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Impact of low-dose chronic exposure to bisphenol A and its analogue bisphenol B, bisphenol F and bisphenol S on hypothalamo-pituitary-testicular activities in adult rats: A focus on the possible hormonal mode of action

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ABSTRACT

Bisphenol A an estrogen-mimic endocrine disrupting chemical, used to manufacture polycarbonate plastics and epoxy resins with toxic effects for male reproduction. Due to its toxicity, industries have started to replace it with other bisphenols. In this study, the toxicity of BPA analogues (BPB, BPF and BPS) was evaluated in a chronic study. We investigated whether the chronic exposure to low bisphenols doses affects spermatogenesis with outcomes on oxidative stress and male reproductive system. Male rats (22 day old) were exposed to water containing 0.1% ethanol for control or different concentrations of BPA and its analogues BPB, BPF and BPS (5, 25 and 50 μg/L) in drinking water for 48 weeks. Results of the present study showed a significant alteration in the gonadosomatic index (GSI) and relative reproductive organs weights. Oxidative stress in the testis was significantly elevated while sperm motility, Daily sperm production (DSP) and number of sperm in epididymis were reduced. Plasma testosterone, LH and FSH concentrations were reduced and estradiol levels were high in 50 μg/L exposed group. These results suggest that exposure to BPA and its analogues for chronic duration can induce structural changes in testicular tissue and endocrine alterations in the male reproductive system.

1. Introduction

Plasticizer such as bisphenol A (BPA) is an environmental pollutant detected in wildlife, humans samples and environment (Corrales et al., 2015). BPA exposure is associated with many human diseases and is suspected to affect many body's physiological functions (Chen et al., 2016a; Chevalier and Fénichel, 2015; Seachrist et al., 2016). Having several concerns for a safer world of BPA there have been several alternatives of BPA introduced into environment known as BPA analogues (Chen et al., 2016a). Bisphenol B (BPB), bisphenol F (BPF) and bisphenol S (BPS) are BPA alternatives which are used for the production of Plastics, epoxy resins, polycarbonates for lining large food containers, water pipes and coatings of Food containers, dyes, paper products and food packaging materials (Chen et al., 2016a; Danzl et al., 2009; Eladak et al., 2015; Goodson et al., 2002; Kinch et al., 2015; Rochester and Bolden, 2015; Yang et al., 2014). BPA analogues have increased concerns regarding emerging environmental pollutants where some of these analogues are detected in concentrations higher than BPA (Caballero-Casero et al., 2016; Chen et al., 2016a). For example, in a study from Italy the concentrations of BPB were higher than BPA in serum samples of healthy women and endometriotic women (Caballero-Casero et al., 2016). Similarly, in another study from Saudi Arabia in the urine of general population the concentrations of both BPS and BPF were higher than BPA (Chen et al., 2016a). In another study food products sold in New York and Albany were analyzed and 75% were detected with bisphenols measurable amounts (Liao and Kannan, 2013). BPS and BPF have been identified up to detectable amounts in food items and paper products (Goldinger et al., 2015; Liao and Kannan, 2014b; Russo et al., 2017). Across the Globe several studies have shown detectable amounts of BPA analogues in the urinary samples, umbilical cord samples and maternal samples (Asimakopoulos et al., 2016; Heffernan et al., 2016; Liu et al., 2017; Lu et al., 2016; Ye

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et al., 2015). BPA and its analogues observed in in vitro studies induced a number of physiological changes in cell lines of red blood cells, pre-adipocytes and testis (Boucher et al., 2016; Desdoits-Lethimonier et al., 2017; Maćczak et al., 2017; Mokra et al., 2017). Studies on rodents show that BPA analogues affects hormone concentrations, testis function, sperm production and sperm DNA damage (Castro et al., 2013; Li et al., 2016; Oliveira et al., 2017; Shi et al., 2017). Many studies of bisphenol A analogues suggest that these chemicals have greater neuroendocrine disruptive effects as BPA where they lead to complex behavioral changes in rodent species (Catanese and Vandenberg, 2016; Kim et al., 2015; Ohtani et al., 2017; Rosenfeld, 2017). Where, these chemicals also affect the gene expression in hypothalamus and other brain areas (Cano-Nicolau et al., 2016; Huang et al., 2016; Qiu et al., 2015, 2018; Zhang et al., 2017, 2018). BPA analogues have also been studied to induce hormonal imbalance in E2 synthesis, thyroid hormone production and testosterone levels (Cano-Nicolau et al., 2016; Kwon et al., 2016; Le Fol et al., 2017; Li et al., 2016).

In vitro and in vivo studies regarding BPA analogues are scare and limited data have shown that these chemicals have reproductive toxicity (Chen et al., 2016a; Naderi et al., 2014). These chemicals also have endocrine disrupting actions in vivo studies and are also estrogenic in nature (Kitamura et al., 2005; Rosenmai et al., 2014; Yamasaki et al., 2004). BPB, BPF and BPS are considered as alternatives to BPA and it is important to understand that whether these compounds are similar or more potent in endocrine disrupting activity than BPA.

In summary the current study provides information about the so called safer alternatives to BPA which have shown similar endocrine disturbances as BPA in animal studies. Most of these disturbances are either steroid or non-steroid pathways. In current study we reported that low concentration of these compounds for a long period of time can impair spermatogenic output and cause changes in the normal spermatogenesis in male rats. The hormonal levels were also altered which suggest that PBA analogues like BPB, BPF and BPS have endocrine disrupting properties by affecting the male reproductive functions in Sprague Dawley rats.

2. Material and methods

2.1. Animals

Male healthy rats (n = 91), weighing (30–40 g) were separated from their mothers on postnatal day 22 (PND 22) and were randomly divided into thirteen groups. Animals were kept in steel cages (7animals/cage) at temperature 22–25 °C and controlled light and dark cycle of 14–10 h light/dark. Animals were fed with laboratory feed (soy and alfalfa free) and water in poly sulfone bottles. All the experimental protocols were approved by the ethical committee of the department of Animal Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

2.2. Experimental design

From PND 23, animals (n = 91) were allocated into thirteen different groups. First served as control and was provided with water containing (0.1% ethanol), while 2nd, 3rd and 4th groups were served with water containing 5, 25 and 50 μg/L BPA respectively. While 5th, 6th and 7th groups were served with water containing 5, 25 and 50 μg/L of BPB. Similarly, 8th, 9th and 10th groups were served with water containing 5, 25 and 50 μg/L of BPF and BPS was also given in water to 11th, 12th and 13th groups at a concentration of 5, 25 and 50 μg/L. All the bisphenols were dissolved in ethanol and the stock solution was diluted with water (final concentration of ethanol in the water was kept below 0.1%). Animals were provided with water alone or water with different concentrations of BPA, BPB, BPF and BPS for the period of 48 weeks. The duration of the exposure was selected according to the OECD test guideline 452 and the doses were selected on the basis of previous studies by (Ji et al., 2013) and (Chen et al., 2017). The BPA, BPB, BPF and BPS solutions in the water bottles was daily replaced with fresh solutions.

After the completion of the experimental period, animals were weighed, and seven animals per group were euthanized by cervical dislocation. Blood was collected from heart through cardiac puncture in heparinized syringes and was subjected to centrifugation at 3000 rpm for 15 min. Plasma was isolated and kept at −20 °C for hormonal assay. Reproductive organs (testis, epididymis, seminal vesicle and prostate) were dissected out and weighed for calculation of gonadosomatic index (GSI) and relative organs weight. Right epididymis and right testis were used for histology while left testis was used for DSP and biochemical analysis. Left epididymis was used for determination of sperm viability, motility and sperm count in the epididymis.

2.3. GSI and relative weight of organs

GSI is an important parameter used for estimation of gonadal maturity in the animals. GSI was obtained for each animal according to the formula used by Barber and Blake (2006).

$$\text{GSI} = \frac{\text{Gonadal weight (g)}}{\text{Body organs weight (g)}} \times 100$$

Relative weight of the organs was determined according to the following formula

$$\text{Relative organ weight} \frac{\text{Organ weight (mg)}}{\text{Body weight (g)}}$$

Relative weights of the organs were expressed as mg/g body weight.

2.4. Biochemical assays

2.4.1. Antioxidant enzymes

Tissues were collected and were processed for the antioxidant enzymes. Tissues were homogenized with automatic homogenizer in phosphate buffer saline and centrifuged at 30,000 g for 30 min. After the centrifugation the supernatant was removed and used for the hormonal analysis, protein estimation and antioxidant enzymes.

2.4.2. Catalase (CAT)

The catalase activity was determined by the method used by (Aebi, 1984) and the change in the absorbance due to H2O2 was measured in the testicular tissues. In this assay 50 ml homogenate was diluted in 2 ml of phosphate buffer with pH of 7.0. After mixing it thoroughly the absorbance was read at 240 nm with an interval of 15 s and 30 s. Change in the absorbance of 0.01 as unit/min was defined as one unit of CAT.

2.4.3. Super-oxidase (SOD)

Superoxide dismutase activity was estimated by the method developed by (Kakkar et al., 1984). In this assay the amount of chromogen formed was measured at 560 nm. The results were expressed in units/mg of protein.

2.4.4. Peroxidase (POD)

POD activity in homogenate was determined by spectrophotometric method of (Carlberg and Mannervik, 1975). In this assay 0.1 ml homogenate was mixed with 0.1 ml of guaiacol, 0.3 ml of H2O2 and 2.5 ml of phosphate buffer and the absorbance was read at 470 nm. Change in the absorbance of 0.01 as unit/min was defined as one unit of POD.

