The Bioscan 7 (3) : 517 - 520, 2012
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

# MATERNAL EXPOSURE TO XENOESTROGEN BISPHENOL A ON EMBRYO FETAL DEVELOPMENT AND TERATOGENIC POTENTIAL IN *RATTUS NORVEGICUS*

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**KEY WORDS**
Bisphenol A
Embryo toxicity
Teratogenicity
Endocrine disruptor
Estrogen

**Received on :**
29.04.2012

**Accepted on :**
27.05.2012

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**ABSTRACT**
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. 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 period of pregnancy in *Rattus norvegicus*. Pregnant rats were administered 600mg/kg of BPA orally using olive oil as a vehicle from days 0 - 15 of gestation. The control group received olive oil only. On completion of the treatment period, the half of the experimental animals were sacrificed under light anesthesia using ether and the other half was allowed to complete their term and deliver their pups. The body weight, the gravid uterine weight, organ weight, number of implantations, number of corpus lutea, litter size, litter weight and growth rate of the viable offsprings were altered after BPA administration. BPA also induced some abnormal changes in gestation such as resorption of fetuses and teratogenicity. The present study suggested that BPA adversely affected the embryo fetal development of the pregnant female rats.

## INTRODUCTION

In recent years, the association between alterations in animal hormonal regulation and exposure to estrogenic endocrine – disrupting chemicals (EEC), such as xenoestrogens has led to increasing public and scientific concern. EEDs reportedly have the potential to produce widespread adverse effects through their endocrine disrupting activity, such as carcinogenicity, neurotoxicity, immunotoxicity, interference with the cardiovascular system, reproductive abnormalities, developmental toxicity, and so on (Kavlock *et al*., 1996; Witorsch, 2002). Many studies in animal models have reported that prenatal exposure to EEDs could induce birth defects (Richter *et al*., 2007; Sone *et al*., 2004), and the embryo toxic and teratogenic potential of EEDs have been of particular concern among researchers. Among the endocrine disrupting chemicals, bisphenol a (BPA, $C_{15}H_{16}O_2$), an estrogen – activity compound has received much attention. Bisphenol A (2, 2-bis (4-hydroxyphenol) propane; BPA), a chemical compound found in plastic products, is being used increasingly in industrial manufacturing materials. Numerous reports state that BPA production was 2, 214, 000 metric tons worldwide per year in 2003 and 3, 200, 000 tons in 2005 (Calafat *et al*., 2005). Because BPA is used to manufacture polycarbonate plastic, epoxy resins and certain dental sealants (Kang *et al*., 2006), humans are frequently exposed to BPA released from plastics and food cans in daily life (Richter *et al*., 2007). Therefore, through these daily exposures BPA potentially affects human health.

Estrogenic activity of BPA has been reported for over 50 years. Krishnan *et al*., (1993) reported BPA is released from autoclaved polycarbonate flasks and estrogenic activity of BPA is mediated via estrogen receptor (ER). Steinmetz *et al*., (1998) indicated that BPA induced the molecular and morphological alterations in the uterus and vagina of adult rats. Low dose of BPA *in utero* accelerated vaginal opening in mice and a large dose of BPA given neonatally induced ovary – independent vaginal epithelial changes (Honna *et al*., 2002). Previous studies through analyses of BPA in the serum of pregnant women and in cord blood collected at birth have indicated that BPA accumulates early in fetuses (Takahashi and Oishi, 2000). Developing fetus is more sensitive to estrogenic chemicals than adults in various induction of abnormalities (Iguchi, 1992). Pregnant rats were orally administered BPA at a dose of 10 mg/kg/day resulting in a decreased number of neonates and decreased survival rate (Tachibana *et al*., 2007). In this study, therefore, we investigated embryo - toxicity and/or foeto - toxicity of BPA through maternal exposure.

## MATERIALS AND METHODS

The study was carried out on healthy, young adult, colony bred pregnant female albino rats (*Rattus norvegicus)* of Charles Foster Strain. These animals weighed 195g - 200g each. They were reared in the Department's animal house at a temperature of 22 $\pm$ 2ºC and exposed to 10-12 hrs of day light. Different experimental groups of the animals were caged separately

and an average of 4 animals per cage was maintained. The control as well as other treated groups of rats was given free access to standard chow and water *ad libitum*. All these animals were housed in wooden cages and provided water in glass bottles.

