Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 1499 - 1510
Received 24 October 2020; Accepted 15 December 2020.

Bisphenol-A Induced Teratogenic Potential in Early Gestation Rats

T. Geetharathan[1], M. Thulasi[3], P. Josthna[2]and K. Peera[3]*

1. Department of Biotechnology, Sri Venkateswara University, Tirupati, Andhra Pradesh,
2. Department of Biotechnology, Sri Padmavati Mahila Visvavidyalayam, Tirupati
3. DBT-BIF Centre, Department of Zoology, SV Univeristy, Tirupati, Andhra Pradesh, India.
Corresponding e-mail: drkutagollapeera2014@gmail.com.

Corresponding Author:
Dr. K. Peera
DBT-BIF Centre,
Department of Zoology,
SV Univeristy,
Tirupati, Andhra Pradesh, India.
e-mail: drkutagollapeera2014@gmail.com.

Abstract:

Bisphenol- A, a chemical compound found in plastic products, is being used increasingly in industrial manufacturing materials. Estrogenic activity of BPA has been reported for over 50 years. The potential teratogenic effects and fetal toxicity of environmental estrogenic endocrine disruptors have become a great concern in recent years, and they have yet to be fully characterized. It accumulates in pregnant adult females and its continued exposure during gestation is likely to have an impact on the development of the fetus. Humans are routinely exposed to bisphenol-A (BPA), an estrogenic compound that leaches from dental materials and plastic bottles and beverage containers. The present study was conducted to evaluate embryo toxic and teratogenic effects of BPA exposure during the early gestation period (Gestational day 8^{th} to 15^{th}) of albino rat. Pregnant rats were administered 300 mg/kg, 400 mg/kg and 500 mg/kg of BPA orally using sesame oil as a vehicle from days 1– 15^{th} of gestation. The control group received sesame oil only. On completion of the treatment period, the half of the experimental animals was sacrificed under light anesthesia using ether and the other half was allowed to complete their term and deliver their pups. The number of implantations, number of corpus lutea, litter size, litter weight and growth rate of the viable offsprings, nervousness, twitching of head, agitation, hazy movement, symmetrical distribution of embryos in both uterine horns were altered and induction of external and skeletal malformations after BPA administration. BPA also induced some abnormal changes in early gestation such as teratogenicity. The present study suggested that BPA adversely affected the reproductive development of the early gestation rats.

Keywords: Bisphenol-A, Teratogenecity, Early gestation rat, Implantations.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 1499 - 1510
Received 24 October 2020; Accepted 15 December 2020.

INTRODUCTION

The toxicological properties of bisphenol-A (BPA) have been a matter of scientific debate and controversy for many years now. BPA is a major high-production chemical, with over 6 billion pounds produced each year and over 100 tons are estimated to be released into the atmosphere (Jimenez-Diaz I,et al.,2010). BPA was first synthesized by Dianin as early as 1891. Numerous studies on the biological effects of BPA have been published, and its potential human health hazards have been extensively summarized (Rubin BS. 2011). Previous studies have linked BPA exposure to abnormalities of the reproductive system and a higher incidence of cardiovascular disease (Lang IA, etal, 2008). Along with recent increases in the prevalence of neurobehavioral disorders (Visser SN, et al., 2014), evidence has been accumulating that BPA can perturb nervous system development. For example, elevated gestational urinary concentrations of BPA have been correlated with adverse behavioral outcomes in children (Harley KG, et al, 2013). However, many uncertainties remain and controversial discussions are still ongoing. The molecular mechanisms mediating the effects of BPA on the nervous system are beginning to be clarified. Disruption of maternal thyroid or gonadal hormones critical for normal development may underlie the effects of BPA. Chevrier et al. found that BPA exposure during pregnancy was associated with decreased total thyroxine (T4) in pregnant women and reduced thyroid stimulating hormone (TSH) in male neonates (Chevrier J, etal.,2013). Furthermore, prenatal exposure to low doses of BPA in pregnant mice alters thyroid receptor expression in the fetal neocortex (Nakamura K, et al, 2006). These findings suggest that perinatal hypothyroxinemia caused by BPA exposure during pregnancy may underlie some of the neurological deficits in offspring. Early life exposure to BPA has been shown to affect the dopamine system (Masuo Y et al., 2011). Gestational exposure to BPA reduces the number of midbrain dopamine neurons in monkeys (Elsworth JD, etal, 2013). It has been reported that tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, is affected by BPA (Ishido M, etal, 2007). Thus, an alteration of the dopaminergic system may account for some of the neurological deficits associated with BPA exposure, such as anxiety-like behaviors (Matsuda S, etal, 2012). Additionally, developmental exposure to BPA alters the organization and function of the oxytocin (OT)/vasopressin (AVP) system, and may thereby impact behaviors regulated by associated pathways (Patisaul HB, etal.,2012). Furthermore, prenatal exposure to BPA affects fetal neocortical development by accelerating neuronal differentiation and migration during the early embryonic stage (Itoh K, etal., 2012). In the female reproductive system, BPA maytarget the mammary gland, the ovary, the oviduct, the uterus and the placenta, also, BPA increased human ovarian cancer cell proliferation in a dose-dependent manner (Jehane, I.E.,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 1499 - 1510
Received 24 October 2020; Accepted 15 December 2020.

