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***Research Article** (Open Access)*

LOW-DOSE IMPACT OF BISPHENOL F EXPOSURE ON STRUCTURAL AND FUNCTIONAL EFFICIENCY OF TESTIS

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ABSTRACT
Bisphenol F (BPF) has replaced bisphenol A (BPA) in manufacturing products containing polycarbonates and epoxy. Regardless of its weaker estrogenic activity, health risks posed by BPF is not evaluated parallel to BPA. Various concerning reports of BPF exposure in association with cardiovascular disease, depression, reproductive defects etc. are surfacing. In the present study low-dose impact of BPF in relation to oxidative stress, germ cell progression, and management of sex hormones and gonadotropins were assessed. Wistar albino male rats were divided into groups based on doses of BPF (0 (Group I), 100 (Group II), 500 (Group III), and 1000 (Group IV)µg/ kg body weight/day) administered for 45 days. An additional Group V was added to assess simultaneous ameliorating effect of Vitamin E while administering 1000 µg/ kg body weight/day of BPF for 45 days. Stereological evaluation indicated significant decline in spermatogonial cells in Group IV (6.29 mil/testis).Histological architect revealed limited but continued production of sperm in all BPF treated rats.Group IV exclusively showed significant decline in testosterone (16%), estrogen (8%) and FSH (4%). Although level of LPO was significantly higher in groups III-V, no parallel change in activities was observed for GSH, SOD and CAT.Concurrent administration of Vitamin E showed minimum variation in level of testosterone and FSH, likewise, retention of normal sperm production was evident in Group V. In conclusion, BPF plays significant role in male reproductive system, by interfering in spermatogenesis, however, low-dose effects are relatively mild. It was also evident that role of Vitamin E was limited in regulating BPF induced oxidative stress in testis.

Keywords: *Spermatogenesis, Bisphenol F, Male infertility, Oxidative Stress, Vitamin E*

INTRODUCTION
Bisphenol F (BPF) is prepared by protonation of methylol glycol that reacts with phenol and formaldehyde.Where acetone is used to make bridging carbon in Bisphenol A (BPA) structure, formaldehyde is used to form the bridging carbon in BPF. It is used in heat resistant laminate, coating, adhesives, insulating materials and reactive intermediates. Due to low viscosity of liquid resins, it is highly suitable for moulding. The global market size of BPF is estimated as $1899.87 million (Business Research Insights, 2023). The market is expected to grow at rate of 2.59% and may reach $2214.69 million. With increase in demand, equally concerning rate of BPF contamination in adults and children has been reported (Lehmler *et al.,* 2018). Previous study has reported BPF as gonadotoxic substance that can induce hormonal imbalance and testicular damage (Odetayo *et al.,* 2023).

Spermatogenesis is the main function of testis, where precursor germ cells turn into mature sperm. The process has many stages, the events of spermatogenesis is cooperated by somatic support cells (SSCs). Sertoli cells are the most abundant type of SSCs in the testis (O'Donnell *et al.,* 2022). These cells harbour germline stem cells (GSCs) which once disassociated become spermatogonium, it eventually becomes sperm following multiple divisions and metamorphosis (Hermo *et al.,* 2010). Interstitial cells, primarily Leydig cells produce androgens which also play crucial role in spermatogenesis. However, its role is

dependent on release of specific hormones guided by anterior pituitary gland such as; luteinizing hormone (LH) and interstitial cells stimulating hormone (ICSH). Most importantly, testosterone is secreted by Leydig cells under controlled stimulus of LH (Ge *et al.,* 2008). It is an important androgen that regulates spermatogenesis by initiating and maintaining production of mature sperm.

Reactive oxygen species (ROS) is present in all physiological processes of cell, it may be beneficial at some occasion and harmful at another. In testis role of ROSs is complex, many studies elaborate that free radicals play important role in germ cell proliferation and maturation (Shi *et al.,* 2010). Nonetheless, high level of ROS generation may also initiate apoptotic, necroptosis, and pyroptosis process, eventually killing the cell through program cell death (Carneiro *et al.,* 2009; Challa and Chan, 2010; Vandenabeele *et al.,* 2010). Situation of such high generation of free radicals are favoured by various processes including inflammatory response, high cellular metabolism, activation of oxidases, activation of xenobiotics, oxygenases etc. Ionizing treatment during chemotherapy also causes generation of high oxidative stress in exposed area. Thus, a homeostasis between generation and neutralization of oxidative radicals must be maintained by the testicular cells to function optimally.

Bisphenol F is a known anti-androgen (Rosenmai *et al.,* 2014) and has shown signs of targeted damages to the tissues by generating excessive oxidative stress (Odetayo *et al.,* 2023). Nonetheless, mechanism by which BPF induce toxicity is still not clear. In the present study, stereological evaluation of germ cell progression in testis of BPF exposed Wistar rats were examined and compared against simultaneous alterations in the level of antioxidants in testicular tissues and sex hormones. Since oxidative toxicity is one of the major factors in damaging of BPF exposed cells, the present study counter evaluated role of Vitamin E in ameliorating the overall impact of BPF on testicular tissues.