**Experimental protocol**

A total of 40 animals, 30 females and 10 males were paired for mating. After 7 days of coupling, we obtained 24 sperm positive female rats. The day on which the sperms were detected in the vagina of females was considered as day '0' of pregnancy and these female rats were taken as the experimental animals. The experimental animals were divided into 2 groups, each group consisting of 12 rats, first group was maintained as control and other group as treated (BPA exposed). Bisphenol A (Batch No: 02077, Product No: 028643, Molecular formula: $C_{15}H_{16}O_2$, Molecular mass: 228.29g / mol) was used for studying embryo toxicity and foeto toxicity in rats. BPA was dissolved in few drops of alcohol and made as micro – crystalline suspension up to desired volume with olive oil. The treatment was given orally using a tuberculin syringe fitted with a cannula. A high dose of 600mg/10ml/ kg body weight of BPA was given to the treated group. The control group received olive oil only. The doses of BPA were selected after finding out the acute $LD_{50}$ value ie.4000mg / kg body weight. The treatment was given from 0 to 15$^{th}$ day of gestational period. The bodyweights of all the experimental animals were recorded every five days of interval.

On the completion of the respective experimental period, half of the pregnant animals were autopsied on the 20$^{th}$ day and the other half were allowed to complete their term and deliver their pups. From the autopsied animals, the organs were quickly excised, cleared off from the adhering fat and blotted free of blood. The absolute weights of the adrenals, kidney, spleen and liver of different experimental animal groups were recorded to the nearest milligram. The gravid uterus was dissected out from each animal, taken its weight and counted the number of implantations, corpus lutea, resorptions, malformed embryos and viable pups. The sex of the pupswere 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.

## RESULTS AND DISCUSSION

Table 1 represents body weight gain, absolute body weight gain and gravid uterine weight (g) of control and treated groups. The body weight gain showed an insignificant increase in the treated groups during the initial period of gestation *i.e.* 0 – 5$^{th}$ and 5$^{th}$ – 10$^{th}$ day when compared to control. However, on 10$^{th}$ – 15$^{th}$ and 15$^{th}$ – 20$^{th}$ day, insignificant decrease in the body weight gain was observed in the treated groups when compared to control. This decrease in the body weight gain was due to decreased gravid uterine weight and increased resorptions after BPA administration. However, the absolute body weight gain of treated female rats showed an increase on 20$^{th}$ day when compared to control. The increase in the

**Table 1: Body weight gain, absolute body weight gain and gravid uterine weight (g) of control and treated groups (n = 6)**

| Parameter | Days | Control | Treated |
|---|---|---|---|
| Body weight gain (g) | 0 – 5$^{th}$ day | 8.33 ± 1.35 | 10. 83 ± 2.74 |
| | 5 - 10$^{th}$ day | 6.66 ±1.35 | 8.33 ± 1.52 |
| | 10 - 15th day | 13.33 ± 3.59 | 8.33 ±1.52 |
| | 15 - 20$^{th}$ day | 15 ± 4.08 | 10 ± 6.12 |
| Absolute body weight gain on 20$^{th}$ day (body weight gain devoid of gravid uterus) | 20$^{th}$ day | 10.92 ± 6.71 | 14.9 ± 3.5 |
| Gravid uterine weight (g) (Uterus with embryos) | 20$^{th}$ day | 23.82 ±5.58 | *12.63 ± 5.37 |

Values are mean ± SE; Significant *p<0.05

$$*\text{Body weight gain (g)} = \frac{\text{body weight of last day - body weight of first day}}{\text{body weight of first day}}$$

$$*\text{Absolute body weight gain on 20}^{th}\text{ day} = \frac{\text{body weight of 20}^{th}\text{ day - gravid uterid weight - body weight of first day}}{\text{body weight of first day}}$$