etal, 2015). Laboratory studies on animals have demonstrated multiple adverse effects of BPA, including the development of the male and female reproductive tracts, obesity and other aspects of metabolic function, development of the brain and neurobehaviors and development of the mammary gland and its response to chemical carcinogens and induction of cancer (Vandenberg, L.N., et al., 2014). However, it may also be argued that low doses of BPA could have adverse effects on human reproductive and developmental health (Goodman, J.E., et al., 2009).

MATERIALS AND METHODS

Chemicals:

Bisphenol-A was purchased from Sd Fine Chemicals, Bombay and dissolved in Sesame oil (vehicle) as stock before administration.

Animals

Adult female Wistar rats, 5-6 months old, weighing 180-250 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 ± 2^0C and relative humidity was ranged between 32 and 65%. Animals were fed with rat pellet diet and free access to safe drinking water ad libitum in glass bottles. The animals were maintained under perfect veterinary supervision.

Experimental protocol

Pregnant females were divided into four groups; each group contained 6 pregnant females which were subjected to the following schedule of dose treatment: rats of the 1st group received vehicle (distillated water and sesame oil) orally. Rats of the 2nd group received BPA, 300 mg/kg (Kim *et al.,* 2001). Rats of the 3rd group received, 400 mg/kg BPA. Rats of the 4th group received, 500 mg/kg BPA (Sharf-EL Deen O *et al.,* 2015)**.** All doses were daily administered orally in dose starting from 8th to 15th day of gestation and sacrificed at 16th day of gestation respectively.

Maternal-Internal examination:

Fetuses were fixed in Bouin's solution for seven days (Barlow, NJ, Foster, 2003). The head of the fetus was removed and head transverse sections were made through the nasal region, the orbital region and between frontal and parietal bones. The abdominal wall of the fetuses was opened and internal organs were examined for any gross malformations. In addition, transverse sections were made through the kidney (Gal AZ, Flaws JA., 2016).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 1499 - 1510
Received 24 October 2020; Accepted 15 December 2020.

Foetal Investigation:

Fetuses were removed from uteri, weighed, sexed and examined for external malformations and measured for crown rump values. Implantation sites, live, dead, resorbed fetuses indices were calculated. After recording all measurements and parameters, foetuses were divided into two groups. The first group was fixed in Bouin's solution for morphological investigations (Sharf-EL Deen et al.,2015), while the second group was placed in Formaldehyde 10% to dissolve the body fats, then transferred to potassium hydroxide (1%) to clear the skeletons and applying Alizarin red S to stain the ossified skeletal bones. Subsequently, macerated in 0.5% KOH and processed in graded series of glycerol (Christian MS.2001). After staining, the fetuses were examined for skeletal anomalies.

$$\text{*Implantation index} = \frac{\text{Number of corpus lutea} \times 100}{\text{Number of implantations}}$$

Pups and litter weight:

The sexes of the pups were noted and their weights were recorded to the nearest milligram. The sex and body weights of the new born viable pups were also recorded from the delivered female rats. The growth rate of the viable off springs was recorded from first day of their birth. A minimum of 6 replicates were taken for each parameter and data was analyzed statistically using student's 't' test.