2.4.5. Lipid per oxidation by (TBARS)

Activity of T-BARS was determined in the homogenate by the method used by (Iqbal et al., 1996) and the results were expressed as TBARS/min/ml of plasma. In this assay 0.1 ml of homogenate was

mixed with 0.29 ml phosphate buffer, 0.1 ml of trichloroacectic acid, 1 ml of trichlorobarbituric acid followed by heating at 95 °C for 20 min and then shifted to ice bath before centrifuging at 2500 rpm for 10 min. The samples were read the help of spectrophotometer at 535 nm.

2.4.6. Reactive oxygen species (ROS)

The assay of reactive oxygen species (ROS) was done according to the method of (Hayashi et al., 2007) and for the presentation of mean values the assay was repeated multiple times. In this assay 5 ml of H2O2 standards and homogenate was mixed with 140 ml of sodium acetate buffer with pH 4.8 in 96 wells plate and incubated at 37 °C for 5 min. After the incubation 100 ml of DEPPD and ferrous sulphate mix sample was added in each well with a ratio of 1:25 and were incubated at 37 °C for 1 min. With an interval of 15 s for 3 min the absorbance was read at 505 nm at micro plate reader.

2.4.7. Total protein content

AMEDA Laboratory diagnostic kit was used for the determination of total protein in tissue. The results of protein were measured by plotting absorbance of the standard against samples. These values were expressed as mg/g of tissue.

2.5. Sperm motility and viability

Immediately after dissection, the cauda epididymis was cut slightly with a scissor in 0.5 ml pre-warmed (at 37 °C) phosphate buffered saline (pH 7.3) containing a drop of nigrosine stain. An aliquot of 50 µL was taken, placed on a pre-cleaned and warmed (at 37 °C) glass slide and was observed under a light microscope at 40X. A total of 100 sperm/sample were analyzed for motility by a technician blinded to the treatment groups. Each sample was analyzed three times and the average values were used as the total sperm motility. For viability, a drop of eosin and nigrosine was added to the sperm sample. A volume of 10 µL was placed on a pre warmed and cleaned glass slide and observed under a microscope at 100 X. Ten fields were analyzed by a person blinded to the treatment groups. A total of 100 sperm/field were checked for eosin staining and numbers of live and dead sperm were estimated. Each sample was repeated three times and average number was reported and expressed as percentage of live sperm.

2.6. Tissue histology

Testicular tissues (Testes and Epididymes) were fixed in formalin for 48 h. Dehydrated with different grades of Alcohol and cleared with help of xylene the paraffin sections (5 µm) were cut and stained with hematoxylin and eosin to assess standard histology and morphometry according to (Ullah et al., 2018). Testicular sections from 10 to 20 per group were digitized under Leica Microscope (New York Microscope company) equipped with digital camera (Canon, Japan).

For the morphometry the images were taken at 20x and 40x and the results were done with Image J software. Area of different sections was calculated with the method of (Jensen, 2013). From 20x images 30 picture per animal were selected and known area of different area of intestinal space, epididymis tubules and seminiferous tubules was measured by the software. Number of different cell types (spermatids, spermatogonia and spermatocytes) and area was calculated and comparison of different groups with control was done.

2.7. Sperm count and daily sperm production

Daily sperm production was done in the testicular tissues, with the help of rotostaor homogenizer (IKA-Werke, Staufen, Germany) the thawed samples were homogenized in 5 ml of solution which contained 0.5% NaCl and 5% triton X-100. The homogenized sample was diluted and samples were transpired to a neubar chamber and 19th stage spermatids were counted under microscope at 40X. Sperm count was done in the testicular tissues as the obtained values by the sperm count in the testes were divided by 6.3 (number of days the spermatids remain in seminiferous epithelium).

2.8. Hormonal analysis

Plasma testosterone and estrogen were determined by Enzymes linked immune sorbent assay (ELISA) kit purchased from Amgenix Inc. USA, while LH and FSH in plasma were determined by ELISA kits purchased from Reddot biotech.

2.9. Statistical analysis

Dunnet's multiple comparison test which followed (ANOVA) was used for the comparison of different groups with control using Graph Pad Prism software (version 5). Values were expressed as Mean ± SEM and were considered significant at $P < 0.05$.

3. Results

3.1. Effect of chronic exposure of different concentrations of BPA and its analogues BPB, BPF and BPS (5, 25 and 50 µg/L) on initial and final body weight and body weight gain of male rats

Initial body weight, final body weight and body weight gain of the control animals and exposed group of different concentrations of BPA and its analogues BPB, BPF, BPS is presented in Table 1. At the start of the experiment all the animals were approximately of the same body weight, however, at the completion of the experiment the body weight of 50 µg/L BPA and its analogues BPB, BPF and BPS exposed groups were significantly high ($P < 0.05$) than control. On the other hand there was no significant difference observed in the final body weight of other treated groups with BPA and it analogues BPB, BPF and BPS when compared to the control. However, the body weight gain was also comparable to the control in the end of the 48 weeks experiment (Table 1).

3.2. Effect of chronic exposure of different concentrations of BPA and its analogues BPB, BPF and BPS (5, 25 and 50 µg/L) on final body weight, GSI and absolute and relative weights of reproductive organs of male rats

Absolute and relative reproductive organs weight, GSI and body weight is represented in Table 2. Significant increase was observed in BPA, BPB, BPF and BPS 50 µg/L ($P < 0.05$) when compared to the

Table 1
Effect of chronic exposure of different concentrations of BPA and its analogues BPB, BPF and BPS (5, 25 and 50 µg/L) on body weight gain of male rats.

Groups	Parameters		
	Initial Body weight (g)	Final Body Weight (g)	Body weight gain
Control	30.63 ± 0.38	541.11 ± 2.02	510.37 ± 2.25
BPA 5 µg/L	32.01 ± 0.31	537.81 ± 1.24	505.81 ± 0.96
BPA 25 µg/L	31.41 ± 0.50	538.40 ± 0.40	507.11 ± 0.44
BPA 50 µg/L	32.41 ± 0.40	549.40 ± 2.65*	517.11 ± 2.30
BPB 5 µg/L	31.98 ± 0.54	535.10 ± 1.44	503.018 ± 1.66
BPB 25 µg/L	31.41 ± 0.74	537.60 ± 1.02	506.21 ± 1.68
BPB 50 µg/L	32.61 ± 0.75	548.60 ± 1.83*	516.11 ± 2.09
BPF 5 µg/L	31.83 ± 0.95	537.80 ± 1.24	505.97 ± 1.12
BPF 25 µg/L	32.54 ± 0.86	538.40 ± 0.40	508.46 ± 1.20
BPF 50 µg/L	32.61 ± 0.67	548.20 ± 2.69*	515.61 ± 2.74
BPS 5 µg/L	32.61 ± 0.92	540.20 ± 2.35	506.41 ± 1.83
BPS 25 µg/L	33.03 ± 0.94	538.60 ± 0.50	507.77 ± 1.01
BPS 50 µg/L	33.26 ± 0.93	548.80 ± 2.28*	515.53 ± 2.98

Values are presented as Mean ± SEM.
*: Indicate significance at $p < 0.05$ vs control.

Table 2
Effect of chronic exposure of different concentrations of BPA and its analogues BPB, BPF and BPS (5, 25 and 50 μg/L) on body and organs weight of male rats.

Groups	Parameters				
	Final body weight (g)	Paired testis (g)	GSI	Absolute Paired Epididymis (g)	Relative epididymis weight (mg/g)
Control	541.11	3.68 ± 0.08	0.69 ± 0.03	1.44 ± 0.03	2.65 ± 0.03
BPA 5 μg/L	537.82	3.54 ± 0.05	0.65 ± 0.02	1.42 ± 0.02	2.62 ± 0.02
BPA 25 μg/L	538.43	3.53 ± 0.05	0.66 ± 0.03	1.40 ± 0.03	2.61 ± 0.03
BPA 50 μg/L	549.41*	3.50 ± 0.03	0.64 ± 0.01*	1.39 ± 0.01	2.55 ± 0.02**
BPB 5 μg/L	535.12	3.53 ± 0.04	0.67 ± 0.04	142 ± 0.04	2.61 ± 0.03
BPB 25 μg/L	537.60	3.55 ± 0.05	0.66 ± 0.03	141 ± 0.03	2.60 ± 0.02
BPB 50 μg/L	548.60*	3.49 ± 0.03	0.65 ± 0.02*	140 ± 0.02	2.54 ± 0.01**
BPF 5 μg/L	537.80	3.54 ± 0.04	0.68 ± 0.04	142 ± 0.03	2.62 ± 0.04
BPF 25 μg/L	538.41	3.53 ± 0.05	0.66 ± 0.03	141 ± 0.04	2.61 ± 0.03
BPF 50 μg/L	548.22*	3.51 ± 0.03	0.64 ± 0.02*	142 ± 0.02	2.55 ± 0.02**
BPS 5 μg/L	540.20	3.55 ± 0.04	0.67 ± 0.04	143 ± 0.05	2.63 ± 0.03
BPS 25 μg/L	538.60	3.54 ± 0.05	0.68 ± 0.03	142 ± 0.04	2.60 ± 0.02
BPS 50 μg/L	548.81*	3.50 ± 0.03	0.65 ± 0.01*	141 ± 0.02	2.56 ± 0.02**

Values are presented as Mean ± SEM.
*: Indicate significance at $p < 0.05$ vs control.
**: Indicate significance at $p < 0.01$ vs control.

control. While, there was no significant difference in the other treatment groups observed when compared to the control. There was no significant difference observed in paired testis when comparison to the control after 48 weeks of exposure to different concentrations of BPA and its analogues BPB, BPF and BPS was done. GSI showed significant ($P < 0.05$) reduction in BPA, BPB, BPF and BPS 50 μg/L exposed groups. While there was no difference observed in the other treated groups when compared to control. There was also no significant difference observed in absolute paired testis of all the treated groups of bisphenols (BPA, BPB, BPF and BPS) when compared to the control, however, relative epididymis weight reduced significantly ($P < 0.01$) in BPA, BPB, BPF and BPS 50 μg/L treated groups. On the other hand, there was difference observed in the other treatment groups but that was not significant to the control (Table 2).