MATERIALS AND METHODS

Chemical compound

Analytical grade Bisphenol F (BPF) or Bis(4-hydroxyphenyl) methane, 4,4'-Methylenediphenol ($CH_2(C_6H_4OH)_2$), CAS Number – 620-92-8, was purchased from Sigma Aldrich-Merck (NJ, USA).

Animals model and approval from ethical committee: Wistar albino male rats (*Rattus norvegicus*) were used in the present study. All rats were of 3-months-old weighing in range of 150-200 g. All animals were maintained under veterinary supervision in the departmental animal facility of Department of Zoology, University of Rajasthan, Jaipur. These animals were housed in polypropylene cages of size 43×27×15 cm. Drinking water was provided *ad libitum* and a 12 h dark and 12 h light schedule was maintained in the animal house.Approval for conducting experiments were procured before commencing the experiments from Institutional Animal Ethics Committee (IAEC). All experiments were carried out in accordance with guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments and Animals (CPCSEA).

Experimental design

Animals were randomly divided into groups containing five animals each. Group I: Vehicle treated control. Based on doses of BPF animal groups were formed Group II: 100 µg/kg body weight/day, Group III: 500 µg/kg body weight/day, and Group IV: 1000µg/kg body weight/day. An additional group was formed to observed amelioration effect of Vitamin E by administrating 80 mg/kg body weight concurrently with 1000µg/kg body weight/day of BPF. BPF treatment were carried out for continuous 45 days, on the 46th day euthanization was carried out according to the CPCSEA guidelines. BPF was dissolved in distilled water to achieve required concentration and orally administered through oral gavage (stainless steel tubes of 2" 18 ga size). All doses used in the study was referred from earlier studies (Wagner *et al.,* 2021; Lee *et al.,* 2022) and were in accordance with OECD guidelines for testing of chemicals (OECD, 2008).

Parameters

Stereological analysis of germ cells and Sertoli cells: Numerical density and count of germ cells were evaluated according to the methods described by Zhengwei *et al.*, (1999) and Wreford (1995). Germ cells were counted in nuclear number on anaxioscopic microscope. Counting of cells were carried out by Image J software (NIH, USA) in still images taken at 40X magnification. Systemic Uniform Random Sampling Scheme (SURSS) was used for field counting (Gundersen and Jensen, 1987). Following formulae were used to calculate numerical density (N_V) and count (N_C) per testis:

N_V = number of cells counted/(area of frame × number of frames × depth) (1)

$N_C = N_V \times$ testis weight (2)

Analysis of testicular micrograph: A small section of testis preserved in 4% paraformaldehyde were dehydrated in graded ethanol. Later cleared in xylene and embedded in paraffin wax. With the help of microtome precision cutting of the embedded tissue block was carried out. A 5 µm thick section was cut and placed over a microscopic slide. The tissue was stained with Harris's haematoxylin and eosin, and later observed under microscope.

Response of antioxidative enzymes: Testicular tissues were prepared by homogenizing a portion of tissue in 10% w/v ice-cold potassium phosphate buffer (pH 7.4). A 0.2 ml of the tissue suspension was used for thiobarbituric acid reactive substance (TBARS) estimation (Ohkawa *et al.*, 1979). Later 1 ml of homogenate was mixed with 10% trichloroacetic acid (TCA) and estimated for GSH estimation (Hissin and Hilf, 1973). For estimation of SOD (Marklund and Marklund, 1974) and CAT (Aebi, 1974), homogenized tissues were centrifuged at 40000 g for 60 min and supernatant was collected. Likewise, activity of glutathione peroxidase (GPx) was measured according to Wood (1970). Protein concentration was estimated according to Bradford (1976).

Level of serum testosterone and gonadotropins: Serum testosterone, estrogen, follicle stimulating hormone (FSH), and luteinizing hormone (LH) were estimated by commercially purchased ELISA kits (ThermoFisher Scientific, MA, USA).

Statistical analysis: All numeric values were represented in Mean±SE, level of significance of variance was evaluated against sham control. Student *t* test (MS-EXCEL, SV, USA) was applied for all paired data. One-way ANOVA (MINITAB, USA) was used in conjunction with Tukey's multiple comparison test to estimated non-parametric data. Level of significance was estimated at confidence intervals (CIs) of 95%, 99% and 99.99%. Two-axis plots were used to comparatively represent volume and count of germ cells. Origin of synthesis of hormones were assessed for induced alterations by two-axis plot (MS-EXCEL, SV, USA). Relatedness between parametric variation under influence of treatment was assessed by regression analysis (R^2) and exponential forecast.