Teratological Examination

The test groups II,III,&IV (full term fetus) 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 (Christian MS.,2001).

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:

Maternal and fetal investigations:

Significant changes were recorded of cesarean females of group-II,III&IV(300mg,400mg & 500mg/kg b.wt) including number of implantation, resorptions, corpus lutea, number of live foetuses, litter size as compared with group-I (control),(Table-1).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 1499 - 1510
Received 24 October 2020; Accepted 15 December 2020.

Pups and litter weight investigation:

Significant changes were recorded of caesarean females of group-II,III&IV(300mg,400mg,500mg/kg b.wt) including male pup weight, total male litter weight, mean female pup weight, total female litter weight and total litter weight (g) of control and treated groups as compared with group-I (control),(Table-2)

Teratological Examination:

Examining the skeletal bones and skull parts, forelimbs and hind limbs, abnormalities were seen in group II,III&IV (5 mg/kg b.wt.) BPA dosed animals in 15th day of pregnancy as compared with group-I (control)(Figure-2h).

Table 1: Number of corpus lutea, number of implantations, foetuses, implantation index and resorptions of control and treated group (n = 6).

	Total number of implantations Mean ± SD	Total Number of corpus lutea Mean ± SD	Implantation index Mean ± SD	Resorptions Mean ± SD	Number of live fetuses Mean ± SD
Control	10.14 ± 1.50	9.45 ± 0.52	76.50 ± 2.09	0	10.14 ± 1.50
300 mg/kg	3.48±4.40	9.5 ± 1.16	60.6 ± 2.69	2.32±0.10	4± 2.75
400 mg/kg	5.33±3.67	7.5 ± 1.18	50.8 ± 4.62	3.80±0.40	2.20± 1.78
500 mg/kg	7.83± 2.48	5.5 ± 1.12	40.6 ± 2.64	4.18±0.33	1.33± 1.72

Values expressed as mean ± standard deviation.

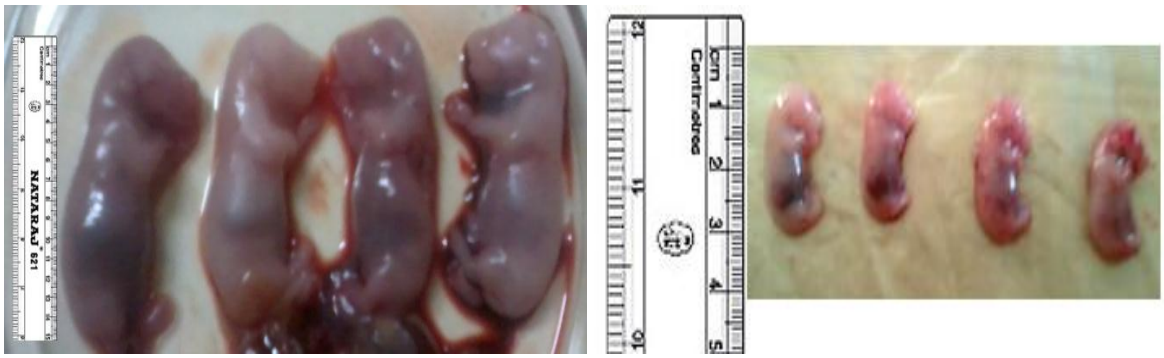
Table-2 : Mean male pup weight, total male litter weight, mean female pup weight, total female litter weight and total litter weight (g) of control and treated groups (n = 6).

Parameter	Control	BPA Treated (300mg/kg/d.b.wt)	BPA Treated (400mg/kg/d.b.wt)	BPA Treated (500mg/kg/d.b.wt)
Mean male pup weight	3.8 ± 0.02	3.0 ± 0.02	1.5 ± 0.08	1.0 ± 0.06
Total male litter weight	6.2 ± 2.4	*3.6 ± 0.15	*2.8 ± 0.06	*2.0 ± 0.02
Mean female pup weight	3.2 ± 0.03	3.7 ± 0.02	2.2 ± 0.03	1.2 ± 0.03
Total female litter weight	12.3 ± 2.6	*3.5 ± 0.03	*1.8 ±0.04	*1.6 ±0.04
Total litter weight	20.5 ±2.5	*6.3 ± 1.3	*2.0 ±0.08	*1.0 ±0.04

Values are mean ± SE* Significant *p<0.05

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 1499 - 1510
Received 24 October 2020; Accepted 15 December 2020.