3.3. Effects of chronic exposure of different concentrations of BPS (5, 25 and 50 μg/L) on absolute seminal vesical weight, relative seminal vesical weight, absolute prostate weight and relative prostate weight of male rats

Seminal vesical weight and prostate weight after 48 weeks of exposure with different treatment groups and control is presented in Table 3. Significant reduction was observed in BPA 25 μg/L ($P < 0.05$), BPA 50 μg/L ($P < 0.01$) when compared to the control. Absolute seminal vesical was reduced significantly ($P < 0.05$, $P < 0.01$) in BPS 25 and 50 μg/L treated groups. Similarly, BPF treatment caused significant reduction ($P < 0.05$ and $P < 0.01$) at does levels of 25 and 50 μg/L. On the other hand, BPS 25 and 50 μg/L significantly reduced ($P < 0.05$ and $P < 0.01$) absolute seminal vesical weight; however other doses of BPA, BPB, BPF and BPS did not reduce absolute seminal vesical weight as compared to the control (Table 3).

Relative seminal vesical weight of different treatment groups of BPA and its analogues BPB, BPF and BPS is presented in Table 3. Significant reduction was observed in BPA 50 μg/L ($P < 0.01$) when compared to the control. Relative seminal vesical weight was reduced significantly ($P < 0.01$) in BPB 50 μg/L treated group. Similarly, BPF treatment caused significant reduction ($P < 0.01$) at 50 μg/L dose level. However, BPF 5 and 25 μg/L did not affect relative seminal weight significantly. BPS 50 μg/L relative seminal vesical weight was significantly reduced ($P < 0.01$), however, other doses did not reduce relative seminal vesical weight as compared to the control (Table 3).

Absolute and relative prostate weight after 48 weeks of exposure with different concentration of BPA and its analogues BPB, BPF and BPS is presented in Table 3. There was no significant difference observed in all the BPA and its analogues BPB, BPF and BPS treated groups as compared to the control. Prostate weight was observed to have reduced in some of the groups exposed to bisphenols but that reduction was not significant to the control (Table 3).

Table 3
Effect of chronic exposure of different concentrations of BPA and its analogues BPB, BPF and BPS (5, 25 and 50 μg/L) on body and organs weight of male rats.

Groups	Parameters			
	Absolute seminal vesicle weight (g)	Relative seminal vesicle weight (mg/g)	Absolute prostate weight (g)	Relative prostate weight (mg/g)
Control	1.90 ± 0.04	3.55 ± 0.04	1.45 ± 0.03	2.71 ± 0.05
BPA 5 μg/L	1.88 ± 0.03	3.48 ± 0.03	1.42 ± 0.03	2.69 ± 0.04
BPA 25 μg/L	1.82 ± 0.02*	3.40 ± 0.04	1.46 ± 0.03	2.66 ± 0.03
BPA 50 μg/L	1.78 ± 0.03**	3.30 ± 0.03**	1.47 ± 0.04	2.65 ± 0.05
BPB 5 μg/L	1.86 ± 0.02	3.47 ± 0.03	1.43 ± 0.03	2.68 ± 0.03
BPB 25 μg/L	1.83 ± 0.03*	3.41 ± 0.04	1.45 ± 0.02	2.67 ± 0.04
BPB 50 μg/L	1.79 ± 0.04**	3.31 ± 0.02**	1.46 ± 0.04	2.65 ± 0.02
BPF 5 μg/L	1.86 ± 0.02	3.46 ± 0.04	1.42 ± 0.03	2.67 ± 0.04
BPF 25 μg/L	1.82 ± 0.02*	3.40 ± 0.03	1.44 ± 0.02	2.66 ± 0.03
BPF 50 μg/L	1.86 ± 0.03**	3.31 ± 0.03**	1.41 ± 0.04	2.64 ± 0.04
BPS 5 μg/L	1.87 ± 0.02	3.49 ± 0.02	1.44 ± 0.03	2.67 ± 0.03
BPS 25 μg/L	1.83 ± 0.03*	3.42 ± 0.04	1.46 ± 0.02	2.68 ± 0.04
BPS 50 μg/L	1.79 ± 0.03**	3.32 ± 0.03**	1.48 ± 0.04	2.64 ± 0.03

Values are presented as Mean ± SEM.
*: Indicate significance at $p < 0.05$ vs control.
**: Indicate significance at $p < 0.01$ vs control.
***: Indicate significance at $p < 0.001$ vs control.

3.4. Antioxidant enzymes, LPO and ROS after chronic exposure to different concentrations of BPA and its analogues BPB, BPF and BPS

Antioxidant enzymes reduced to a significant level while ROS and LPO levels increased in rats testicular tissues after chronic exposure to different concentrations of BPA and its analogues BPB, BPF and BPS as presented in Table 4. CAT activity was expressed as units/mg tissue and in BPA 25 μg/L and BPA 50 μg/L significant ($P < 0.05$) reduction was observed in exposed groups as compared to control. Similarly, significant reduction was also observed in BPB 25 μg/L ($P < 0.05$) and BPB 50 μg/L ($P < 0.01$) groups when compared to the control group. On the other hand, CAT activity was significantly reduced in BPF 50 μg/L ($P < 0.05$) as compared to control. In BPS exposed group only significant reduction was observed in BPS 50 μg/L ($P < 0.05$) when compared to the control group. While there was no significant difference observed in the other exposed groups of BPA, BPB, BPF and BPS as

Table 4
Effect of chronic exposure of different concentrations of BPA and its analogues BPB, BPF and BPS (5, 25 and 50 μg/L) on oxidative stress in the testicular tissues of male rats.

Groups	Parameters				
	CAT (U/mg protien)	SOD (U/mg protien)	POD (U/mg protien)	LPO (U/mg protien)	ROS (U/mg protien)
Control	7.47 ± 0.15	32.34 ± 0.29	6.04 ± 0.15	7.72 ± 0.24	98.70 ± 0.29
BPA 5 μg/L	6.71 ± 0.41	32.09 ± 0.68	5.74 ± 0.07	7.62 ± 0.27	99.15 ± 0.18
BPA 25 μg/L	6.43 ± 0.25*	31.38 ± 0.43	5.60 ± 0.09*	7.73 ± 0.02	104.5 ± 1.67
BPA 50 μg/L	6.38 ± 0.25*	30.66 ± 0.33**	5.40 ± 0.10**	8.43 ± 0.07**	122.7 ± 3.53***
BPB 5 μg/L	7.11 ± 0.35	32.16 ± 0.30	5.65 ± 0.04	7.49 ± 0.07	98.35 ± 0.42
BPB 25 μg/L	6.38 ± 0.30*	31.34 ± 0.31	5.50 ± 0.13*	7.57 ± 0.08	105.0 ± 2.73
BPB 50 μg/L	6.09 ± 0.28**	30.81 ± 0.20*	5.42 ± 0.07**	8.60 ± 0.22**	122.6 ± 3.34***
BPF 5 μg/L	7.13 ± 0.13	32.32 ± 0.24	5.65 ± 0.05	7.38 ± 0.06	98.70 ± 0.42
BPF 25 μg/L	6.46 ± 0.27	31.14 ± 0.30	5.54 ± 0.11*	7.54 ± 0.09	105.4 ± 1.12
BPF 50 μg/L	6.17 ± 0.24**	30.42 ± 0.11**	5.41 ± 0.13**	8.59 ± 0.14**	122.0 ± 4.06***
BPS 5 μg/L	7.08 ± 0.26	32.59 ± 0.17	5.62 ± 0.09	7.48 ± 0.10	98.84 ± 0.40
BPS 25 μg/L	6.46 ± 0.20	31.63 ± 0.16	5.45 ± 0.09*	7.56 ± 0.08	105.4 ± 1.37
BPS 50 μg/L	6.36 ± 0.16*	30.57 ± 0.15**	5.44 ± 0.11**	8.60 ± 0.03**	121.5 ± 3.28***

Values are presented as Mean ± SEM.
*: Indicate significance at $p < 0.05$ vs control.
**: Indicate significance at $p < 0.01$ vs control.
***: Indicate significance at $p < 0.001$ vs control.

Table 5
Effect of chronic exposure of different concentrations of BPA and its analogues BPB, BPF and BPS (5, 25 and 50 μg/L) on plasma testosterone and estradiol concentrations in male rats.