RESULTS

Stereological evaluation of germ cells and Sertoli cells

Similar pattern in alteration of counts of germ cells were noted in stereological analysis. Among all three evaluated germ cells i.e. spermatogonia ($p=0.003^{-3}$), spermatocytes (p=0.001), and spermatids (p=0.0005), indicated highly significant decline in volume/cm^3 and count/testis of Group IV. The decline appeared to be dose dependent in all three investigated germ cells, indicating direct impact of BPF administration (Figure 1). Although, the decline in germ cells count in other groups of BPF treated animals were also significant comparing to control, nonetheless, the level of significance for Group II (p=0.017) was narrow and thus values were close to control. Besides, Group III and V showed highly significant decline in counts when compared against control (Figure 1). In contrast, response to BPF administration on Sertoli cell count were completely deviated from responses recorded for germ cells. Group II and V showed non-significant alteration in the count of Sertoli cells, whereas, Group III indicate only narrow variation when compared with control (Figure 1). Regardless, decline in number of Sertoli cells per testis was highly

significant in Group IV, which indicates robust effect of BPF exposure on treated animals.

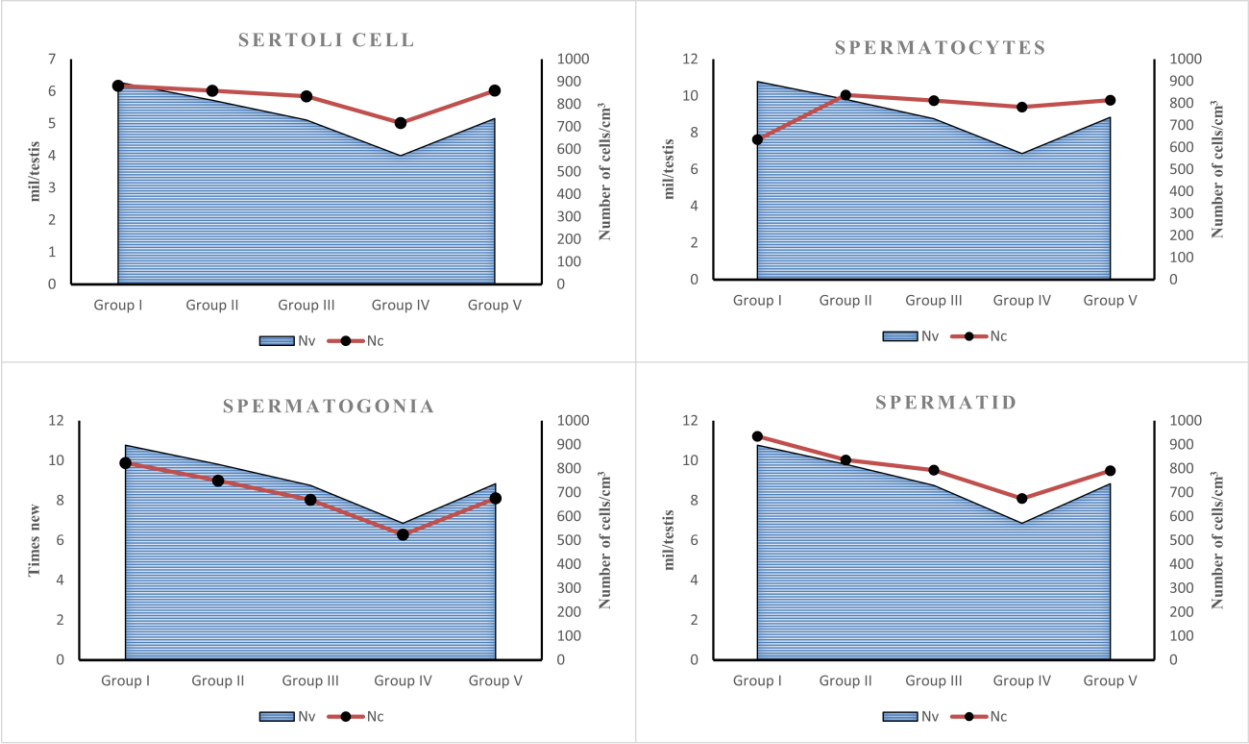

Figure 1: Stereological evaluation of testicular cells in control and BPF administered groups.

Histological architecture of testicular tissues

Histological attributes of testis of Group I showed normal architecture revealing proper spermatogenic events. Lumen of seminiferous tubules was filled with newly formed sperms. Spermatogonia, spermatocytes and spermatids were resting on the basal lamina and epithelial layer showed all stages of spermatogenesis (Figure 2A). Similarly, Group II showed normal spermatogenesis, lumen of the seminiferous tubules was adequately filled with mature sperms. However, thin basal lamina was apparent in the histological architecture. Loss of cells were also found in the interstitial spaces (Figure 2B). Remarkably, the disassociation between seminiferous tubules were almost complete in Group III. Though spermatogenic event were present in the amount of sperm in the lumen was significantly lower comparing to control (Figure 2C). spermatogenic stages in seminiferous tubules were partially disoriented in Group III. Nevertheless, the extent of disorganization of germ cell progression in Group IV was significantly higher. Lumen was partially filled with mature sperms, epithelial layer showed minimum cell progression. Likewise, basal lamina was thin, appearance of vacuolization was observed at few occasions, indicating potential toxicity. Regardless, spermatogenesis in Group IV was apparent, production rate however was positively affected (Figure 2D). Animals simultaneously treated with Vitamin D besides BPF showed limited but normal spermatogenesis. Epithelial layer contained all stages of spermatogenic events, however, interstitial space was still disoriented.

