

Effects of in utero di-butyl phthalate and butyl benzyl phthalate exposure on offspring development and male reproduction of rat

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Abstract The study was conducted to assess the effects of in utero di-butyl phthalate (DBP) and butyl benzyl phthalate (BBP) exposure during late gestation on offspring's development and reproductive system of male rats. Pregnant rats were treated orally with DBP (2, 10, 50 mg/kg), BBP (4, 20, 100 mg/kg), and diethylstilbestrol (DES) 6 µg/kg (positive control) from GD14 to parturition. A significant reduction in dams' body weight on GD21 in DBP-, BBP-, and DES-treated groups was observed. The gestation length was considerably elevated in the treated groups. Decline in male pups' body weight was significant at PND75 in DBP- (50 mg/kg), BBP- (20,100 mg/kg), and DES-treated groups. The weight of most of the reproductive organs and sperm quality parameters was impaired significantly in DBP- (50 mg/kg) and BBP- (100 mg/kg) treated groups. Further, a non-significant decline in testicular spermatid count and daily sperm production was also monitored in treated groups. A significant reduction in serum testosterone level in BBP (100 mg/kg), whereas the testicular activity of 17β-HSD was declined non-significantly in the treated groups with respect to control. The data suggests that DBP and BBP exposure during late gestation period might have adverse effects on offspring's development, spermatogenesis, and steroidogenesis in adult rats.

Keywords Di-n-butyl phthalate · Butyl benzyl phthalate · Diethylstilbestrol · In utero · Testis · Gestation

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Introduction

Phthalate esters are a class of widely distributed industrial chemicals, used as plasticizers to soften the polyvinyl chloride-based products. Phthalates have become ubiquitous environmental contaminants because large-scale production, widespread use, and their high concentration in cosmetics and personal care products, toys, pharmaceuticals, medical devices, food packaging, vinyl flooring, and building materials (Hernandez-Diaz et al. 2009). Phthalates are generally lipophilic, which influences their leaching and environmental partitioning characteristics. They cannot covalently bind to the polymers matrix. Therefore, migration from the products into the environment is likely to occur (Schettler, 2006). The potential route of human exposure to phthalates is via ingestion, inhalation, and dermal contact. Phthalates and their metabolites have been detected in different human body fluids such as maternal urine (Koch et al. 2006), milk (Lottrup et al. 2006), and amniotic fluid (Silva et al. 2004). Phthalates can be transferred from maternal blood into the developing fetus via placenta (Mose et al. 2007) and into neonates via breast milk (Main et al. 2006). Thus, the possibility of these compounds entering into the biological system has generated concern about their reproductive and developmental toxic potential in humans. Reports are available on in vitro estrogenic potential of di-butyl phthalate (DBP) and butyl benzyl phthalate (BBP) in estrogen-responsive human breast cancer cells (Jobling et al. 1995; Harris et al. 1997) and in recombinant yeast screen assay (Coldham et al. 1997). Thus, exposure to these chemicals during this critical period of development might affect offspring development and function. The declining trends in semen quality reported from different parts of the world, especially from industrialized countries, suggest a possible role of some of the industrial chemicals. The declining trend in sperm quality in men during the last few decades

could be the result of several environmental factors (Miguel et al. 2006).

There are reports of the adverse effects of DBP such as pathological changes in testes and male reproductive accessory glands, hypospadias, cryptorchidism, retention of nipples, reduced anogenital distance, and reduced sperm production in animals (Duty et al. 2004; Lyche et al. 2009). Zhang et al. (2004) reported that in utero DBP exposure might cause a significant reduction in the litter size, pups body weight at birth, anogenital distance (AGD) in male pups, organ weight, and sperm quality. Further, BBP was also shown to be developmentally toxic in mice and rats (Nagao et al. 2000). BBP in utero exposure showed a decrease in size of testis and a reduction in daily sperm production in adult rats (Sharpe et al. 1995). Owing to these, this study was conducted to investigate the effects of DBP and BBP exposure during late gestation period on offspring's development and male reproductive system of rats.

Material and methods

Animals

Albino rats were obtained from the institute's animal house facility. They were maintained on standard rat diet (Amrit Feed, Pune, India) and tap water ad libitum. Virgin female rats, about 10 weeks old, were mated overnight with male of the same age. The day when sperm were detected in the vaginal smear was considered gestation day zero (GD0). Successfully mated females were divided into nine groups randomly with a minimum of six rats in each group.

