnancy failure at 1000 mg/kg but no toxicity observed at 100 mg/kg dose of BPA.[23]

Regarding maternal and fetal investigation, the present data, finds no significant changes in number of implantation sites, number of resorptions, corpus lutea, weight of litters and litter size. Kim et al (2001) also reports that no significant changes induced by BPA in the number of corpora lutea and implantation sites but increase in foetal deaths and resorption in offspring, reduced litter size and weight of fetuses of pregnant Sprague-Dawley rats when administered with a high BPA level during the entire gestational period but at low dose of BPA, no adverse sign of maternal toxicity were observed.[23] On the contrary, the data obtained by Deen et al (2015) revealed that pregnant rats treated with Bisphenol-A induced reduction in implantation sites, corpus lutea and increased resorption rates. The litters size and weight were also significantly decreased at300 & 600 mg BPA/kg/day on both 15th and 19th days of gestation.[5] Also, this phenomenon of decrease in number of implantation sites and resorption when pregnant rats and mice treated with BPA (600 mg/kg) and (10.125 mg/mouse/day, ~400 mg/kg/day) from day 0 - 15th of gestation.[24,25]

In the present investigation, we did not find any kind of impairments in skeletal system at the oral dose of 5 mg/kg b.wt./week of BPA. In support with, no embry of etaldysmorphogenesis at an oral exposure level of 100 mg BPA/ kg and 1000 mg BPA/kg.[23] In contrast, severe skeletal anomalies were seen at 300 & 600 mg BPA/kg/day on both 15th and 19th days of gestation.[5] Teratogenicity also get affected when pregnant rats and mice with the treatment of 600 and 400 mg/kg/day from day 0 - 15th of gestation.[24, 25]

Effect on overall body weight of BPA treated females comes up with controversial results. In present study, no significant difference in body weight of BPA-treated female rats in cesarean and significant changes in non pregnant were observed. Similarly, Delclos and his colleagues found that BPA doses ≤ 2,700 µg/kg bd.wt./day did not affect gestational body weight gain of rat.[26]Cabaton et al (2011) also reports no statistical difference in the dam weights by treatment groups of BPA exposed perinatally in CD-1 mice.[27] Early prepubertal exposure to BPA (10 and 100 mg/kg) in mice significantly decreased body weight from postnatal day 18 to 30 but 0.1 and 1 mg/kg of BPA treated groups showed no significant differences in body weight.[28] But Sharf-ElDeen et al(2015) observed that body weight of pregnant females showed remarkable reduction at both 15th and 19th days of gestation when fed with 300 & 600 mg BPA/kg/day.[5] In another study, at 300 and 1000 mg BPA/kg, suppression of maternal body weight wasobserved.[23]

CONCLUSION

The study infers no teratological deformities in F_1 generation following BPA treatment at 5 mg BPA/kg b.wt./week orally for 3 months at which the physiological behavior of females was not affected.

Acknowledgements: The authors express their sincere gratitude to Head of the Department of Zoology, University of Rajasthan, Jaipur, Rajasthan for providing necessary research facilities for the successful completion of this study.

REFERENCES

1. Biedermann S, Tschudin P, Grob K. Transfer of bisphenol A from thermal printer paper to the skin. Analytical and Bioanalytical Chemistry 398(1), 2010, 571-576.
2. Braun J, Kalkbrenner A, Calafat A, Bernert J, Ye X, Silva M, Barr D, Sathyanarayana S, Lanphear B. Variability and predictors of urinary bisphenol A concentrations during pregnancy. Environmental Health Perspectives 119, 2010, 131-137.
3. Akingbemi B, Sottas C, Koulova A, Klinefelter G, Hardy M. Inhibition of testicular steroidogenesis by the xenoestrogenbisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. Endocrinology 145(2),2004, 592-603.