Figure- 1: Teratogenic effect of (BPA) doses on the rat embryos at 15th stages:



(A) (B)

Plate 1: A & B photograph showing a lateral view of fetus's size obtained from control and treated pregnant rat after 15th day of gestation

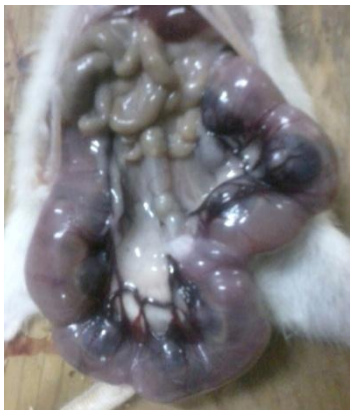
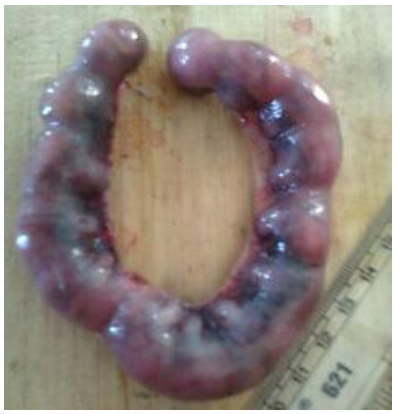


Fig.1a - Showing rat uterus with fetus and implantation sites



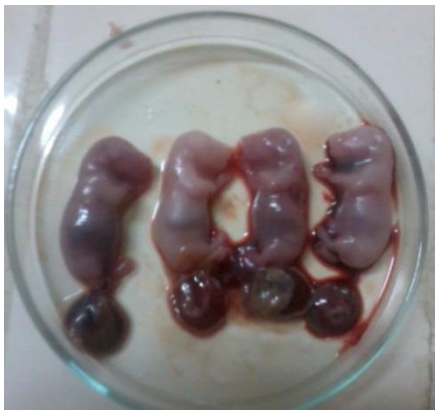


Fig.1b - Showing fetus with placenta

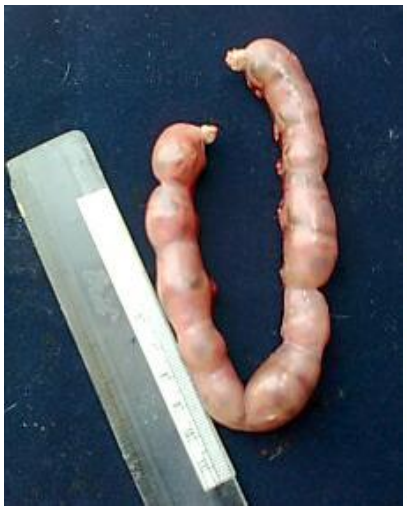


Fig.2a- Gravid uterus of control female rat (Uterus having normal embryos)

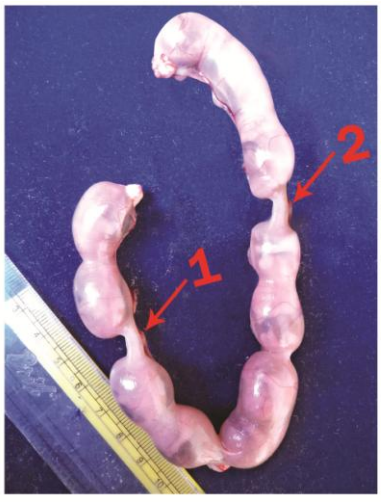


Fig.2b- Gravid uterus of BPA (300 mg/kg) exposed female rat. Uterus with reduced number of pups

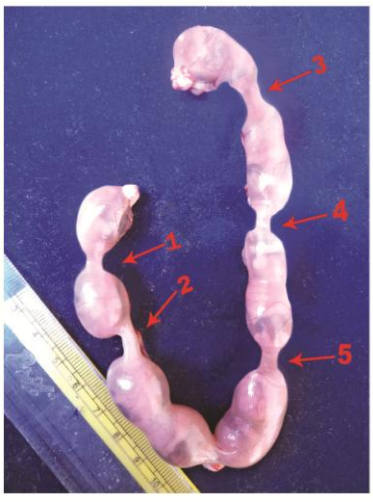


Fig.2c- Gravid uterus of BPA (400 mg/kg) exposed female rat. Uterus with reduced number of pups

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 1499 - 1510
Received 24 October 2020; Accepted 15 December 2020.