Chemicals and treatment

The pregnant rats were treated orally with 20, 100, and 500 times of reference dose (RfD)* of DBP (i.e., 2, 10, and 50 mg/kg) and BBP (i.e., 4, 20, and 100 mg/kg), procured from M/s. Greyhound chromatography and allied chemicals, UK in corn oil by gavage from GD14 up to parturition. Positive control (diethylstilbestrol (DES) 6 µg/kg obtained from M/s. Sigma chemicals, USA); vehicle control (corn oil M/s. MP Biomedical, France), and control group were also maintained in similar fashion. The volume of each dose was adjusted to 1 ml/kg based on animal body weight.

Observations in dams and F1 offsprings

Pregnant rats were examined daily for obvious signs of toxicity, weight gain, and gestation length. The litter size, live and dead pups were recorded from each dam at postnatal day (PND1) (live birth index), PND4 (viability index), and PND21 (weaning index) and sex ratio (PND1) from each

dam were recorded. Pups were examined for gross external abnormalities. The physical developmental landmarks, i.e., eye opening, fur formation, pinna detachment, and testis descend were also recorded. Anogenital distance (the distance between the anus and the genital tubercle) was measured for each offspring in all the groups at PND5 and PND25.

Pups body weight was recorded on PND1, PND21, and thereafter, biweekly until necropsy (PND75). Representative number of male animals from each dam was sacrificed on PND75. Reproductive organs (viz. testes, epididymis, prostate, vas deference, and seminal vesicle) and vital organs (viz. liver, kidney, and adrenal gland) were dissected out, cleaned, and weighed using digital balance.

Sperm quality parameters

The sperm quality parameters, i.e., sperm motility (Linder et al. 1986), sperm count (Wyrobek et al. 1983; Kumar et al. 1999), testicular spermatid count (Thayer et al. 2001), daily sperm production (Robb et al. 1978), and sperm head shape abnormality (Feuston et al. 1989) were evaluated.

(RfD*, Reference dose - An estimate, with uncertainty spanning perhaps an order of magnitude, of a daily oral exposure to the human population, including sensitive subgroups that are likely to be without an appreciable risk of deleterious effects during a lifetime) (www.epa.gov/tln/atw/hlref/hapglossaryrev.html).

17 β-Hydroxy steroidhydrogenase (HSD)

For the estimation of 17β-HSD, the testis was homogenized (1:10) in chilled Tris-KCl buffer, (pH 7.4), containing 0.05 % Triton X- 100 and the homogenate was centrifuged at 8,000 RPM for 30 min at 4 °C by ultracentrifuge (Sigma, Germany). The supernatant was used for HSD estimation (Jana et al. 2006).

Serum testosterone

The serum testosterone concentration was measured using commercial available ELISA kits according to manufacturer's instructions (Testosterone Cat. N0. 582701, M/S Cayman Chemical Company, 1180 E. Ellsworth Road, USA).

Statistical analysis

Study variables of experimental animals were compared for significant differences from control. Data are expressed as mean ± SE. Statistical analysis was performed using Graph pad prism software (V. 5.01). In order to evaluate the significance level between control and exposed groups, unpaired *t* test was used.

Results

Effects of DBP and BBP exposure on dams' body weight gain and gestation length

There was no mortality recorded in any of the groups. Dams' body weight gain increased steadily from their initial body weight in all the treated and non-treated groups. However, the weight gain (percentage) was significantly lower in DBP-, BBP-, and DES-treated groups on GD21 as compared to control. The gestation length was considerably elevated in all the treated groups with respect to control (Fig. 1).

Effects of DBP and BBP exposure on rat offsprings development

No significant difference was observed in the litter size, live pups, fetal mortality rate, and sex ratio (M/F) between treated and non-treated groups. The body weight of male pups at birth and PND21 (weanling) decreased significantly in all the DBP-, BBP-, and DES-treated groups, but was non-significant in 2 mg/kg DBP-treated group at birth and their respective control (Table 1). Anogenital distance in male pups was slightly lower in both DBP- and BBP-treated groups and it was significant at PND25 in DES-treated group. The data on developmental indices, i.e., live birth, viability, and weanling indices showed slight alteration between treated and non-treated groups (Table 1). The data on the age of physical developmental landmarks (i.e., pinna detachment, fur formation, eye opening, testis descend) indicated that these landmarks were slightly delayed in higher doses of DBP, BBP, and also in DES-treated groups as compared to control (Table 2).