Parameters				
Groups	Testosterone (ng/ml)	Estradiol (pg/ml)	LH (ng/ml)	FSH (mIU/ml)
Control	12.02 ± 0.98	2.81 ± 0.33	1.79 ± 0.07	0.79 ± 0.07
BPA 5 μg/L	11.68 ± 0.43	3.64 ± 0.24	1.68 ± 0.08	0.75 ± 0.02
BPA 25 μg/L	10.61 ± 020	3.72 ± 0.40	1.55 ± 0.08	0.67 ± 0.04
BPA 50 μg/L	09.76 ± 0.36**	4.20 ± 0.34*	1.52 ± 0.03*	0.59 ± 0.05*
BPB 5 μg/L	11.05 ± 0.23	3.47 ± 0.19	1.62 ± 0.04	0.76 ± 0.07
BPB 25 μg/L	10.90 ± 0.21	3.93 ± 0.22	1.55 ± 0.03	0.63 ± 0.06
BPB 50 μg/L	09.36 ± 0.41***	4.55 ± 0.33**	1.48 ± 0.02*	0.58 ± 0.05*
BPF 5 μg/L	11.49 ± 0.37	3.53 ± 0.19	1.59 ± 0.08	0.73 ± 0.04
BPF 25 μg/L	10.43 ± 0.33	3.86 ± 0.26	1.54 ± 0.05	0.64 ± 0.01
BPF 50 μg/L	09.40 ± 0.05***	4.48 ± 0.29**	1.49 ± 0.07*	0.59 ± 0.02*
BPS 5 μg/L	11.39 ± 0.11	3.43 ± 0.31	1.63 ± 0.06	0.74 ± 0.03
BPS 25 μg/L	10.31 ± 0.63*	3.82 ± 0.16	1.56 ± 0.06	0.60 ± 0.02
BPS 50 μg/L	09.45 ± 0.33***	4.39 ± 0.29**	1.49 ± 0.02*	0.58 ± 0.03*

Values are presented as Mean ± SEM.
*: Indicate significance at $p < 0.05$ vs control.
**: Indicate significance at $p < 0.01$ vs control.
***: Indicate significance at $p < 0.001$ vs control.

compared to control.

SOD activity was expressed as (mU/mg protein) and in BPA 50 μg/L significant ($P < 0.01$) reduction was observed as compared to control. Similarly, BPB 50 μg/L exposed group caused significant ($P < 0.05$) reduction as compared to the control. On the other hand, BPF 50 μg/L significantly reduced ($P < 0.01$) SOD concentration in the rat testicular tissues. BPS high dose group 50 μg/L also ($P < 0.01$) reduced SOD concentration. However, 5 μg/L and 25 μg/L exposed groups did not show significant reduction in the SOD activity after chronic exposure with BPA, BPB, BPF and BPS.

POD activity was expressed as (U/mg protein) in the testis after chronic exposure, significant reduction in BPA 25 μg/L and 50 μg/L ($P < 0.05$ and $P < 0.01$) was observed as compared to the control. Significant reduction was observed in BPB 25 μg/L ($P < 0.05$) and BPB 50 μg/L ($P < 0.01$) when compared to the control. POD activity was reduced significantly ($P < 0.05$ and $P < 0.01$) in BPF 25 μg/L and BPF 50 μg/L treated groups. Similarly, BPS treatment caused significant reduction ($P < 0.05$ and $P < 0.01$) at dose levels of 25 and 50 μg/L. However BPA, BPB, BPF and BPS 5 μg/L did not affect POD activity significantly.

LPO activity in the different treatment groups and control after chronic exposure is presented in Table 4. Significant increase ($P < 0.01$) in BPA 50 μg/L was observed as compared to the control. All the high doses of BPB, BPF and BPS (50 μg/L) caused significant increase ($P < 0.01$) in the LPO activity as compared to control. However, there was no significant difference observed in 5 μg/L and 25 μg/L groups of BPA, BPB, BPF and BPS as compared to the control.

ROS in the testicular tissues of animals exposed to different concentrations of BPA, BPB, BPF and BPS for 48 weeks is presented in Table 4. Significant increase was observed in BPA 50 μg/L ($P < 0.001$) when compared to the control. ROS activity increased significantly ($P < 0.001$) in BPB 50 μg/L treated groups. Similarly, BPF treatment caused significant increase ($P < 0.001$) at 50 μg/L dose level. However, BPS 50 μg/L significantly increased ($P < 0.001$) ROS activity as compared to control. On the other hand, all the other doses (5 μg/L and 25 μg/L) of BPA, BPB, BPF and BPS did not cause significant reduction in the ROS activity as compared to the control.

3.5. Plasma testosterone, LH, FSH and estradiol concentrations in the animals after chronic exposure of 48 weeks to different concentrations of BPA and its analogues BPB, BPF and BPS

Plasma testosterone (ng/ml), Luteinizing hormone (ng/ml), Follicle-stimulating hormone (mIU/ml) and estradiol concentrations (ph/ml) are presented in Table 5. Significant reduction was observed in BPA 50 μg/L ($P < 0.01$) when compared to the control. Testosterone concentration reduced significantly ($P < 0.001$) in BPB 50 μg/L treated group. Similarly, BPF caused significant reduction ($P < 0.001$) at dose level 50 μg/L. On the other hand, BPS 25 μg/L and 50 μg/L significantly reduced ($P < 0.05$, $P < 0.001$ respectively) testosterone in the plasma, however other doses of BPA, BPB, BPF and BPS did not reduced plasma testosterone as compared to the control.

Plasma estradiol concentrations in the animals exposed to BPA 50 μg/L were significantly ($P < 0.05$) increased than control group. Estradiol concentration increased significantly ($P < 0.01$) in BPB 50 μg/L treated group. Similarly, BPF treatment caused significant increased ($P < 0.001$) at dose level of 50 μg/L, however, BPF 5 μg/L and 25 μg/L did not affect estradiol concentration significantly. On the other hand BPS 50 μg/L significantly increased ($P < 0.001$) estradiol concentration; however other groups did not increase estradiol concentration as compared to the control.

Plasma LH concentrations in the treatment groups were reduced as compared to the control (Table 5). Significant reduction was observed in BPA 50 μg/L ($P < 0.05$) when compared to the control. LH concentrations were reduced significantly ($P < 0.05$) in BPB 50 μg/L treated groups. Similarly, BPF treatment caused significant reduction ($P < 0.05$) at dose level of 50 μg/L. BPS 50 μg/L significantly reduced ($P < 0.05$) plasma LH concentration, However other doses did not reduce plasma LH concentrations as compared to the control.

Plasma FSH concentrations in the treatment groups were found reduced as compared to the control group (Table 5). Significant reduction in plasma FSH levels ($P < 0.05$) was noted in the highest concentration (50 μg/L) exposed group of BPA when compared to the control. FSH concentration was reduced significantly ($P < 0.05$) in BPB 50 μg/L when compared to the control. Similarly, BPF treatment caused significant reduction ($P < 0.05$) at dose level of 50 μg/L. On the other hand, PBS 50 μg/L significantly reduced ($P < 0.05$) FSH concentration in plasma. However, other treatment groups of BPA, BPB, BPF and BPS plasma FSH levels were reduced but were not statistically significant.

3.6. Sperm parameters, DSP and number of sperms in different parts of epididymis after chronic exposure to different concentrations of BPA, BPB, BPF and BPS

Exposure to different concentrations of BPA and its analogues BPB, BPF and BPS for 48 weeks caused no significant reduction in the percentage of motile sperm. However, Exposure to BPA highest concentration (50 μg/L) for 48 weeks caused significant ($P < 0.05$) reduction in motile sperm percentage but did not show effect on viable sperm percentage. Significant reduction was observed in BPB 50 μg/L ($P < 0.01$) when compared to control. Motile sperm percentage was reduced significantly ($P < 0.05$, $P < 0.01$) in BPF 25 and 50 μg/L. On the other hand, PBS 25 and 50 μg/L significantly reduced ($P < 0.05$, $P < 0.01$) percentage of motile sperms after exposure for 48 weeks of chronic exposure. However, in the different concentrations of BPA, BPB, BPF and BPS where no significant difference observed when compared to control (Table 6).

DSP in the different treatment groups and control is presented in Table 6. Significant reduction was observed in BPA 50 μg/L ($P < 0.01$) when compared to control. DSP was reduced significantly ($P < 0.01$) in BPB 50 μg/L treated group. Similarly, BPF treatment caused significant reduction ($P < 0.01$) at dose level of 50 μg/L. BPS 50 μg/L also caused significant reduction ($P < 0.01$) in the treated groups. On the other hand, BPA, BPB, BPF and BPS 5 and 25 μg/L treated groups did not affect DSP significantly.

Sperm number in caput epididymis was significantly reduced in the BPA 25 μg/L ($P < 0.05$) and BPA 50 μg/L ($P < 0.01$) exposed groups. Significant reduction was observed in BPB 25 μg/L ($P < 0.05$) and BPB 50 μg/L ($P < 0.01$) when compared to the control. Similarly, BPF treatment caused reduction ($P < 0.05$, $P < 0.01$) at dose levels of 25 and 50 μg/L. In BPS 25 and 50 μg/L caused significant reduction ($P < 0.05$, $P < 0.01$) in the caput epididymis sperm number when compared to the control. However, some of the BPA, BPB, BPF and BPS did not reduce sperm number in the caput epididymis as compared to the control.

Sperm number in the cauda epididymis in different treatment groups and control is presented in Table 6. Significant reduction was observed in BPA 50 μg/L treated group ($P < 0.05$) when compared to the control. Cauda epididymis sperm number was reduced significantly ($P < 0.05$) in BPB 50 μg/L treated group. Similarly, BPF treatment caused significant reduction ($P < 0.05$) at dose level of 50 μg/L. BPS 50 μg/L also significantly reduced ($P < 0.05$) cauda epididymis sperm number as compared to control. On the other hand, there was no significant difference observed in BPA, BPB, BPF and BPS 5 and 25 μg/L treated groups when compared to the control.