4. Lee H, Chattopadhyay S, Gong E, Ahn R, Lee K. Antiandrogenic effects of bisphenol A and nonylphenol on the function of androgen receptor. Toxicological Sciences 75(1), 2003, 40-46.
5. Sharf-EL Deen O, Bakry S, Shaeir WAA, Mohammed FE, Adel M. Teratogenicity of Bisphenol-A (BPA) in Pregnant rat. American-Eurasian Journal of Toxicological Sciences 7(4), 2015, 229-238.
6. Salian S, Doshi T, Vanage G. Perinatal exposure of rats to bisphenolA affects the fertility of male offspring. Life sciences 85, 2009, 742–752.
7. Palanza P, Howdeshell K, Parmigiani S, VomSaal F. Exposure to a low dose of bisphenol A during fetal life or in adulthood alters maternal behavior in mice. Environmental Health Perspectives 110, 2002, 415-422.
8. Adewale H, Todd K, Mickens J, Patisaul H. The impact of neonatal bisphenol-A exposure on sexually dimorphic hypothalamic nuclei in the female rat. Neurotoxicology 32(1), 2011, 38-49.
9. Somm E, Schwitzgebel V, Toulotte A, Cederroth C, Combescure C, Nef S, Aubert M, Hüppi P. Perinatal exposure to bisphenol A alters early adipogenesis in the rat. Environmental Health Perspectives 117(10), 2009, 1549-1555.
10. Palanza P, Gioiosa L, VomSaal F, Parmigiani S. Effects of developmental exposure to bisphenol A on brain and behavior in mice. Environmental Research 2, 2008, 150-157.
11. Mendiola J, Jørgensen N, Andersson A, Calafat A, Ye X, Redmon J, Drobnis E, Wang C, Sparks A, Thurston S. Are Environmental Levels of Bisphenol A Associated with Reproductive Function in Fertile Men? Environmental Health Perspectives 118(9), 2010, 1286-1291.
12. Li D, Zhou Z, Qing D, He Y, Wu T, Miao M, Wang J, Weng X, Ferber J, Herrinton L. Occupational exposure to bisphenol-A (BPA) and the risk of Self-Reported Male Sexual Dysfunction. Human Reproduction 2010, 1-9.
13. Meeker J, Ehrlich S, Toth T, Wright D, Calafat A, Trisini A, Ye X, Hauser R. Semen Quality and Sperm DNA Damage in Relation to Urinary Bisphenol A among Men from an Infertility Clinic. Reproductive Toxicology 30(4), 2010, 532-539.
14. Mok LE, Ehrlich S, Williams P, Petrozza J, Wright D, Calafat A, Ye X, Hauser R. Urinary bisphenol A concentrations and ovarian response among women undergoing IVF. International Journal of Andrology 2, 2010, 385-393.
15. Balakrishnan B, Henare K, Thorstensen EB, Ponnampalam AP, Mitchell MD. Transfer of bisphenol A across the human placenta. American Journal of Obstetrics & Gynecology 202(4), 2010, 393.
16. Nishikawa M., Iwano H, Yanagisawa R, Koike N, Inoue H. Placental transfer of conjugated bisphenol A and subsequent reactivation in the rat fetus. Environmental Health Perspectives 118, 2010, 1196-1203.
17. Kalyan CC, Catherine AVV, Matthew LS, Barrie DR, Gordon KM. Maternal bisphenolA exposure impacts the fetal heart transcriptome. PLoS ONE 9(2), 2014, e89096.
18. CPCSEA guidelines For Laboratory Animal Facility, 2010, 1-39.
19. Christian MS. Test methods for assessing female reproductive and developmental toxicology. In: Hayes, A. W. (ed.). Principles and Methods of Toxicology.4th edn, Taylor and Francis, Philadelphia. 2001, 1301-1381.
20. Taylor P. Practical Teratology. Academic Press, New York. 1986.
21. Gal AZ, Flaws JA. Evidence for Bisphenol A induced female infertility: a review (2007-2016). Fertility and Sterility 106(4), 2016, 827-856.
22. Monje L, Varayoud J, Munoz de Toro M, Luque EH, Ramos JG. Neonatal exposure to bisphenol A alters estrogen-dependant mechanisms governing sexual behavior in the adult female rat. Reproductive Toxicology 28(4), 2009, 435-442.
23. Kim JC, Shin HC, Cha SW, Koh WS, Chung MK, Han SS. Evaluation of developmental toxicity in rats exposed to environmental estrogenBisphenol-A during pregnancy. Life Science 69(22), 2001, 2611-2625.
24. Roy GK, Malini NA. Maternal exposure to xenoestrogenBisphenol-A on embryo fetaldevelopmentandteratogenic potential in Rattusnorvegicus. The Bioscan an InternationalQuarterly Journal of Life Sciences 7(3), 2012, 517-520.
25. Varayoud J, Ramos JG, Bosquiazzo VL, Lower M, Muñoz-DE-Toro M, Luque EH. Neonatal exposure to bisphenol-A alters rat uterine implantation-associated gene expression and reduces the number of implantation sites. Endocrinology 152(3), 2011, 1-11.
26. Delclos KB, Camacho L, Lewis SM, Vanlandingham MM, Latendresse JR, Olson GR, Davis KJ, Patton RE, Dacosta GG, Woodling KA, Bryant MS, Chidambaram M, Trbojevich R, Juliar BE, Felton RP, Thorn BT. Toxicity evaluation of bisphenol A administered by gavage to Sprague-Dawley rats from gestation day 6 through postnatal day 90. Toxicological Sciences 139(1), 2014, 174-197.
27. Cabaton NJ, Wadia PR, Rubin BS, Zalko D, Schaeberle CM, Askenase MH, Gadbois JL, Tharp AP, Whitt GS, Sonnenschein C, Soto AN. Perinatal exposure to environmentally relevant levels of bisphenol A decreases fertility and fecundity in CD-1 mice. Environmental Health Perspectives 119, 2011, 547-552.
28. Nah WH, Park MJ, Gye MC. Effects of prepubertal exposure to bisphenol A on the onset of puberty, ovarian weights, and estrous cycle in female mice. Clinical and Experimental Reproductive Medicine 38(2), 2011, 75-81.

Source of Support: Nil, **Conflict of Interest:** None.