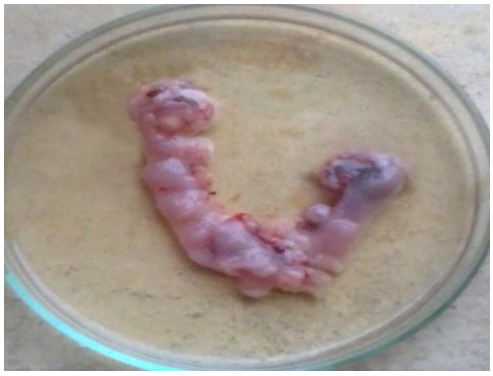


Fig.2d- Gravid uterus of BPA (500 mg/kg) exposed female rat. Resorptions were observed in uterus and no normal embryos were seen

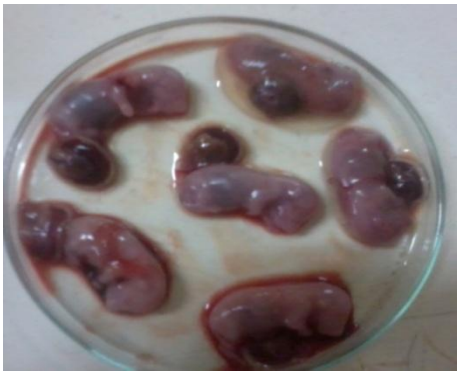


Fig.2e- Malformed embryo of BPA exposed female rat. Embryo with abnormal snout morphology

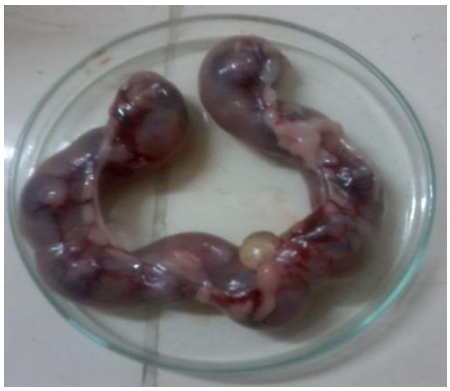


Fig.2f- Photomicrograph of control uterus on 15th day of gestation respectively, showing normal distribution of embryos.

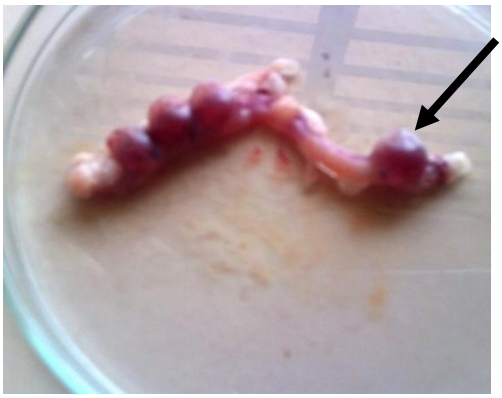
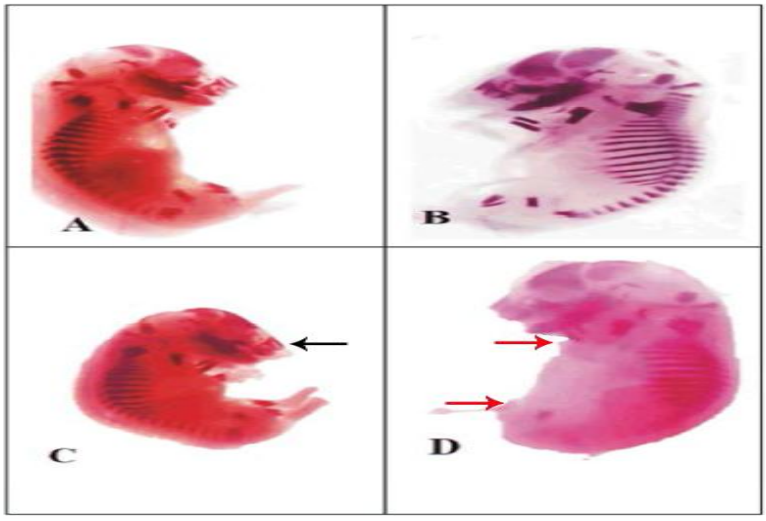