3.7. Histological and planimetry changes of testicular tissue in adult male rats exposed to different concentrations of BPA, BPB, BPF and BPS for 48 weeks

Histological study of the microscopic slides of the testicular tissues revealed normal morphology of the structures in the control and 5 μg/L exposed groups. The seminiferous tubules were compactly arranged with sperm filled lumen and the interstitial space was relatively thin in these groups. In the groups exposed to 25 μg/L and 50 μg/L of BPA and its analogues BPB, BPF and BPS the tubules were relatively small with larger interstitial spaces and less filled lumen. Cellular arrest at spermatogoneal stage and at round spermatids were more evident in the highest concentration (50 μg/L) exposed group. In 25 μg/L exposed group, cellular arrest was observed but was less than 50 μg/L exposed group (Fig. 1).

Planimetry results showed significant ($P < 0.05$) reduction in the height of epithelium in the group exposed to 50 μg/L of BPA for weeks. Significant reduction was observed in BPB 50 μg/L ($P < 0.01$) when compared to the control. Epithelial height was reduced significantly ($P < 0.01$) in BPF 50 μg/L treated group. Similarly, BPS treatment caused significant reduction ($P < 0.05$) at dose level of 50 μg/L. However, BPA, BPB, BPF and BPS 5 and 25 μg/L groups did not affect epithelial height significantly. On the other hand, there was no significant difference observed in area of seminiferous tubules, area of interstitium and in diameter of seminiferous tubules of all treated groups of BPA, BPB, BPF and BPS as compared to the control (Table 7).

3.8. Number of different cells types in seminiferous tubules in the testis of adult rats exposed to different concentrations of BPA and its analogues BPB, BPF and BPS for 48 weeks

Number of different cells in the seminiferous tubules of male rats testis are presented in Table 8. Significant reduction in the number of spermatogonia was observed in the group exposed to BPA 50 μg/L ($P < 0.05$) than control. Significant reduction was also observed in BPB 50 μg/L ($P < 0.05$) treated group when compared to the control. Similarly, BPF treatment caused significant reduction ($P < 0.05$) at dose level of 50 μg/L. On the other hand, BPS 50 μg/L significantly reduced ($P < 0.05$) number of spermatogonia as compared to control. However, BPA, BPB, BPF and BPS 5 and 25 μg/L did not reduce significantly the number of spermatogonia as compared to control.

In the number of spermatocytes significant reduction was observed in BPA 50 μg/L ($P < 0.05$) when compared to the control.

Table 6
Effect of chronic exposure of different concentrations of BPA and its alternatives BPB, BPF and BPS (5, 25 and 50 μg/L) on sperm parameters and sperm number in epididymis of rats.

Groups	Parameters				
	Viable sperms (%)	Motile sperms (%)	DSP (x 106)	Caput epididymis sperm number (× 106/g organ)	Cauda epididymis sperm number (× 106/g organ)
Control	93.92 ± 0.48	79.56 ± 0.54	53.34 ± 0.6	303.16 ± 1.38	598.15 ± 2.46
BPA 5 μg/L	93.87 ± 0.65	77.72 ± 1.74	52.22 ± 0.3	296.62 ± 3.88	590.57 ± 0.22
BPA 25 μg/L	93.52 ± 0.92	77.01 ± 1.69	50.56 ± 1.4	291.78 ± 2.03*	589.28 ± 4.88
BPA 50 μg/L	92.01 ± 0.89	77.27 ± 0.89*	48.44 ± 0.3**	291.88 ± 4.11**	583.38 ± 1.64*
BPB 5 μg/L	93.95 ± 0.84	78.08 ± 0.68	52.34 ± 0.7	295.04 ± 2.10	592.18 ± 2.10
BPB 25 μg/L	93.13 ± 0.74	75.97 ± 0.51	51.04 ± 1.5	293.92 ± 2.04*	590.38 ± 5.06
BPB 50 μg/L	92.33 ± 0.86	74.17 ± 0.42**	48.32 ± 0.5**	290.16 ± 1.12**	580.98 ± 0.94*
BPF 5 μg/L	93.49 ± 0.97	78.33 ± 0.34	52.14 ± 0.6	295.14 ± 2.05	592.46 ± 2.02
BPF 25 μg/L	93.13 ± 1.09	75.33 ± 0.38*	50.68 ± 1.1	293.28 ± 0.75*	589.36 ± 2.66
BPF 50 μg/L	92.19 ± 0.91	74.70 ± 0.30**	48.58 ± 0.7**	288.86 ± 0.96**	583.14 ± 1.66*
BPS 5 μg/L	93.57 ± 1.07	78.12 ± 0.51	52.24 ± 0.5	295.52 ± 1.55	590.74 ± 5.07
BPS 25 μg/L	93.32 ± 1.01	75.27 ± 1.10*	50.32 ± 0.8	293.48 ± 1.77*	589.94 ± 4.88
BPS 50 μg/L	92.99 ± 0.97	74.28 ± 0.74**	48.22 ± 0.5**	291.12 ± 1.70**	584.64 ± 1.68*

Values are presented as Mean ± SEM.
*: Indicate significance at $p < 0.05$ vs control.
**: Indicate significance at $p < 0.01$ vs control.

Spermatocytes number was reduced significantly ($P < 0.05$) in BPB 50 μg/L treated group. Similarly, BPF 50 μg/L treatment caused significant reduction ($P < 0.05$) at dose level of 50 μg/L. BPS 50 μg/L treated group significantly reduced ($P < 0.05$) the number of spermatocytes when compared to the control. On the other hand, the other doses of BPA, BPB, BPF and BPS did not reduce number of spermatocytes as compared to the control.

Number of spermatids in different treatment groups and control is presented in Table 8. Significant reduction was observed in BPA 50 μg/L ($P < 0.01$) when compared to the control. Spermatids number reduced significantly ($P < 0.01$) in BPB 50 μg/L treated group. Similarly, BPF treatment caused significant reduction ($P < 0.01$) at dose level of

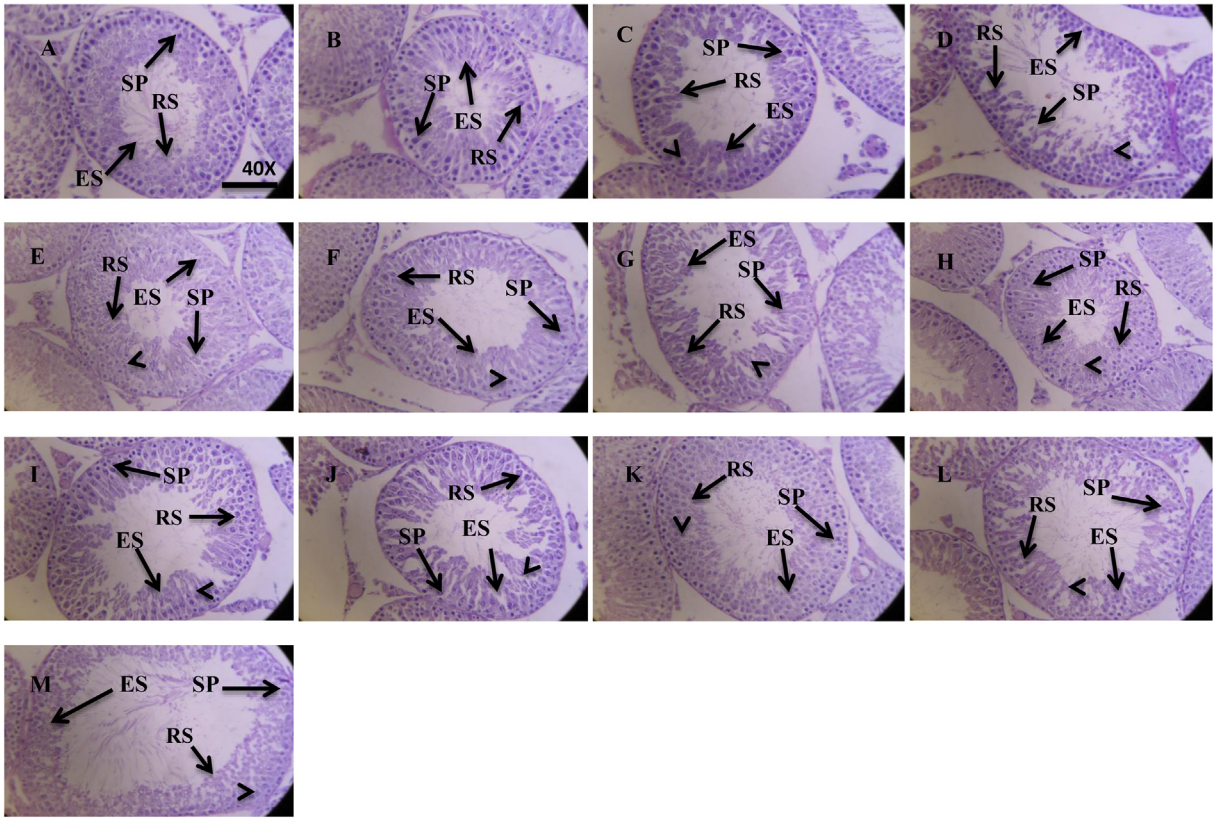

Fig. 1. Photomicrograph from testicular tissue showing (A) control; having thick epithelium with normal spermatogonia (SP), Round spermatids (RS), Elongated spermatids (ES) and filled lumen with sperm (B, C and D); BPA (5, 25 and 50 μg/L) treated presenting seminiferous tubules with epithelium (Line without arrow head) and spermatids (White arrow); (E, F and G) BPB (5,25 and 50 μg/L) treated presenting seminiferous tubules with epithelium (Line without arrow head) and elongating spermatids (White arrow); (H, I and J) BPF (5, 25 and 50 μg/L) treated presenting seminiferous tubules with epithelium (Line without arrow head) and elongating spermatids (White arrow); (K, L and M) BPS (5, 25 and 50 μg/L) treated presenting seminiferous tubules with epithelium (Line without arrow head) and spermatids (White arrow). H&E (40x).