Alteration in levels of hormones related to spermatogenesis

Estimation of serum testosterone indicated no alteration in Groups II and III, indicating minimum or not

impact of BPF. Nonetheless, Group IV and V showed significant decline in the level testosterone (Table 1). In contrast, level of estrogen was mostly immune to BPF exposure regardless of doses. Although minor reduction was noted in Group IV, which statistically found significant (p=0.032), nonetheless, the

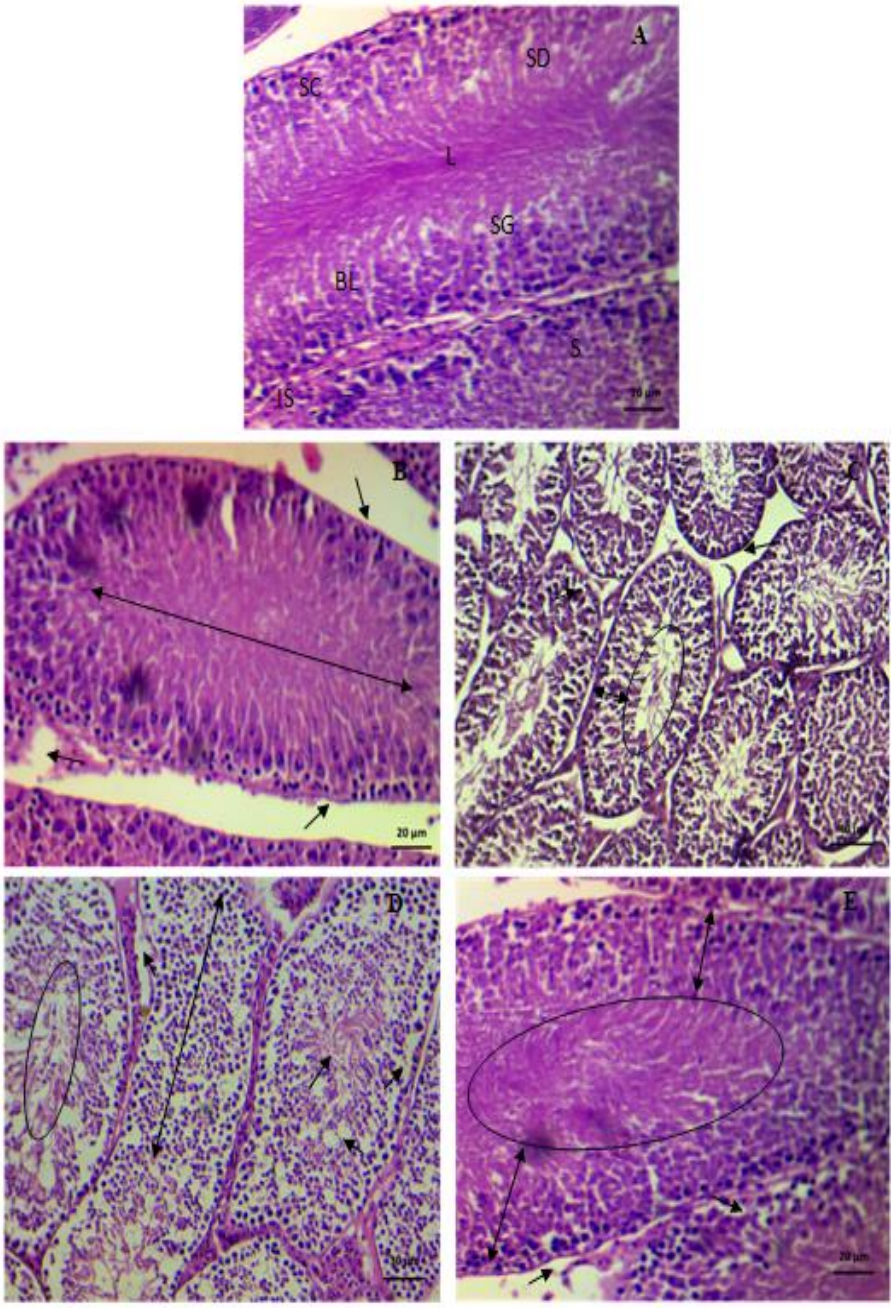

Figure 2: Testicular histology showing normal cellular architecture in Group I (A). While loss of interstitial space was evident in Group II (B) sperm production appeared nominal. Major adversities were recorded in Groups III (C) and IV (D), loss of Leydig cells and interstitial space was common. Higher disorientation in germ cell progression was apparent in Group IV, also presence of vacuolization was witnessed. Group V (E) showed normal cellular architecture however, loss of interstitial cells was still appeared.

deviation was very minor and may be influenced by other factors. In the two-axis plot it appeared that though dependence of variables on each other for unique cause was low (based on origin of synthesis), in Group IV both variations were intercepted, thus linking a possible common factor (Figure 3A). Impact of BPF on pituitary driven gonadotrophs were limited as no test groups except for Group IV showed any significant variation when compared with control (Table 1). FSH was significantly decline in Group IV (p=0.020), whereas, other test groups for lower doses reveal no impact. On the other hand, LH showed no significant decline in the serum concentration, revealing minimum or least influence of BPF. Still the difference in concentration of both hormones indicates indirect or selective regulation of gonadotrophs (Table 1). The lack of dependence in response to BPF led variation was highly conspicuous indicating FSH linkage completely independent of LH concentration in serum (Figure 3B).