Fig.2g- Uteri from BPA treated rats from 15th day of gestation showing Shortness of horn



A-Photomicrograph showing a lateral view of fetus's skeleton obtained from control on 15th day of gestation, B,C&D- treated rat embryos after 15thdays of gestation, showing abnormal ossification of the skull parts, forelimbs and hind limbs (black arrows)at 15th stage

Fig. 2h-Teratogenic effect of (BPA) doses on the skeletal system of the rat embryos at 15th stages.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 1499 - 1510
Received 24 October 2020; Accepted 15 December 2020.

DISCUSSION:

In the present developmental toxicity study, the oral administration of BPA at the doses of 300mg/kg/b.wt, 400mg/kg/b.wt, 500mg/kg/b.wt, produced a dose dependent adverse effect on fertility index and number of implantation sites in the uterine horns of the female rats by virtue of an increase in the percentage of the post implantation embryonic loss. These results are agreement with those of Roy George, K. and N.A. Malini, 2012. Treatment of the pregnant rats with Bisphenpl-A from day 8^{th} up to 15^{th} of gestation resulted in several deleterious effects not only on the pregnant rats but also on their offspring.

Various parameters evaluated in present study are useful indices to access the toxicity of BPA. While cytotoxic agents can be disrupt pregnancy possibly by interfering with the mitotic division of the fetus, chemical insults both before and after the implantation process can be result in pre and post implantation embryonic loss.

The resorption index and implantation loss establishes correlation between the number of implanted blastosysts and those that have not developed (Almedia FG and Lemonacia I P., 2000). Further, the dose dependent increase in the resorption index by the BPA in the present study is an indication of failure in the development of embryo. Such occurrences of foetal resorption suggest that interruption of pregnancy occurred after implantation of the foetus. Increase the rate of resorption is in agreement with results obtained by Kim, J.C (2001), who reported increase in foetal death and resorption in offspring of pregnant Sprague-Dawley rats administrated a high BPA level during the entire gestational period. Also, this phenomenon of decrease in number of implantation sites, resorption and teratogenicity is confirmed when pregnant rats and mice treated with BPA (600mg/kg) and (10.125 mg/mouse/day, ~400 mg/kg/day) from day 0 - 15^{th} of gestation (Varayoud, J., et al., 2011).

Table- 2: represents mean male pup weight, total male litter weight, mean female pup weight, total female litter weight and total litter weight (g) of control and treated groups of female albino rats. Total male litter weight, total female litter weight and total litter weight were significantly ($p<0.05$) decreased in BPA exposed groups due to reduced litter size when compared to control. However there is no significant difference in mean male pup weight and mean female pup weight of control and treated groups. Kim et al. (2001) reported that administration of a high BPA level (500 mg/kg) during the entire gestational period in Sprague-Dawley rats reduced the weight of the fetuses. Maternal exposure in sheep at BPA levels of 30 to 50 ng/mL during days 30 to 90 of gestation resulted in low birth weight in

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 1499 - 1510
Received 24 October 2020; Accepted 15 December 2020.

offspring (Savabieasfahani et al., 2006).

Regarding skeletal malformations resulted in this study; BPA doses administrated to pregnant rats significantly increased the incidence of fetal skeletal malformations. BPA inhibits follicle growth and decreases steroidogenesis. In vitro follicle culture system which resulting in decrease in estradiol, estrone, testosterone androstenedione, dehydroepiandro-sterone sulfate and progesterone levels produced by the follicles which impair the reproductive axis physiologically reported by Peretz, J.,et al.(2011). Also, BPA in Vitro can influence cell growth and morphological differentiation resulting in malformed embryos with small forebrain and midbrain, small fore limb bud and abnormal optic and abnormal flexion. These irregularities clearly demonstrate that BPA is developmentally toxic to rats. In addition, it was suggested that exposure of placental cells to low doses of BPA may cause detrimental effects, leading In vivo to adverse pregnancy outcomes such as preeclampsia, IUGR, prematurity and pregnancy loss (Benachour, N. and A. Aris, 2009).