Table 7
Effect of chronic exposure of different concentrations of BPA and its alternatives BPB, BPF and BPS (5, 25 and 50 μg/L) on planimetry of testis in rats.

Groups	Parameters			
	Area of seminiferous tubules (%)	Area of Interstitium (%)	Seminiferous tubule diameter (μm)	Epithelial height (μm)
Control	85.02 ± 1.95	16.42 ± 0.72	207.90 ± 1.77	71.22 ± 1.90
BPA 5 μg/L	82.64 ± 0.23	17.80 ± 0.95	201.08 ± 3.13	67.88 ± 1.02
BPA 25 μg/L	82.06 ± 0.67	16.22 ± 1.32	205.08 ± 1.55	65.74 ± 1.28
BPA 50 μg/L	82.17 ± 1.72	16.66 ± 1.38	203.97 ± 1.48	61.58 ± 2.17*
BPB 5 μg/L	82.73 ± 1.05	17.68 ± 0.38	205.87 ± 1.60	69.18 ± 1.29
BPB 25 μg/L	81.64 ± 0.56	15.90 ± 1.49	207.46 ± 1.47	68.13 ± 1.31
BPB 50 μg/L	83.71 ± 1.38	15.69 ± 1.37	203.24 ± 1.25	60.02 ± 2.72**
BPF 5 μg/L	84.58 ± 1.54	16.26 ± 1.63	204.81 ± 1.59	68.06 ± 2.10
BPF 25 μg/L	82.44 ± 0.71	15.65 ± 1.29	203.53 ± 1.72	66.35 ± 1.75
BPF 50 μg/L	84.46 ± 1.26	17.02 ± 1.51	205.46 ± 1.22	60.83 ± 2.15**
BPS 5 μg/L	83.51 ± 0.82	18.20 ± 0.52	205.24 ± 1.24	66.26 ± 2.65
BPS 25 μg/L	82.30 ± 0.69	17.86 ± 0.66	204.86 ± 1.58	64.44 ± 1.87
BPS 50 μg/L	83.28 ± 0.71	19.04 ± 0.78	204.35 ± 1.63	61.96 ± 2.72*

Values are presented as mean ± SEM.
*: Indicate significance at $p < 0.05$ vs control.
**: Indicate significance at $p < 0.01$ vs control.
***: Indicate significance at $p < 0.001$ vs control.

Table 8
Effect of chronic exposure of different concentrations of BPA and its analogues BPB, BPF and BPS (5, 25 and 50 μg/L) on number of different cell types in the testis of rats.

Groups	Parameters		
	Spermatogonia (n)	Spermatocytes (n)	Spermatids (n)
Control	65.66 ± 0.62	77.10 ± 1.06	257.26 ± 1.79
BPA 5 μg/L	63.14 ± 0.75	75.40 ± 1.29	250.54 ± 2.67
BPA 25 μg/L	63.56 ± 0.83	73.32 ± 1.97	248.10 ± 2.71
BPA 50 μg/L	60.62 ± 0.72*	72.18 ± 1.20*	245.58 ± 2.42**
BPB 5 μg/L	63.98 ± 1.36	74.32 ± 0.94	250.32 ± 1.80
BPB 25 μg/L	63.68 ± 1.03	73.54 ± 1.41	248.36 ± 2.20
BPB 50 μg/L	61.26 ± 1.13*	71.82 ± 1.29*	245.40 ± 2.50**
BPF 5 μg/L	63.72 ± 1.13	73.64 ± 1.35	250.10 ± 2.87
BPF 25 μg/L	63.20 ± 1.16	72.64 ± 1.24	248.22 ± 2.34
BPF 50 μg/L	61.34 ± 0.84*	71.50 ± 1.26*	245.16 ± 1.97**
BPS 5 μg/L	63.40 ± 1.05	74.74 ± 1.30	250.04 ± 2.77
BPS 25 μg/L	63.64 ± 1.15	73.84 ± 1.23	248.32 ± 2.52
BPS 50 μg/L	61.58 ± 0.87*	72.12 ± 1.24*	244.02 ± 2.01**

Values are presented as mean ± SEM.
*: Indicate significance at $p < 0.05$ vs control.
**: Indicate significance at $p < 0.01$ vs control.

50 μg/L. BPS 50 μg/L group was also observed with significantly reduced ($P < 0.01$) number of spermatids as compared to the control. However, there was no significant difference observed in BPA, BPB, BPF and BPS 5, 25 μg/L groups when compared to the control.

3.9. Planimetry and morphological changes in the caput region of epididymis of rats exposed to different concentrations of BPA, BPB, BPF and BPS for 48 weeks

Epididymis Caput region Planimetry results did not show significant reduction in the tubular diameter in the groups exposed to different concentrations of BPA, BPB, BPF and BPS after 48 week chronic exposure. There was also no significant difference observed in the other parameters as lumen diameter, epithelial height and area covered with epithelium and lumen of different treatment groups when compared to the control (Table 9, Fig. 2).

There was very slight difference observed in the morphological difference of caput region of epididymis among the different treatment groups of BPA and its analogues BPB, BPF and BPS and control. In the different treatment groups of 50 μg/L of BPA, BPB, BPF and BPS slightly reduced number of sperm in the lumen was observed when compared to the control. There was no significant difference observed in the other exposed groups in comparison to the control (Fig. 2).

3.10. Planimetry and morphological changes in the cauda region of epididymis of rats exposed to different concentrations of BPA and its analogues BPB, BPF and BPS for 48 weeks

Planimetry of the cauda region of the epididymis showed no significant alteration in the tubular diameter in the groups exposed to different concentrations of BPA and its analogues BPB, BPF and BPS than control after 48 weeks of exposure. Similarly, other parameters like lumen diameter, epithelial height, area covered by epithelium and area covered by lumen did not show any significant alterations compared to the control (Table 10, Fig. 3). Morphological difference observed in the cauda region of epididymis showed only a slightly reduced number of sperms in the lumen of 50 μg/L exposed groups with different concentrations of BPA, BPB, BPF and BPS for 48 weeks of chronic exposure. No significant alterations were obvious in other groups in comparison with control (Fig. 3).

4. Discussion

A growing number of studies recently have reported the adverse toxic effects of bisphenol A involvement in many chronic diseases. Therefore, the concern of many environmental agencies and government security groups has led to the development of many substitutes for BPA such BPB, BPF and BPS. These all analogues leaching from plastic containers have been shown to a lesser extent; though it has been detected in a small amount in the food samples across the globe (Liao and Kannan, 2013, 2014a; b; Viñas et al., 2010; Yamazaki et al., 2015). Although there is very little data on the effects of low dose of BPA and its analogues BPB, BPF and BPS which are widely used to replace BPA. Widespread use of bisphenols caused growing concern over the adverse effects provoked by these substances on human health (Song et al., 2014). In vitro, in vivo studies and epidemiological surveys have shown that BPA and its analogues exhibits neurotoxic potential, hepatotoxic, cancer development risks and endocrine toxicity (Cabaton et al., 2009; Catanese and Vandenberg, 2016; Grignard et al., 2012; Rochester and Bolden, 2015; Soto et al., 2013; Ullah et al., 2018). There has been less attention given to BPA analogues and its toxicological effects on reproductive system.

The postnatal period is also a sensitive exposure period for certain endocrine disruptors to have a direct effect on the intra-testicular environment and adversely affect spermatogenesis. During the late fetal

Table 9
Effect of chronic exposure of different concentrations of BPA and its analogues BPB, BPF and BPS (5, 25 and 50 μg/L) on planimetry of caput epididymis in rats.

Groups	Parameters				
	Tubular diameter (μm)	Lumen daimeter (μm)	Epithelial height (μm)	Epithelium (%)	Lumen (%)
Control	366.40 ± 1.34	292.01 ± 2.76	34.05 ± 1.03	33.25 ± 2.37	70.75 ± 4.70
BPA 5 μg/L	358.80 ± 1.75	290.60 ± 2.61	33.40 ± 2.43	32.05 ± 1.50	69.75 ± 1.94
BPA 25 μg/L	356.20 ± 3.21	288.02 ± 1.90	30.04 ± 2.79	31.51 ± 0.49	68.55 ± 2.00
BPA 50 μg/L	357.20 ± 3.05	287.20 ± 2.22	29.40 ± 1.01	29.25 ± 2.49	64.25 ± 2.86
BPB 5 μg/L	359.04 ± 2.19	290.60 ± 1.70	33.04 ± 0.44	32.98 ± 1.06	69.55 ± 4.33
BPB 25 μg/L	358.40 ± 4.99	288.20 ± 1.48	31.40 ± 2.26	31.65 ± 0.48	68.75 ± 4.67
BPB 50 μg/L	357.80 ± 3.03	287.80 ± 0.95	30.75 ± 2.49	29.16 ± 1.13	65.75 ± 2.78
BPF 5 μg/L	358.40 ± 0.74	290.80 ± 1.96	33.05 ± 2.42	32.65 ± 2.17	67.95 ± 1.70
BPF 25 μg/L	359.20 ± 1.57	288.60 ± 0.24	31.40 ± 1.75	31.05 ± 1.83	65.25 ± 0.98
BPF 50 μg/L	356.80 ± 3.27	287.20 ± 2.47	30.05 ± 0.88	29.20 ± 1.13	64.35 ± 3.58
BPS 5 μg/L	357.60 ± 1.27	290.60 ± 2.98	33.60 ± 1.81	33.40 ± 1.58	68.95 ± 1.42
BPS 25 μg/L	355.80 ± 2.19	289.80 ± 4.63	31.20 ± 3.07	30.50 ± 2.39	66.05 ± 0.72
BPS 50 μg/L	354.40 ± 3.13	287.80 ± 3.02	30.40 ± 2.47	28.50 ± 1.49	65.75 ± 1.60