	Testosterone (ng/ml)	Estrogen (pg/ml)	FSH (mUI/ml)	LH (mUI/ml)
Group I	**2.37±0.01**	**1.30±0.01**	**3.25±0.03**	**4.02±0.05**
Group II	**2.36±0.02**	**1.24±0.04**	**3.19±0.03**	**3.69±0.18**
Group III	**2.31±0.04**	**1.25±0.03**	**3.26±0.05**	**3.82±0.16**
Group IV	**1.98±0.05****	**1.19±0.03***	**3.11±0.05***	**3.86±0.16**
Group V	**2.22±0.04***	**1.23±0.02**	**3.27±0.04**	**3.93±0.12**

Table 1: Estimation of testosterone, estrogen and gonadotrophs in control and test groups. Level of significance was measured against Group I. *p<0.05, **p<0.01.

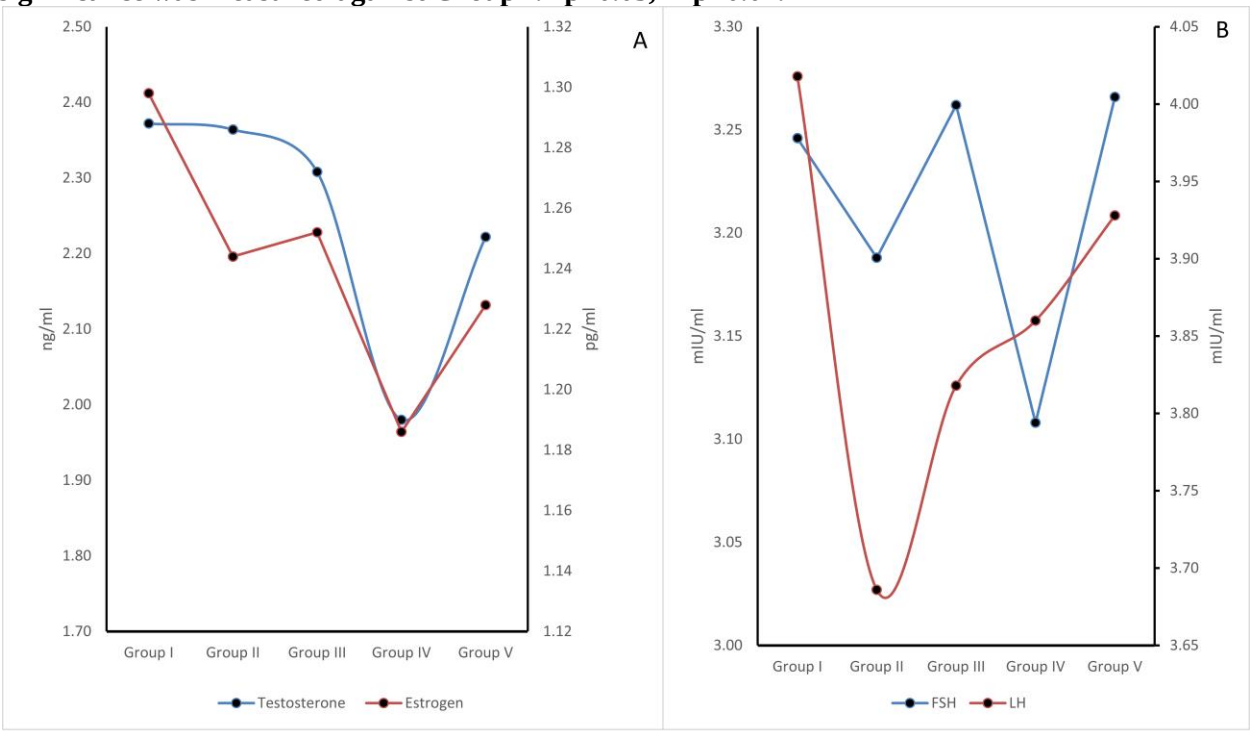

Figure 3: Dependence of hormonal level in serum under influence of BPF based on origin of synthesis. A. Leydig cell synthesized testosterone and estrogen. B. Pituitary synthesized LH and FSH.

Oxidative stress and antioxidative response of testicular tissue

Evaluation of lipid peroxidation indicated dose dependent increase in the testicular tissues (Figure 4A). Group II showed best toleration against the BPF doses, nevertheless, highly significant decline was noted in level of SOD (p=0.001). Activity of SOD was also substantially declined in Group III (p=0.041) (Figure 4D). Activities of CAT was normal in all groups except for Group III (p=0.037) which revealed significant decline (Figure 4E). Activity of GPx was reduced substantially in Group III (p=0.003), IV (p=0.004), and V (p=0.016). Whereas, activities of GSH was mostly unaltered in Groups II, IV, and V (Figure 4C). Activities of antioxidants showed higher imbalance in activities of GPx and SOD comparing to CAT, and GSH, most of which were found in Groups II and III. Results showed dose-based biases in response of antioxidants. In the relatedness plot all four test groups (II (R^2=0.834), III (R^2=0.802), IV (R^2=0.829), and V (R^2=0.846)) showed extreme similarities to the control. However, closest resemblance between Groups II and V indicated negligible low dose response of BPF and ameliorating effect of vitamin E. Regardless, the extent of generation of oxidative radicals (in response to BPF treatment) in the testicular tissues were appeared to be adequately managed by the in-house antioxidants (Figure 5).