Body and organ weight changes in F1 adult male rats

The male rats were sacrificed on PND75; data on body and organ weight are shown in Table 3. A significant decline in body weight was observed in DBP- (50 mg/kg) BBP- (20 and 100 mg/kg), and in DES-treated groups; whereas in DBP- (2 and 10 mg/kg) and BBP- (4 mg/kg) treated groups, the alterations were statistically non-significant with respect to the control (Table 3).

The data on organ weight showed a declining trend in all the treated groups. The weight of epididymis and prostate significantly decreased in DBP- (50 mg/kg), BBP- (100 mg/kg), and in DES-treated group. The testis, adrenal gland, and seminal vesicle weight was decreased in DBP- (50 mg/kg) and in DES-treated groups. However, the kidney weight was significantly decreased in DBP- (2 and 50 mg/kg) and BBP- (100 mg/kg) treated groups. The liver weight also significantly declined in DBP- (50 mg/kg) treated group with respect to the control (Table 3).

Epididymal sperm count, motility, testicular spermatid count, and daily sperm production in F1 adult rats

The cauda epididymal sperm count and sperm motility (percentage) were significantly reduced in DBP-(50 mg/kg), BBP- (100 mg/kg), and in DES-treated groups. Further, the testicular spermatid count and daily sperm production (DSP) also showed a declining trend (statistically non-significant) in the treated groups as compared to the control (Table 4). Different types of sperm head shape abnormalities (i.e., amorphous, hook absent, bent neck, straight head, small hook, banana shape, pin head/two tailed) were observed in treated and non-treated animals using phase contrast microscope (Zeiss

Fig. 1 Dams body weight gain on GD21 and gestation length after DBP and BBP exposure. Compared to control ($*p < 0.05$; $**p < 0.001$)

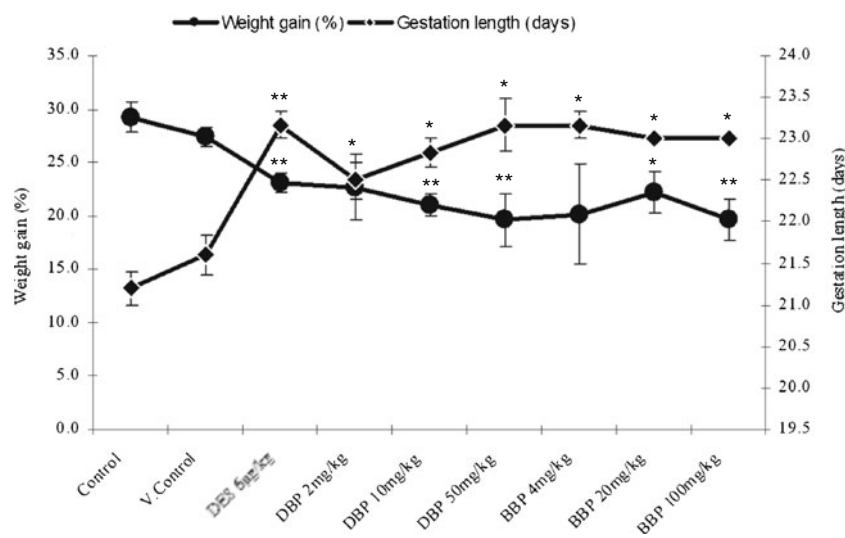


Table 1 Pregnancy outcome after DBP and BBP in utero exposure (GD14)