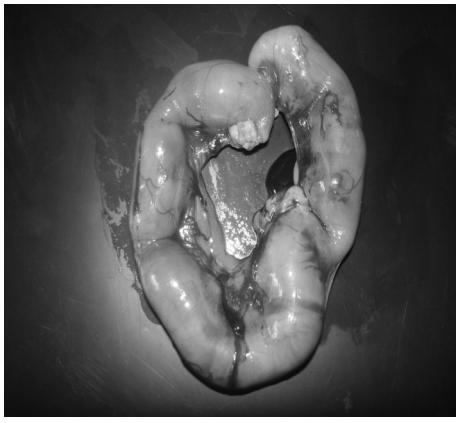

**Figure 1: Gravid uterus of control female rat.(Uterus having normal embryos)**

body weight gain during initial period of gestation and absolute body weight gain can be attributed to the general metabolism of the body which is a steroid sensitive parameter (Akingbemi *et al.*, 2004). Bisphenol A stimulated growth especially in female rats due to its estrogenic properties. Some of the earlier reports suggested that developmental exposure to BPA and other environmental chemicals with endocrine – disrupting effects is associated with obesity in mice after they reach puberty and throughout maturity (Miyawaki *et al.*, 2007). How prenatal exposure to BPA may exert lasting effects on body weight remains to be determined. Most recently Bisphenol A was shown to increase gene expression of adipogenic transcription factors in 3T3-L1 preadipocytes (Phrakonkham *et al.*, 2008). If similar actions of BPA occur *in vivo*, they would be expected to contribute to increased adiposity and increased body weight. The gravid uterine weight was significantly ($p<0.05$) decreased in treated groups (Fig. 2A and 2B) compared to control (Fig. 1) due to reduced number of viable off springs and increased resorptions after BPA exposure. These results indicate that BPA can readily cross blood placental barrier

**Table 2: Number of corpus lutea, number of implantations, implantation index and resorptions of control and treated group (n = 6)**

| Parameter | Control | | | Treated | | |
|---|---|---|---|---|---|---|
| | Right | Left | Total | Right | Left | Total |
| Number of corpus lutea | 4.66 ± 0.27 | 4.86 ± 0.27 | 9.45 ± 0.52 | 5.16 ± 0.27 | 4.33 ± 0.89 | 9.5 ± 1.14 |
| Number of implantations | 3 ± 0.81 | 4 ± 0.47 | 7 ± 0.94 | 3.16 ± 0.64 | 3.33 ± 0.68 | 6.5 ± 1.15 |
| Implantation index | 64.37 ± 1.86 | 85.83 ± 2.36 | 76.50 ± 2.09 | 62 ± 5.7 | 54 ± 6.0 | *60.6 ± 2.69 |
| Resorptions | 0 | 0.5 ± 0.3 | 0.5 ± 0.3 | 2.5 ± 0.69 | 2.66 ± 0.84 | *4.33 ± 1.45 |

Values are mean ± SE*Significant *p<0.05

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

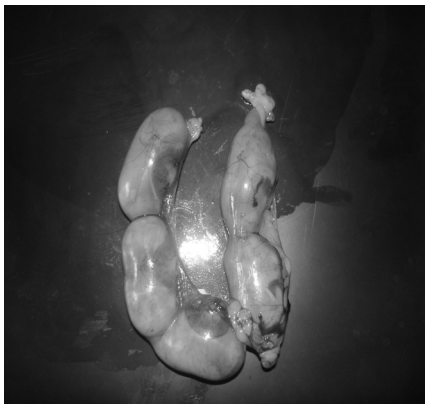

**Figure 2A: Gravid uterus of BPA exposed female rat. Uterus with reduced number of pups**

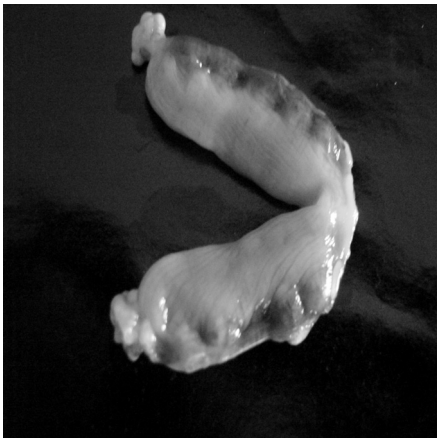

**Figure 2B: Gravid uterus of BPA exposed female rat. Resorptions were observed in uterus and no normal embryos were seen**