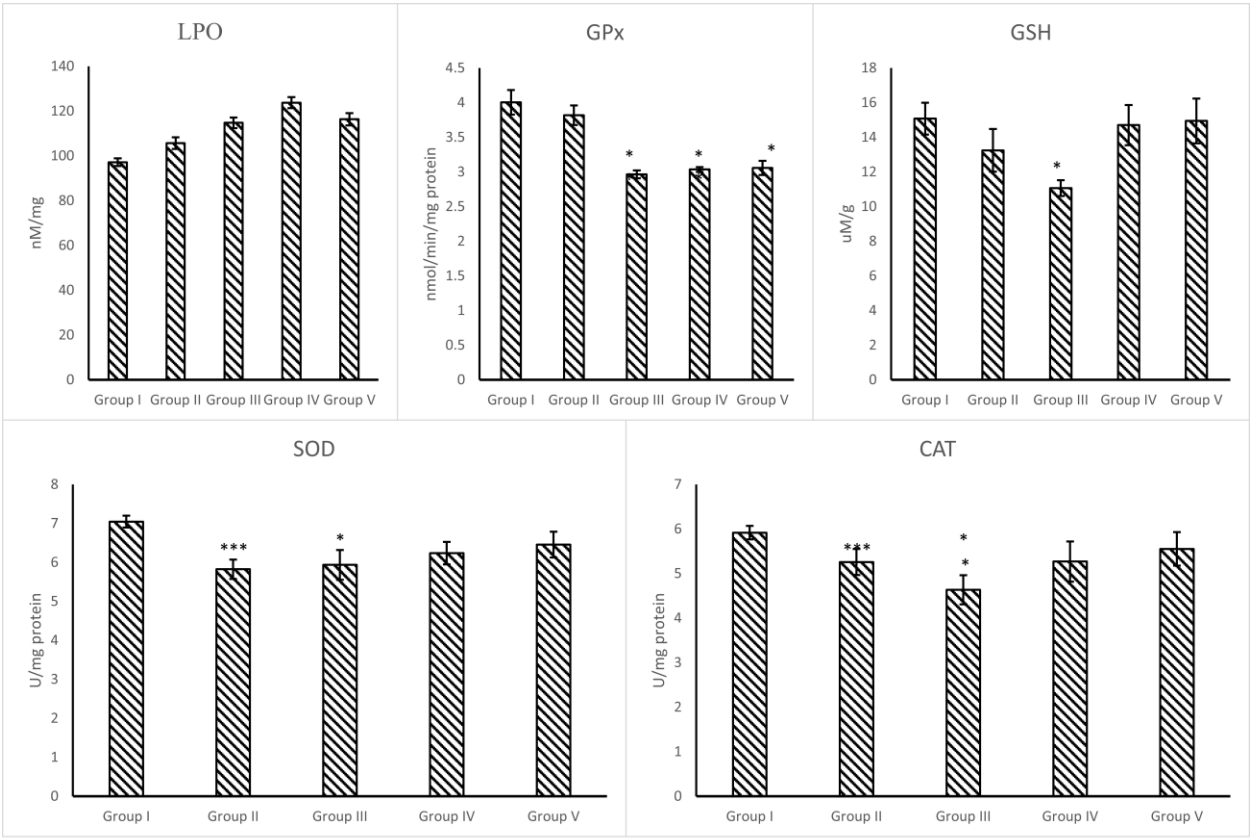

Figure 4: Activity of antioxidant in testicular tissues in rats following administration of BPF in comparison with control. Level of significance was measured against Group I. *p<0.05, **p<0.01, ***p<0.001.

DISCUSSION

Phasing out of BPA from mainstream market and reducing its contamination required a suitable alternative. Credentials of the candidate compound must have similar properties, but with minimum or no adversities to human health or the environment. Many countries have decided to abolish use of BPA in highly concerning products and replacing with BPF and BPS instead (Song *et al.*, 2017). Despite global

efforts, BPA substitutes-based products could only become strong candidates for replacement.