Findings	Control 0	V. Control 1 ml/kg	DES 6 µg/kg	DBP		BBP			
				2 mg/kg	10 mg/kg	50 mg/kg	4 mg/kg	20 mg/kg	100 mg/kg
Litter size	8.40±1.29	8.80±1.02	10.83±0.79	8.50±1.20	8.17±1.17	8.83±1.19	8.33±1.12	8.00±0.71	9.67±0.71
Live pups/litter	8.00±1.22	8.80±1.02	10.17±0.75	8.00±1.21	7.83±1.30	8.17±1.01	8.17±1.19	7.40±0.93	8.83±0.40
Fetal mortality (%)	4.00±4.0	0.00±0.00	5.81±2.98	6.57±3.33	7.41±5.49	6.24±3.26	2.78±2.78	8.21±5.64	7.47±3.53
Sex ratio (M/F)	0.47:0.53	0.53:0.47	0.54:0.40	0.41:0.59	0.43:0.57	0.51:0.49	0.52:0.48	0.55:0.45	0.59:0.41
L.B.I (%) PND1	96.00±4.0	100.0±0.0	94.19±2.98	93.43±3.33	92.59±5.49	93.76±3.26	97.22±2.78	91.79±5.64	92.53±3.53
V.I (%) PND4	94.00±6.0	100.0±0.0	94.19±2.98	93.43±3.33	90.93±5.29	93.76±3.26	93.17±3.17	89.79±5.25	89.19±3.69
W.I (%) PND21	94.00±6.0	100.0±0.0	94.19±2.98	93.43±3.33	90.93±5.29	92.37±3.70	80.48±8.01	81.21±13.4	89.19±3.69
AGD-M (PND5)	0.51±0.02	0.49±0.01	0.48±0.01	0.49±0.02	0.47±0.01	0.48±0.01	0.48±0.01	0.48±0.01	0.47±0.01
AGD-M (PND25)	1.74±0.01	1.73±0.01	1.66±0.01*	1.72±0.01	1.72±0.01	1.72±0.02	1.73±0.01	1.72±0.01	1.71±0.01
Pups wt. M (gm)									
PND1	6.02±0.07	5.97±0.05	5.86±0.03*	5.94±0.12	5.70±0.11*	5.87±0.04*	5.82±0.05*	5.78±0.06*	5.71±0.08*
PND21	32.42±0.93	32.33±0.66	30.72±0.61	30.43±0.46*	27.15±1.25*	28.04±0.55*	28.22±0.56*	25.23±0.60**	27.22±0.42*

Values are expressed in mean ± SE

LBI live birth index, VI viability index, WI Weanling index, AGD-M anogenital distance-male, V vehicle

**p*<0.05 compared to control

Table 2 Age (days) of developmental landmarks in F1 offsprings after DBP and BBP exposure

Treatment	Pinna detachment	Fur formation	Eye opening	Testis descend
Control	4.60±0.24	5.60±0.24	12.40±0.24	21.40±0.24
V. Control	4.60±0.24	5.40±0.24	12.60±0.40	21.40±0.24
DES 6 µg/kg	4.83±0.17	5.83±0.31	13.00±0.26	21.81±0.31
DBP 2 mg/kg	4.67±0.21	5.67±0.33	12.50±0.22	21.50±0.34
DBP 10 mg/kg	4.83±0.17	5.50±0.22	12.50±0.22	21.67±0.49
DBP 50 mg/kg	5.00±0.26	5.83±0.31	12.83±0.31	21.83±0.40
BBP 4 mg/kg	4.50±0.22	5.50±0.22	12.33±0.21	21.50±0.22
BBP 20 mg/kg	4.60±0.24	5.60±0.24	12.40±0.24	21.60±0.40
BBP100 mg/kg	4.83±0.31	5.67±0.21	12.50±0.22	21.33±0.21

Values are expressed in mean ± SE

CAXIO-Scope A1, 10X). These abnormalities were higher in treated groups with respect to control. The percentage of sperm abnormalities elevated significantly in DBP- (50 mg/kg), BBP- (100 mg/kg) and DES-treated groups as compared to control (Table 4).

Effects of DBP and BBP exposure on testicular 17β-Hydroxysteroid dehydrogenase activity

DBP and BBP exposure showed a declining trend in the testicular activity of steroidogenic enzyme (i.e., 17β-HSD) in all the DBP-, BBP-, and DES-treated groups. But, the alteration was more pronounced (statistically non-significant) in BBP-treated groups as compared to DBP-treated groups (Fig. 2).

Effects of DBP and BBP exposure on serum testosterone levels in F1 adult rats

The data on serum testosterone revealed that the testosterone levels were decreased in all the DBP-, BBP-, and DES-treated groups, but it was significantly declined in BBP higher dose (100 mg/kg) treated group with respect to the control (Fig. 3). However, at a dose of 2 mg/kg did not reveal any significant changes.