We have noticed in the present work that, the day-to-day administration of the BPA at the first stage of gestation in female rats led affect implantation particularly at the dose treatment when compared to control. This effect of the aqueous extract of BPA on implantation is marked by the presence of resorption sites through the uterine horns of rats sacrificed on the 15th day of gestation and by the absence of pups to those left to term. These results agree with those of Yakubu *et al*. (2010) who observed a reduction in the number of embryonic implantation sites. It is known that, implantation takes place normally three to four days after fertilizationin female rats (Vaissaire, J.P. 1977), which suggests that administration of the BPA from the early gestation would have hindered the process of implantation at dose 300 mg/kg and 400mg/kg and even blocked it at dose 500 mg/kg.

Prevention or minimize exposure to bisphenol-A in the following ways:

- Use glass, stainless steel, or polyethylene bottles (PETE, PET, or #1; HDPE or #2; LDPE or #4) instead of polycarbonate (PC or #7) bottles.
- Avoid using polycarbonate higher temperatures, Use glass (or) ceramic containers instead.
- Cut back on consumption of canned foods to reduce exposure to bisphenol-A contamination from the interior coating of the container. Also, avoid canned foods with higher fat content, which may have higher levels of bisphenol-A.
- Before getting dental sealants, check with your dentist about the ingredients in the products they use, as some formulations may leach bisphenol-A.

Conclusion:

In conclusion, this study also, sends several warnings to the mankind to exert more efforts at national and international levels to informing the world community about the deleterious effects of BPA as one of the endocrine disruptors agents not only on the pregnant dams but also on their offspring and should be make rules and precautions from the contamination and ingestion of such compounds to save ourselves and our offspring from the serious harmful effects.

Acknowledgments:

Authors are thankful to the Department of Biotechnology and Department of Zoology, Sri Venkateswara University, Tirupati for providing necessary lab facilities.

REFERENCES:

1. Benachour, N. and A. Aris, 2009. Toxic effects of low doses of Bisphenol-A on human placental cells. Toxicology and Applied Pharmacology, 241(3): 322-328.
2. Barlow, NJ, Foster(2003), PMPathogenesis of male reproductive tract lesions from gestation through adulthood following in utero exposure to Di(*n*-butyl) phthalate Toxicol Patho. l200331397410Google Scholar, Abstract, ISI.
3. 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.
4. Chevrier J, Gunier RB, Bradman A, et al. Maternal urinary bisphenol a during pregnancy and maternal and neonatal thyroid function in the CHAMACOS study. Environ Health Perspect. 2013; 121: 138-144.
5. Gal AZ, Flaws JA. Evidence for Bisphenol A induced female infertility: a review (2007-2016). Fertility and Sterility 106(4),2016, 827-856.
6. Elsworth JD, Jentsch JD, Vandevoort CA, et al. Prenatal exposure to bisphenol A impacts midbrain dopamine neurons and hippocampal spine synapses in non-human primates. Neurotoxicology.2013; 35: 113-120.
7. Goodman, J.E., R.J. Witorsch, E.E. McConnell, I.G. Sipes, T.M. Slayton and C.J. Yu, 2009. Weight-of-evidence evaluation of reproductive and developmental effects of low doses of bisphenol-A. Crit. Rev. Toxicol., 39: 1-75.
8. Harley KG, Gunier RB, Kogut K, et al. Prenatal and early childhood bisphenol A concentrations and behavior in school-aged children. Environ Res. 2013; 126: 43-50.