and early neonatal period, estrogenic substances can alter estrogen receptor (ER) expression in the testis, which will influence the ability of Leydig cells to function, and which will delay the eventual onset and progression of puberty (Sharpe et al., 2003). Bisphenols like BPA, BPB, BPF and BPS are toxicants that can cause a hypothyroid state in neonatal rats and is associated with increased number of Leydig cells, reduced size of Leydig cells, and decreased steroidogenic function of Leydig cells (Kim et al., 2001; Mendis-Handagama and Ariyaratne, 2004). Therefore, the reason of selecting rats of PND 20 was that to know about the effect of these bisphenols on the onset of puberty and how the later stage after puberty is disturbed after chronic exposure to low dose of these chemicals.

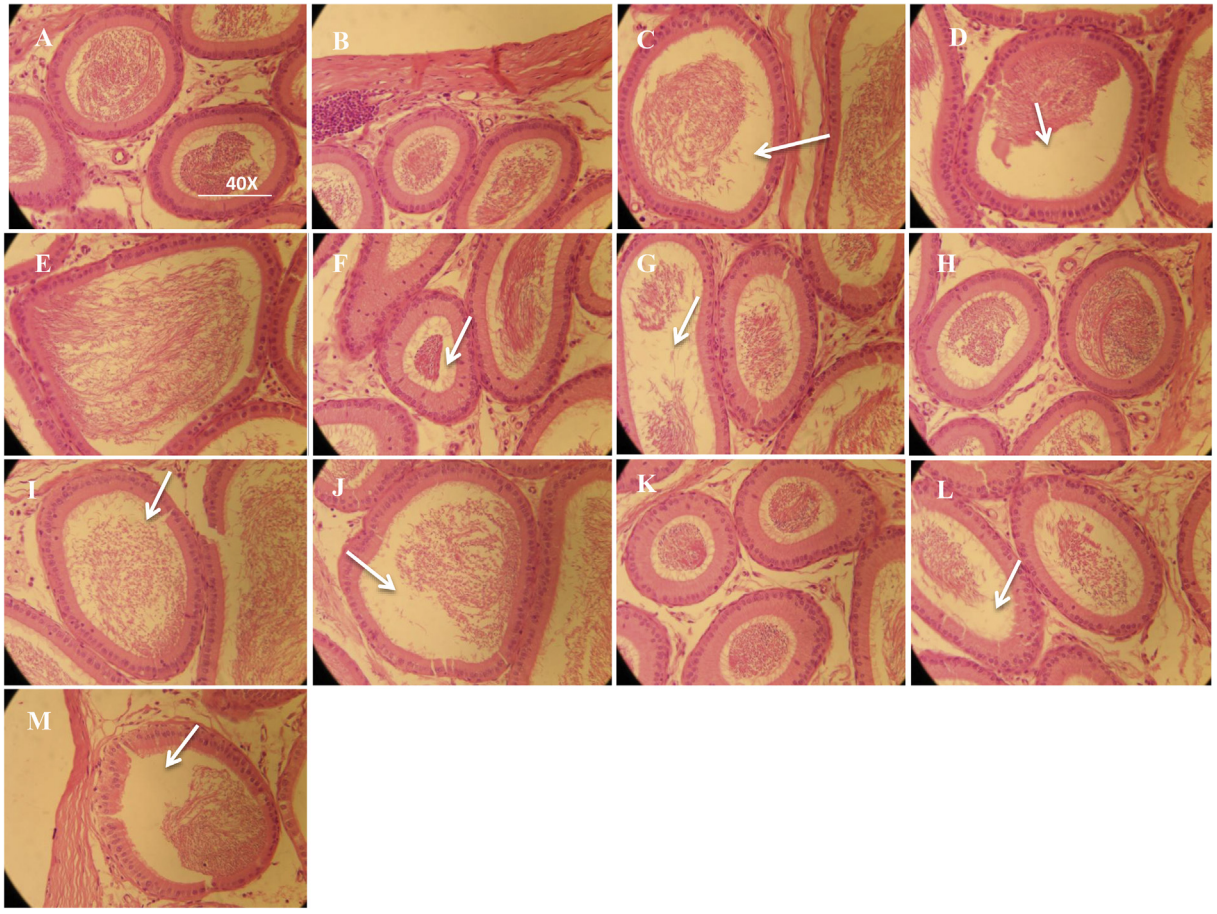

Fig. 2. Photomicrograph of caput epididymis tissue showing (A) control; with compact arrangement of caput tubules with sperm filled lumen (B) BPA (5 μg/L) exposed group, presenting normal caput tubules like in the control (C), BPA (25 μg/L) exposed group showing seminiferous tubules with less number of sperm in the lumen (Arrow) and (D) BPA (50 μg/L) exposed group presenting caput tubules with empty lumen (Arrow). Similarly, (E) BPB (5 μg/L) exposed group, presenting normal caput tubules, (F) BPB (25 μg/L) exposed group showing less number of sperms in the lumen, (G) BPB (50 μg/L) exposed group showing less number of sperms and empty lumen (Arrow). (H) BPF (5 μg/L) exposed group, presenting normal caput tubules, (I) (25 μg/L) exposed group showing seminiferous tubules with less number of sperm in the lumen (Arrow) and (J) BPF (50 μg/L) exposed group showing less number of sperms and empty lumen (Arrow). K, L BPS (5, 25 μg/L) exposed groups showing caput tubules with less number of sperms in the lumen and (M) BPS (50 μg/L) exposed group presenting less number of sperms and empty lumen. H& E (40x).

Table 10
Effect of chronic exposure of different concentrations of BPA and its analogues BPS, BPF and BPS (5, 25 and 50 μg/L) on planimetry of cauda epididymis in rats.

Groups	Parameters				
	Tubular diameter (μm)	Lumen diameter (μm)	Epithelial height (μm)	Epithelium (%)	Lumen (%)
Control	443.61 ± 1.67	415.60 ± 2.13	28.65 ± 1.05	33.25 ± 2.94	67.75 ± 1.97
BPA 5 μg/L	440.81 ± 0.72	412.60 ± 1.38	27.53 ± 1.46	31.51 ± 2.08	68.11 ± 0.88
BPA 25 μg/L	440.61 ± 3.91	411.11 ± 2.98	26.72 ± 0.86	28.91 ± 0.70	67.31 ± 1.68
BPA 50 μg/L	439.81 ± 2.32	410.10 ± 2.98	26.22 ± 1.75	27.75 ± 6.66	70.05 ± 1.69
BPB 5 μg/L	439.81 ± 0.95	413.40 ± 1.73	27.62 ± 1.45	29.51 ± 0.72	68.31 ± 2.27
BPB 25 μg/L	440.81 ± 2.95	415.60 ± 2.35	26.28 ± 1.68	27.25 ± 1.13	68.75 ± 1.87
BPB 50 μg/L	439.81 ± 3.11	414.60 ± 1.96	25.62 ± 2.10	26.75 ± 2.00	70.45 ± 1.27
BPF 5 μg/L	440.01 ± 0.54	414.40 ± 0.91	27.82 ± 2.45	31.51 ± 2.29	68.51 ± 2.00
BPF 25 μg/L	439.81 ± 1.22	413.20 ± 1.80	26.82 ± 2.39	29.75 ± 6.36	70.25 ± 1.67
BPF 50 μg/L	439.81 ± 1.13	413.12 ± 1.90	26.21 ± 1.00	27.51 ± 6.36	70.51 ± 3.55
BPS 5 μg/L	440.61 ± 2.13	414.13 ± 4.32	27.21 ± 2.19	29.51 ± 2.39	68.51 ± 2.54
BPS 25 μg/L	440.21 ± 1.05	413.80 ± 1.63	26.80 ± 3.10	27.75 ± 1.26	68.85 ± 1.17
BPS 50 μg/L	441.81 ± 1.75	413.40 ± 1.73	25.60 ± 3.24	27.51 ± 1.17	70.51 ± 3.55

In this study we have shown that BPB, BPF and BPS have many properties in common to BPA where we observed reduction in GSI, relative weights of reproductive organs, testosterone, LH and FSH concentrations and alterations in tissue histology in groups exposed to higher concentrations of BPA and its analogues BPB, BPF and BPS. Oxidative stress in the testicular tissue was induced and the DSP was reduced in the higher concentration exposed group than control. Our results were not very different from some of these studies done in past with BPA and its analogues where Meeker et al., 2009 in his study explained that BPA concentrations 1.3 (< 0.4–36.4) ng/mL in urine are in relation with reproductive hormones like testosterone and follicle stimulating hormone (FSH). Similarly, In another study Rubin 2011 explained the relation of BPA with reproductive hormones similar concentration with our results. On the other hand, Volkel et al., 2002 in