Discussion

The study indicated that DBP and BBP exposure during late gestation interferes in the development of offsprings as evidenced by a reduction in the body weight of F1 male rats, and might have also affected the male reproductive system of adult rats as observed by reduction in reproductive organs weight, deterioration in sperm quality parameters and also in testosterone levels. However, no marked difference in developmental landmarks and indices were found between DBP-, BBP-treated, and non-treated groups. Ema and

Miyawaki (2002) reported that BBP at 500 and 1,000 mg/kg significantly decreased the maternal (body weight gain) and food consumption. Dams treated with BBP 300, 600, and 900 mg/kg from GD 8–18, showed a significant reduction in body weight. DBP at 50 mg/kg also caused a reduction in dams body weight gain (Howdeshell et al. 2008). The present study also indicated that dams exposed to DBP and BBP at late gestation may have adverse effect on body weight gain and developing fetus.

The gestation length was significantly elevated in all DBP, BBP, and DES-treated groups. A recent study on di-n-hexyl phthalate exposure during GD6–20, showed an increase in the gestation length (Saillenfait et al. 2009). Exposure to DBP (GD14–18 at 750 mg/kg) also delayed the gestation period in rats. Wistar rats treated with 1 % of DBP in the diet from GD6 to PND28 prolonged the gestation length and decreased the body weight of male and female rats (Zhu et al. 2009). The results on litter size and live pups/litter are in accordance with the findings of Nagao et al. (2000). They reported that chronic BBP exposure at 20, 100, and 500 mg/kg showed no marked alteration in the litter size, live pups, and sex ratio. DBP exposure (GD1–PND21) at 50 and 250 mg/kg showed minor variations in litter size but at 500 mg/kg, it was significantly reduced (Zhang et al. 2004). In the present study, DBP and BBP exposure slightly increased the rate of fetal mortality. Howdeshell et al. (2008) also showed an increase in fetal mortality in a dose-dependent manner in DBP- and BBP-treated groups. The data on developmental landmarks (i.e., pinna detachment, fur formation, eye opening, and testes descend) and indices (i.e., live birth, viability, and weanling index) did not show any significant alteration. No marked difference in the age of pinna detachment, eye opening, or incisor eruption in the pups' of DBP-treated groups was also reported (Li et al. 2009). However, Nagao et al. (2000) reported that the chronic BBP exposure at

Table 3 Effects of DBP and BBP exposure on body and organ weight (gm) of F1 adult male rats

Findings	Control 0	V. control 1 ml/kg	DES 6 µg/kg	DBP			BBP		
				2 mg/kg	10 mg/kg	50 mg/kg	4 mg/kg	20 mg/kg	100 mg/kg
Body weight	212.30±1.36	210.2±1.10	199.63±1.65**	209.07±1.45	208.79±1.49	202.33±1.53**	209.82±1.07	207.38±1.37*	203.03±1.56**
Epididymis	0.304±0.013	0.301±0.005	0.263±0.009*	0.298±0.004	0.293±0.006	0.267±0.007*	0.295±0.006	0.291±0.004	0.264±0.004*
Testis	1.265±0.009	1.263±0.011	1.202±0.007*	1.262±0.005	1.258±0.014	1.230±0.008*	1.251±0.008	1.257±0.006	1.238±0.020
Prostate	0.251±0.008	0.249±0.005	0.200±0.004**	0.248±0.012	0.245±0.009	0.213±0.006*	0.245±0.014	0.245±0.008	0.221±0.005*
Seminal vesicle	0.752±0.032	0.748±0.013	0.666±0.027	0.744±0.020	0.743±0.017	0.658±0.027*	0.743±0.020	0.748±0.011	0.668±0.028
Adrenal gland	0.022±0.001	0.022±0.0005	0.019±0.001*	0.022±0.001	0.021±0.001	0.019±0.001*	0.021±0.001	0.021±0.001	0.021±0.001
Liver	7.57±0.202	7.35±0.123	7.18±0.179	7.33±0.156	7.31±0.212	7.02±0.174*	7.35±0.097	7.33±0.016	7.11±0.225
Kidney	0.783±0.009	0.778±0.011	0.770±0.009	0.748±0.012*	0.772±0.018	0.729±0.014*	0.772±0.016	0.772±0.010	0.700±0.012**

Values are expressed in mean ± SE

p*<0.05; *p*<0.001 compared to control

20 and 100 mg/kg significantly delayed the pinna opening or eyelid opening in male and female pups. Further, Ema et al. (2000) found that the duration of testis descent significantly increased in DBP 1,500 mg/kg treated group. The difference in the time of testis descent in the present study might be due to different doses used in both the studies. The results on viability index and weanling index, is in agreement of the study of Giribabu et al. (2012). They observed that DBP exposure caused a slight reduction in pups' survival. However, Zhang et al. (2004) reported that DBP exposure at 50 and 250 mg/kg showed no marked difference in pups' survival on PND1 while it was significantly reduced in 500 mg/kg treated group.