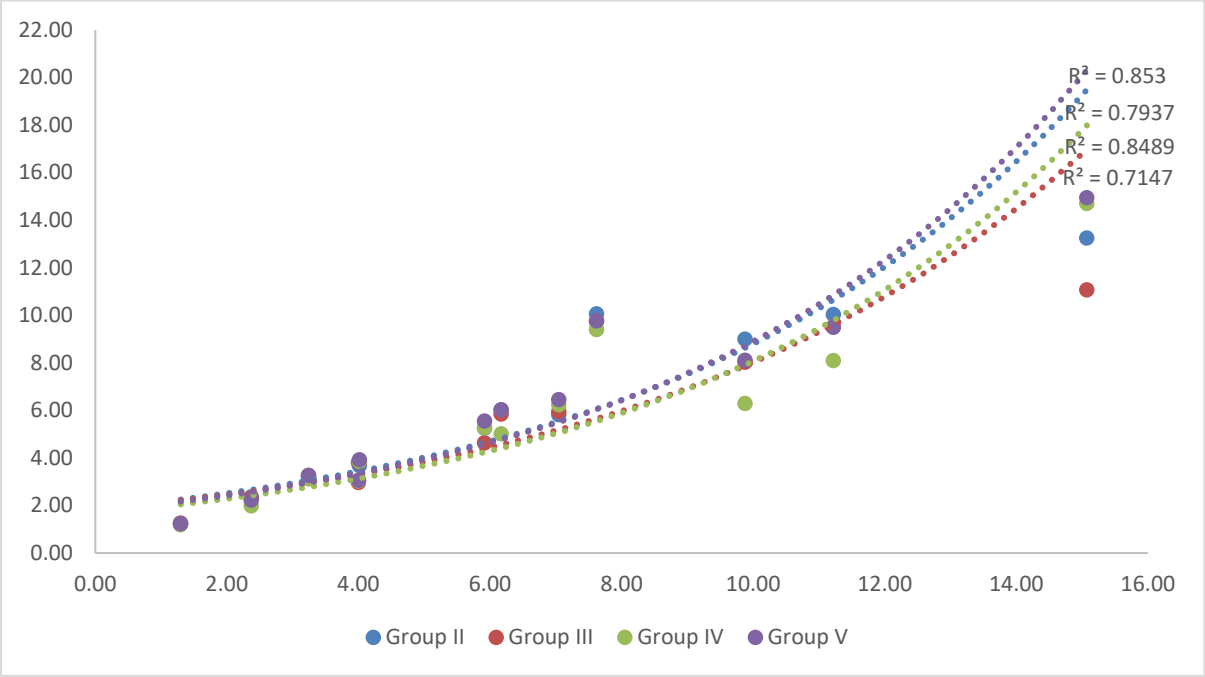

Figure 5: Relatedness in variations of parametric variables (antioxidant activities, hormonal levels, and germ cell counts) against control under influence of BPF.

Notwithstanding, Kojima *et al.*, (2019) reported significantly lesser extent of endocrine disruption by BPS and BPF comparing to BPA. Although this study still claims both agonistic and antagonistic activities of BPF and BPS against androgen receptor (AR), estrogen receptor (ER)-alpha and beta. A study by Martinez *et al.*, (2020) reported that BPS has more endocrine disrupting ability comparing to BPF and to the greater extent BPA. Thus, BPF leads the candidature by significant margin. However, it cannot be ruled out that level of research to establish BPA as toxic and harmful to health, took decades of rigours work. In the present study an attempt was made to evaluate potential antifertility activity of low-dose BPF exposure in male. Special attention was given to spermatogenesis and hormonal balance under influence of BPF. The histological micrograph indicted little or no disparity between control and group of animals administered with 100 µg/ kg body weight of BPF. Interestingly, production of sperms continued in all test groups, regardless of daily doses of BPF. It is however, important to note that although production of sperm continued among all groups, reduction in production of sperm was evident in groups of animals treated with 500 and 1000 µg/ kg body weight of BPF. A study by Ullah *et al.*, (2019) reported that high-dose exposure of BPF significantly reduces spermatogenesis. Unlike BPA and BPS reproductive toxicity in form of vacuolization (Vijaykumar *et al.*, 2017; Mas *et al.*, 2021) and pyknotic cells in the seminiferous tubules were significantly low and were only present in animal treated with 1000 µg/ kg body weight of BPF. A study by Odetayo *et al.*, (2023) reported that 10, 30, and 50 mg/kg BPF could induce apoptosis in germ cells and promoted coagulative necrosis. Therefore, the present study hypothesizes that higher doses BPF may cause similar adversities in seminiferous tubules as shown earlier by BPA and BPS. Simultaneously, low dose exposure of BPF are most likely to pose minimum damage to the testicular cells, adversities posed by the low dose may reverse with countertreatment or upon withdrawal. The present study revealed that administration of Vitamin E to animals exposed to 1000 µg/ kg body weight of BPF tolerated BPF induced injuries, significantly. Stereological evaluation