(Markey *et al*., 2010) and that maternal BPA exposure transferred to the fetus can cause resorptions, reduced birth outcomes, developmental abnormalities and other adverse health effects in the offspring (Somm *et al*., 2009) suggesting embryo toxicity and foetotoxicity. Observations in pregnant Fischer rats have reported that BPA, administered in a single oral dose, is able to rapidly traverse the placenta and distribute

**Table 4 : 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 | Treated |
|---|---|---|
| Mean male pup weight | 3.5 ± 0.02 | 3.6 ± 0.02 |
| Total male litter weight | 7.2 ± 2.2 | *3.7 ± 0.15 |
| Mean female pup weight | 3.4 ± 0.03 | 3.5 ± 0.02 |
| Total female litter weight | 10.3 ± 2.4 | *3.6 ± 0.03 |
| Total litter weight | 20.7 ±2.5 | *7.3 ± 1.3 |

Values are mean ± SE*Significant *p<0.05

**Table 5: The organ weight (g) of control and treated groups (n = 6)**

| Organ weight (g) | Control | Treated |
|---|---|---|
| Liver(g) | 5.99 ± 0.054 | 6.85 ± 0.142 |
| Spleen(g) | 0.246 ± 0.024 | 0.455 ± 0.024 |
| Adrenals(mg) | 0.0150 ± 0.0011 | 0.0152 ± 0.0018 |
| Kidney(g) | 5.35 ± 0.12 | 4.23 ± 0.024 |

**Table 6: The growth rate (g) of viable off springs of control and treated groups (n = 6)**

| Experimental groups | Control | Treated |
|---|---|---|
| 1st day | 5.83 ± 0.15 | *11.6 ± 1.2 |
| 5th day | 8.33 ± 0.60 | *15 ± 1.6 |
| 10th day | 16.66 ± 0.951 | 20 ± 1.4 |
| 15th day | 21.66 ± 0.95 | 26.6 ±1.35 |
| 20th day | 30 ±1.17 | 35 ±1.6 |
| 25th day | 36.66 ±1.51 | 44.57 ± 0.8 |

Values are mean ± SE *Significant *p<0.05

within fetal organs. Following a single oral dose of 1g/kg BPA to rats, the chemical was found to reach a maximal concentration within fetal organs by 20 minutes; after 40 minutes the concentration of BPA was higher in the fetus than in the maternal blood (Takahashi and Oishi, 2000).

Table 4: 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 (300 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

**Table 3: Number of malformed embryos and number of viable off springs of control and treated groups (n = 6)**

| Parameter | Control | | | Treated | | |
|---|---|---|---|---|---|---|
| | Right | Left | Total | Right | Left | Total |
| Number of viable off springs | 3.33 ± 0.72 | 3 ± 1.25 | 6.02 ± 1.44 | 1.16 ± 0.72 | 1.66 ± 0.76 | *2.3 ± 1.18 |
| Number of malformed embryos | 0 | 0 | 0 | 1 ± 0.36 | 0.8 ± 1.1 | 1.8 ± 0.38 |

Values are mean ± SE*Significant *p<0.05

**Figure 3: Malformed embryo of BPA exposed female rat. Embryo with abnormal snout morphology**

to 50 ng/mL during days 30 to 90 of gestation resulted in low birth weight in offspring (Savabieasfahani *et al.*, 2006).

Table 5 represents the organ weight (g) of control and treated groups. The organ weights didn't alter significantly in control and BPA exposed groups. Table 6 represents the growth rate (g) of viable off springs of control and treated groups. The growth rate was significantly ($p < 0.05$) increased in BPA exposed groups in all intervals when compared to control. These data confirm the advancement in the onset of puberty in off springs of BPA exposed groups. In rodents, BPA has been shown to readily traverse the placenta and to bind a -fetoprotein with negligible affinity relative to estradiol; this results in its enhanced bioavailability during neonatal development (Takahashi and Oishi, 2000). Further, it is present in the mouse fetus and amniotic fluid during maternal exposure in higher concentrations than that of maternal blood. In conclusion BPA demonstrates embryo toxicity and teratogenicity in *Rattus norvegicus*.

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