AGD is a developmental landmark for the differentiation of the external genitalia and is commonly used as hormonally sensitive parameter of sex differentiation in rodent. Masculinization of external genitalia in males is controlled by dihydrotestosterone, a metabolite of testosterone (Imperato-Mcginley et al. 1985; Clark et al. 1990). In the present study, DBP and BBP exposure slightly reduced the AGD at PND5 and PND25 in male pups. Earlier studies reported that BBP at 20 and 100 mg/kg showed no change in AGD at PND0 in male pups but it was significantly reduced at 500 mg/kg (Nagao et al. 2000). Exposure to DBP resulted in decreased AGD in male pups at PND4 which was statistically significant in 250 and 500 mg/kg treated groups (Zhang et al. 2004). Ema and Miyawaki (2002) also showed that BBP exposure at 250 mg/kg (GD15–17) slightly reduced the AGD while at BBP 500 and 1,000 mg/kg caused a significant reduction in the AGD of male fetuses. The data on pups' body weight revealed that it was lower at PND1 and PND21 in all the treated groups, and it was significant in some of the DBP- and BBP-treated groups at PND75. Recently, Liu et al. (2012) reported that DBP exposure (GD14–18 at 750 mg/kg) caused a significant reduction in body weight of male fetuses at PND. Similarly, Giribabu et al. (2012) showed that DBP in utero exposure caused a reduction in the weight of fetuses at birth. Gray et al. (2000) also suggested that di(2-ethylhexyl)phthalate (DEHP) and BBP exposure (GD14 - PND3 at 750 mg/kg) significantly decreased the pups' body weight at birth. Later, Zhang et al. (2004) also reported that exposure of DBP at 250 and 500 mg/kg to pregnant rat caused a dose related reduction in pup's body weight at birth. Ema et al. (1998) showed a significant reduction in live fetus weight in BBP 500, 750, and 1,000 mg/kg (GD0-8) treated groups. These, coupled with the data obtained, suggest that prenatal DBP and BBP exposure affects the fetus development.

Table 4 Effects of DBP and BBP exposure on sperm quality parameters of F1 adult rats

Treatment	Sperm count ($10^6/\text{ml}$)	Motile sperm (%)	Spermatid count ($10^6/\text{g tissue}$)	DSP ($10^6/\text{g tissue}$)	Sperm abnormalities (%)
Control	35.25±0.96	83.86±1.42	82.47±4.9	13.52±0.81	1.12±0.22
V. Control	34.35±0.68	82.00±1.41	81.59±4.7	13.38±0.78	1.14±0.14
DES 6 $\mu\text{g}/\text{kg}$	31.65±0.61*	78.14±1.33*	77.84±4.0	12.76±0.66	1.68±0.13*
DBP 2 mg/kg	33.80±0.88	81.06±1.47	80.88±3.4	13.26±0.56	1.38±0.17
DBP 10 mg/kg	33.20±0.70	80.99±1.60	79.95±5.6	13.11±0.92	1.39±0.17
DBP 50 mg/kg	31.70±0.41*	79.42±0.92*	79.49±3.4	13.03±0.56	1.66±0.11*
BBP 4 mg/kg	33.15±1.00	80.94±1.72	81.14±5.4	13.30±0.90	1.54±0.14
BBP 20 mg/kg	32.60±1.35	81.43±2.10	80.73±5.2	13.23±0.85	1.47±0.11
BBP 100 mg/kg	31.83±0.95*	78.80±1.34*	78.77±3.5	12.91±0.58	1.72±0.13*