9. Ishido M, Yonemoto J, Morita M. Mesencephalic neurodegeneration in the orally administered bisphenol A-caused hyperactive rats. Toxicol Lett. 2007; 173: 66-72.
10. Itoh K, Yaoi T, Fushiki S. Bisphenol A, an endocrine-disrupting chemical, and brain development. Neuropathology. 2012; 32: 447-457.
11. Jehane, I.E., M.E. Shaymaa and A.E.G. Akmal, 2015. Bisphenol A induces oxidative stress and DNA damage in hepatic tissue of female ratoffspring. The Journal of Basic & Applied Zoology, 71: 10-19.
12. Jimenez-Diaz I, Zafra-Gomez A, Ballesteros O, et al. Determination of Bisphenol A and its chlorinated derivatives in placental tissue samples by liquid Chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2010; 878: 3363-3369.
13. Lang IA, Galloway TS, Scarlett A, et al. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. JAMA. 2008; 300: 1303.
14. Masuo Y, Ishido M. Neurotoxicity of endocrine disruptors: possible involvement in brain development and neurodegeneration. J Toxicol Environ Health B Crit Rev. 2011; 14: 346-349.
15. Matsuda S, Matsuzawa D, Ishii D, et al. Effects of perinatal exposure to low dose of bisphenol A on anxiety like behavior and dopamine metabolites in brain. Prog Neuropsychopharmacol Biol Psychiatry. 2012; 39: 273-279.
16. Nakamura K, Itoh K, Yaoi T, et al. Murine neocortical histogenesis is perturbed by prenatal exposure to low doses of Bisphenol A. J Neurosci Res. 2006;84: 1197-1205.
17. Patisaul HB, Sullivan AW, Radford ME, et al. Anxiogenic effects of developmental bisphenol A exposure are associated with gene expression changes in the juvenile rat amygdala and mitigated by soy. PLoS One. 2012; 7: e43890.
18. Peretz, J., R.K. Gupta, J. Singh, I. Hernández Ochoa and J.A. Flaws, 2011. Bisphenol-A Impairs Follicle Growth, Inhibits Steroidogenesis and Downregulates Rate-Limiting Enzymes in the Estradiol Biosynthesis Pathway. Toxicological Sciences, 119(1): 209-217.
19. Roy George, K. and N.A. Malini, 2012. Maternal exposure to xenoestrogen Bisphenol-A on embryo fetal developmentand teratogenic potential inRattusnorvegicus. The Bioscan an InternationalQuarterly Journal of Life Sciences, 7(3): 517-520.
20. Richter, C. A., Birnbaum, L. S., Farabollini, F., Newbold, R. R., Rubin, B. S., Talsness, C. E., Vandenbergh, J. G., Walser-Kuntz, D. R., and vomSaal, F. S. (2007). In vivo effects of bisphenol A in laboratory rodent studies.Reprod.Toxicol. 24, 199–224.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 24, Issue 2, 2020, Pages. 1499 - 1510
Received 24 October 2020; Accepted 15 December 2020.

21. Rubin BS. Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. J Steroid Biochem Mol Biol. 2011; 127: 27-34.
22. Sharf-EL Deen O, Bakry S, Shaeir WAA, Mohammed FE, AdelM. Teratogenicity of Bisphenol-A (BPA) in Pregnant rat.American-Eurasian Journal of Toxicological Sciences 7(4), 2015, 229-238.
23. Steel, R.G. and J.H. Torrie, 1981. Principle and Procedure of Statistics. A Biometric Approach, Second ed. McGraw- Hill Book Company, New York, USA.
24. Vandenberg, L.N., R.G. Roy, K. Kurunthachalam, A.T. Julia, B. Van- B. Richard, A.D.C.L. Carrie, Y. Yang, R.N. Retha, P. Vasantha, S.V.S. Frederick and J.W. Tracey, 2014. A round robin approaches to the analysis of bisphenol a (BPA) in human blood samples. Environmental Health, 13: 25.
25. Varayoud, J., J.G. Ramos, V.L. Bosquiazzo, M. Lower, M. Muñoz-de-Toro and E.H. Luque, 2011. Neonatal Exposure to Bisphenol-A Alters Rat Uterine Implantation-Associated Gene Expression and Reduces the Number of Implantation Sites. Endocrinology, 152(3): 1-11.
26. Visser SN, Danielson ML, Bitsko RH, et al. Trends in the parent-report of health care provider-diagnosed and medicated attention-deficit/hyperactivity disorder: United States, 2003-2011. J Am Acad Child Adolesc Psychiatry. 2014; 53: 34-46.