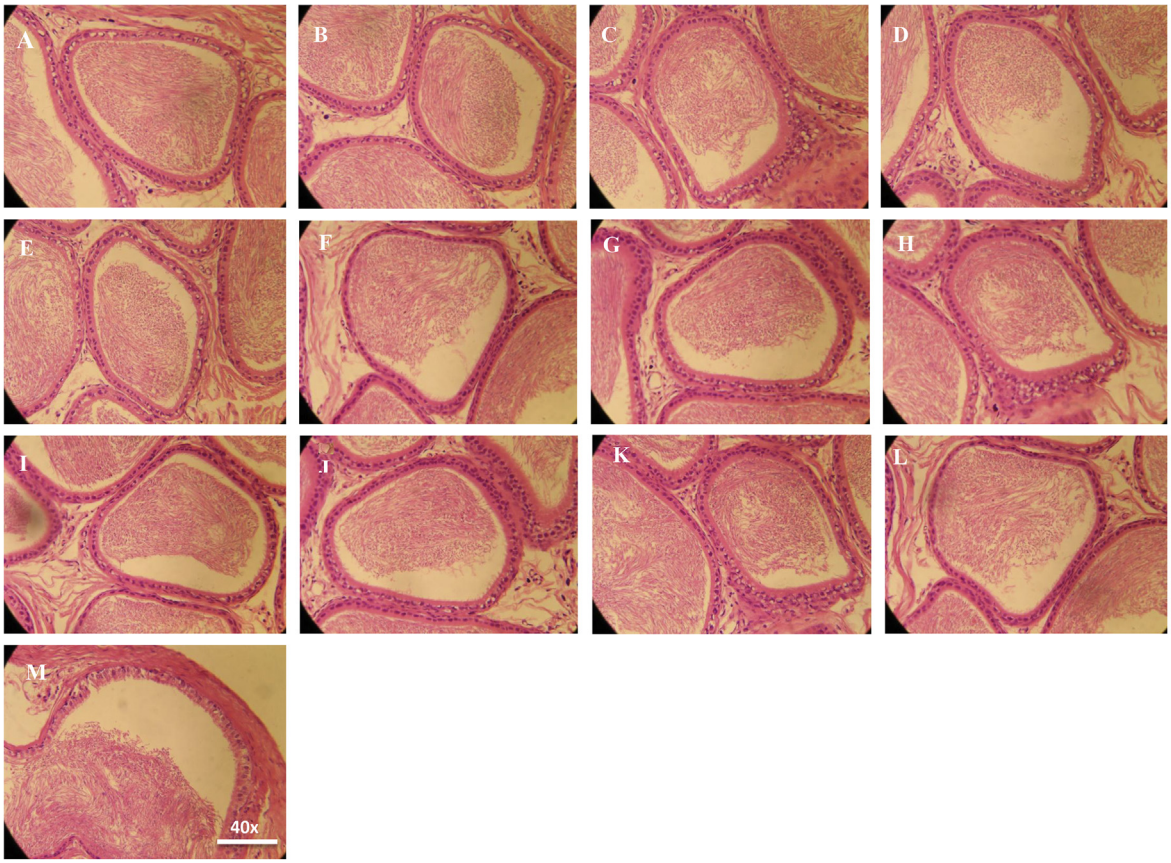

Fig. 3. Photomicrograph of cauda epididymis tissue showing (A) control; with compact arrangement of cauda tubules with sperm filled lumen (B) BPA (5 μg/L) exposed group, presenting normal caput tubules like in the control (C) BPA (25 μg/L) exposed group, presenting cauda tubules with sperm filled lumen (D) BPA (50 μg/L) exposed group presenting cauda tubules with less sperm in the lumen. Similarly, (E) BPB (5 μg/L) exposed group, presenting normal caput tubules like in the control (F) BPB (25 μg/L) exposed group, presenting cauda tubules with sperm filled lumen (G) BPB (50 μg/L) exposed group presenting cauda tubules with less sperm in the lumen. Likewise, (H)BPF (5 μg/L) exposed group, presenting normal caput tubules like in the control (I) BPF (25 μg/L) exposed group, presenting cauda tubules with sperm filled lumen (J) BPF (50 μg/L) exposed group presenting cauda tubules with less sperm in the lumen. In the same way, (K) BPS (5 μg/L) exposed group, presenting normal caput tubules like in the control (L) BPS (25 μg/L) exposed group, presenting cauda tubules with sperm filled lumen (M) BPS (50 μg/L) exposed group presenting cauda tubules with less sperm in the lumen. H&E (40x).

his study about BPA metabolic kinetics said that low dose (5 mg) of BPA in humans orally lead to altered reproductive hormones (Meeker et al., 2009; Rubin, 2011; Shi et al., 2015; Völkel et al., 2002).

In the present study hormones were disturbed of all the exposed groups to BPA and its analogues like BPB, BPF and BPS. Where we observed that both LH and FSH concentrations were inhibited and the concentration of testosterone had decreased in the exposed groups. However, the concentrations of estradiol in higher concentrations exposed groups had increased which suggests that either the gonadotropin secretions were inhibited at the level of pituitary or the secretions of GnRH from hypothalamus were affected which resulted in reduced levels of testosterone which needs further studies to be elucidated. This can also be because of disturbed testosterone machinery which produces testosterone and the disturbance resulted by prolonged oxidative stress in the testicular tissues. In the previous studies it was reported that oxidative stress induced by BPA and some of its analogues result into disturbed hormones in the different organisms (Feng et al., 2016; Hassan et al., 2012; Moghaddam et al., 2015; Naderi et al., 2014; Yang et al., 2017). In different studies previously it was reported that BPA and BPS exposure lead into oxidative stress in the peripheral blood mononuclear cells and testis and also lead into lipids and protein degradation in vitro (Michałowicz et al., 2015; Mokra et al., 2015; Ullah et al., 2016, 2017). The results of our study about inhibition of testosterone and anti-androgenic effects of these chemicals are in line with studies of Molina-Molina et al. (2013) an in-vitro study with low doses of BPA and BPS came across disturbed androgens levels after exposure to bisphenols and Rochester and Bolden 2015 also showed that bisphenol A analogues BPB and BPF have the potency to be in the same order of magnitude and in similar actions as BPA regarding androgens in both in vivo and in vitro studies (Molina-Molina et al., 2013; Rochester and Bolden, 2015). Testosterone reduced concentrations might be a result of suppression of GnRH transcripts in the hypothalamus which also suggest that suppressed GnRH lead in reduced gonadotropin secretion (Ji et al., 2013; Roelofs et al., 2015). However, increased estrogen levels seem to be due to estrogenic mode of action of bisphenol A and its analogues BPB, BPF and BPS (Liao and Kannan, 2013; Sui et al., 2012; Yamazaki et al., 2015).

Poor developments of reproductive organs lead into reduction in the daily sperm production, reduction in the GSI of male rats and alteration in the seminiferous tubules. The reduction of these parameters in our study were accompanied by arrest in spermatogoneal cells and round spermatids, which seem to have resulted because of reduced DSP, reduced number of sperm in the epididymis and epithelial height. Our results are in relation with multiple studies with BPA and some of its analogues where LH and FSH reduced levels supported the histological alterations in the testis and reduction in sperm production as in a by Brown et al. (2008) in the male rainbow trout exposed to 10 ng of EE2/l for 50 days showed altered reproductive hormones and it troubling embryonic aneuploidy whereas, Eladak et al., 2015 in his studies on BPA, BPF and BPS showed that 10 nmol/L-100 nmol/L of these compounds are involved in decreasing testosterone concentrations and alter physiological functions of reproductive organs (Brown et al., 2008; Chen et al., 2013; Eladak et al., 2015; Somm et al., 2009). Previous literature has also shown that estrogenic compounds do have effect on the reducing weight of the reproductive organs in the adulthood. The main reason for the reduction in weight and spermatogenesis is the presence of androgen and estrogen receptors in these organs that paly critical role in the spermatogenesis. On the other hand, gonadotropin receptor is also considered very important in the synthesis of androgens and spermatogenesis. It has been reported in several studies that any sort of alteration in these receptors lead into alteration in the testis physiology and spermatogenesis (Blake and Ashiru, 1997; Delfosse et al., 2014; Liang et al., 2016; Pelletier, 2000; Yang et al., 2017).

In the current study we observed that BPA and its analogues BPB, BPF and BPS at different concentrations not only resulted in potential hazardous effects on spermatogenesis but also lead into oxidative stress in the reproductive organs of male rats by reducing the DSP and altering seminiferous tubule epithelium. The results highlight the potential toxic effect of BPA and some of its analogues in different organisms tested in in-vitro and in-vivo studies where researchers observed the toxic effect of these compounds on male reproductive system (Chen et al., 2016b; Liang et al., 2016; Maćczak et al., 2016; Ullah et al., 2016, 2017, 2018; Zhang et al., 2016).

5. Conclusion

On the basis of the results from the present study, it can be concluded that exposure for a long period of time to low concentrations of BPA and its analogues BPB, BPF and BPS are capable of suppressing gonadotropins secretions from pituitary, exhibiting estrogenic and antiandrogenic effects in the mammals, inducing oxidative stress in the testicular tissues and affecting spermatogenesis by causing arrest at spermatogoneal stage as well as at the stage when spermatids can be seen. Further molecular studies need to be done to identify the exact mechanism of action of BPA and its analogues BPB, BPF and BPS through which it exhibits potential hazardous effects on the male reproductive tissues of mammals.

Declaration of interest

The authors report no declarations of interest.

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