Values are expressed in mean \pm SE

DSP daily sperm production

* $p < 0.05$ compared to control

In utero DBP exposure produced several abnormal responses in male reproductive organs and these effects may be due to disruption of the stage-specific expression of genes related to androgen-dependent organs development (Kim et al. 2010). The weight of reproductive organs (i.e., epididymis, testis, prostate, and seminal vesicle) and vital organs (i.e., liver, kidney, and adrenal gland) was found to be lower in DBP (50 mg/kg) and BBP (100 mg/kg) higher dose-treated groups after prenatal exposure. Gray et al. (2000) showed that DEHP and BBP in utero exposure (GD14–PND3) caused a significant reduction in testis, seminal vesicle, prostate, and epididymis weight but the weight of liver, kidney, and adrenal gland reduced non-significantly. Nagao et al. (2000) reported that BBP exposure at 500 mg/kg significantly decreased the weight of epididymis, testis, and ventral prostate.

Further, DBP at 500 mg/kg (GD1–PND21) caused a significant reduction in epididymis, liver, and kidney weight while prostate weight decreased non-significantly in DBP 250 mg/kg treated group.

Experimental studies showed a decrease in sperm production and increase in morphological abnormalities following prenatal and/or neonatal exposure to DBP in rodents (Wang et al. 2005; Mahood et al. 2007; Auharek et al. 2010). Earlier, Jeng and Yu (2008) also reported that phthalates exposure might cause a significant reduction in sperm motility and daily sperm production in rats. In our study, sperm count and motility showed a decrease while sperm head shape abnormalities increased significantly in DBP (50 mg/kg) and BBP (100 mg/kg) higher dose-treated groups (Fig. 4). A declining trend was also observed in testicular spermatid count and DSP in the treated groups. These data lends

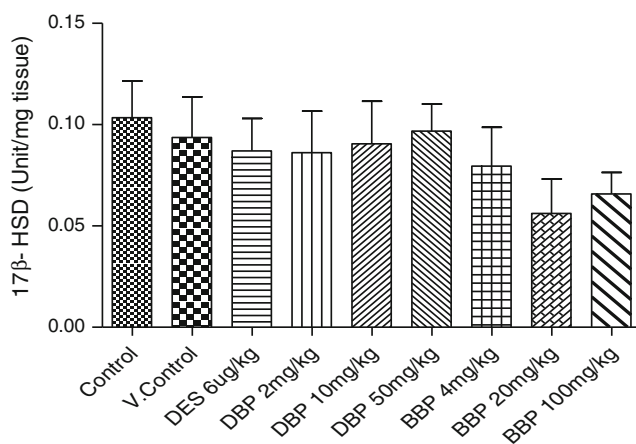


Fig. 2 Effects of DBP and BBP exposure on testicular $17\beta\text{-HSD}$ activity in F1 adult rats; V vehicle

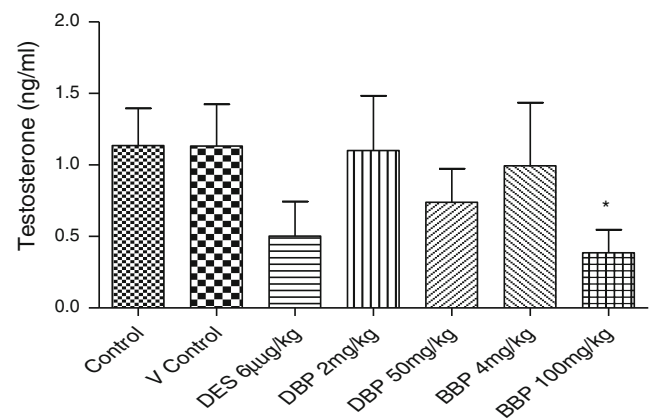


Fig. 3 Effect of DBP and BBP exposure on serum testosterone levels in F1 adult rats; compared to control (* $p < 0.05$)

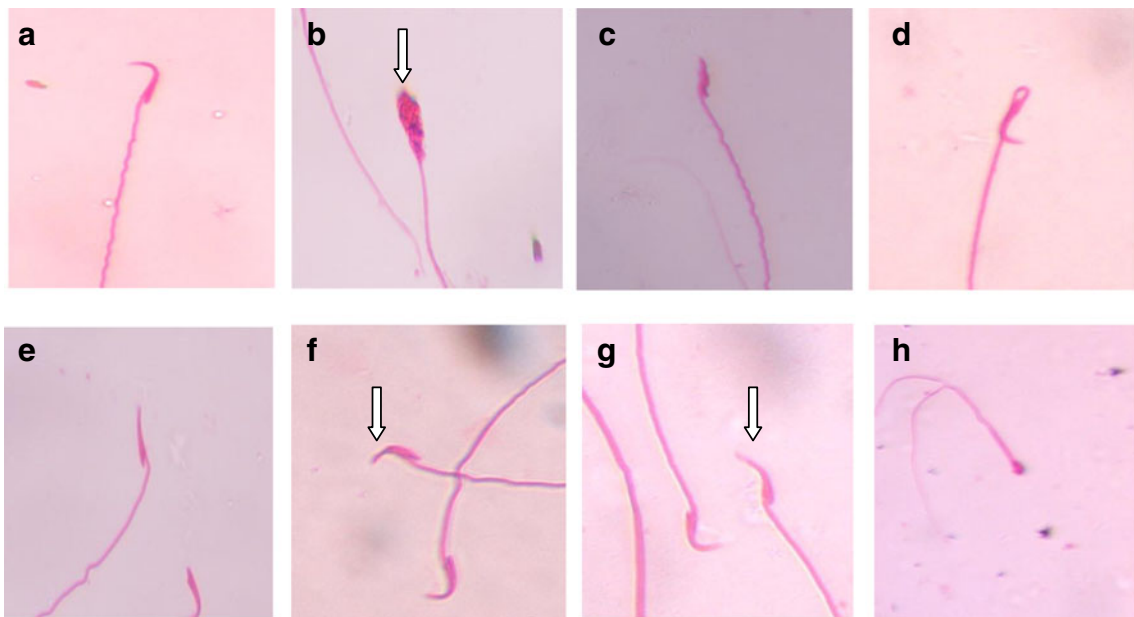


Fig. 4 Rat sperm head shape abnormalities after DBP, BBP, and DES exposure, i.e., **a** Normal sperm. **b** Amorphous. **c** Hook absent. **d** Bent neck. **e** Straight head. **f** Small hook. **g** Banana shape. **h** Pin head/two tailed

support to the notion that DBP and BBP exposure affects the programming of spermatogenesis, resulting in lower sperm density and inferior sperm quality at adulthood. Recently, Giribabu et al. (2012) reported that DBP exposure from GD1, 7, and 14 caused a significant reduction in sperm count, motility, viability, hypoosmotic swelling coil tailed, and increase in abnormal sperm number. It is known that testicular 17β-HSD is a gonadotropin-dependent enzyme (Murono and Payne, 1979), thus 17β-HSD activities might be dependent upon the gonadotropin secretion. Earlier, Steinberger (1971) reported that the increased and decreased in the activity of 17β-HSD is due to the increase and decrease in pituitary gonadotropin secretion, as LH plays an important role in testicular androgenesis. In the present study, the testicular activity of 17β-HSD enzymes declined in all the DBP-, BBP-, and DES-treated groups; this might be due to the reduction in gonadotropin secretion. Recently, Giribabu et al. (2012) also reported that prenatal DBP exposure caused a significant reduction in the testicular activity of 3 and 17β-HSD. In the present study, DBP, BBP, and DES exposure also caused a reduction in serum testosterone levels. These results coincide with previous studies, which showed decreased serum testosterone levels and impairment of steroid hormone metabolism after DBP exposure (Hirosawa et al. 2006; Xiao-feng et al. 2009). The data on serum testosterone is also supported by the Lehmann et al. (2004) study, which showed decrease in testosterone level after prenatal DBP exposure. Xiao-

feng et al. (2009) suggested that DBP inhibits the testosterone production through a glucocorticoid-mediated pathway in pre-pubertal male rats. The present result also corroborates the observation of Kim et al. (2010). They reported that DBP exposure from GD 10–19, caused a reduction in dihydrotestosterone and testosterone level in F1 male rats. The observed reduction in serum testosterone level in DBP- and BBP-exposed rats might be responsible for alterations in the reproductive functions. It is known that inside the Sertoli cells, testosterone is selectively bound to the androgen receptor and activation of receptor will result in initiation and maintenance of spermatogenic process and inhibition of germ cell apoptosis (Dohle et al. 2003).

Conclusion

Prenatal exposure to DBP and BBP at late gestation might have adverse effects on offspring development and reproductive health, as evidenced by decline in offspring’s body and tissues weight and impairment in spermatogenesis and steroidogenesis.

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