confirmed earlier assumptions made in the present study. The study revealed only major decline in group of animals administered with 1000 µg/ kg body weight of BPF, that too was restricted to spermatogonia and too some extent spermatids. It appeared that decline in spermatids was consequential event of low spermatogonial cell count. Low spermatogonial cell is indicative of irregular proliferation due to poor mitotic index (Ehmcke and Schlatt, 2006). There could be number of factors that may interfere with spermatogonial cell count, such as genetic disorder, chemotherapy, treatment related necrosis and or anatomical abnormalities. Apoptosis and necrosis are normal phenomenon in testicular tissues to regulate number of germ cell (Napoletano *et al.,* 2017). It is possible that exposure of BPF may engaged with factors influencing regulation of spermatogonial cell counts. It may also be assumed that other external factors, such as; excess generation of oxidative radicals may force spermatogonial cells to undergo apoptosis. The present study showed normal concentration of testosterone, estrogen, FSH and LH in groups of animals administered with 100 and 500 µg/ kg body weight of BPF. However, significant decline in levels of testosterone and FSH was observed in high dose group (1000 µg/ kg body weight). The present result was in accordance with study carried out by Ullah *et al.,* (2019), they also reported decline in level of testosterone and FSH when administered high dose of BPF (1, 5, 25, 50, and 100 mg/kg body weight). Since in the present study maximum dose of BPF was 1 mg/kg body weight results deviated slightly form the study carried out by Ullah *et al.,* (2019). The referred study showed significant decline in the level of LH along with FSH which varied in the present study as no change in concentration of LH was recorded in any test groups regardless of doses of BPF. Strikingly, the present study noted significant decline in the level of estrogen. It is to be noted that estrogen level affect sperm count and motility (Aschim *et al.,* 2005; Guarducci *et al.,* 2006). Thus, decline in the level of estrogen under influence of BPF accounts for a separate mechanism linking to reproductive insufficiency in male. Adequate estrogen concentration is also important for bones, low level of estrogen is associated with osteoporosis. Interestingly, a study by Kim *et al.,* (2021) revealed that bisphenols have role in creating imbalance between osteoclasts and osteoblasts. The claim made by Kim *et al.,* (2021) also confirmed role BPF in alteration of estrogen concentration in serum.Despite significant impact on testosterone, FSH and estrogen following administration of 1000 µg/ kg body weight of BPF, its role in lower dose groups were irrelevant. The present study showed that impact of 1000 µg/ kg body weight of BPF on testosterone and estrogen were alike, nonetheless, in lower dose groups, variation in concentrations of both hormones were independent from each other indicating, damage of Leydig cells under influence of BPF is dependent on dose threshold. Unlike testosterone, and estrogen, level of FSH and LH were completely independent from each other ruling out any direct association between BPF and hypothalamic-pituitary-gonadal (HPG) axis.Since FSH and LH function together the imbalance may affect testosterone synthesis and eventually the spermatogenesis. A study by Gordetsky *et al.,* (2012) reported that abnormal FSH in male are associated with abnormal semen profile. Previous studies have noted that low FSH in male is linked with negative feedback to the pituitary gland (Orlowski and Sarao, 2023; Kazmi and Can, 2023). Activities of antioxidants in testicular tissue of animals treated with BPF indicated depletion. Among investigated antioxidants, activities of GPx showed significant decline in animals exposed to 100, 500, 1000 µg/ kg body weight of BPF. This observation was in accordance to Linillos-Pradillo *et al.,* (2023), the study reported significant decline in the activities of GPx in group of animals administered with 0.036, 3.6 mg/kg body weight/day of BPF. It was important to note that response of antioxidants to BPF were substantially varied with respect to doses. Authors of this study revealed that higher dose of BPF were less depleted in activities comparing to lower dose. The present study also noted dissimilarities in response of antioxidants in response to 100, 500, 1000 µg/ kg body weight of BPF. Lowest activities of SOD, GSH, and CAT were observed in animals treated with 500 µg/ kg body weight of BPF, whereas, animals treated with upper and lower doses showed better toleration. Regardless of extreme contrast in antioxidant responses, the common factor was rate of lipid peroxidation in testicular tissues.

Measurements of LPO were dose dependent and significantly higher in the test groups, indicating robust generation of oxidative radicals. The present study observed minimum impact of Vitamin E doses in animal exposed with 1000 µg/ kg body weight of BPF. It was noted that despite maximum dose of BPF in the study, no significant alterations were found for activities of SOD, CAT, and GSH when compared with control. Activities of these antioxidants were alike in both groups of 1000 µg/ kg body weight of BPF, with or without treatment of Vitamin E. Similarly, when GPx showed significant variation in group of animals administered with 1000 µg/ kg body weight of BPF, concurrent administration of Vitamin E could not alter much in the activity. Relatedness between variations in antioxidant activities, hormonal alteration and stereological evaluation showed extreme linearity (>0.8). The exponential forecast linked all three parameters as extremely related under isolated event, in the present case this event could most likely be the exposure of BPF. It reveals that BPF has dynamic impact on spermatogenesis, and testicular functions. Based on previous reports produced against other bisphenol analogues (Xi *et al.,* 2011; Mínguez-Alarcón *et al.,* 2017), the adversities were not too intense at low dose exposure. However, long-term and high-dose exposure are highly likely to cause reproductive adversities in male. Relatedness among variations in animals treated with Vitamin E under influence of BPF, showed closest resemblance to control, indicating positive tolerance against BPF induced alterations.

CONCLUSION

The present study concludes that BPF plays important role in modulation of testicular function and spermatogenesis. However, low dose exposure is less likely to significantly affect fertility. It appeared that role of Vitamin E in regulating damages in testicular tissues were limited, but effective in retaining the functional abilities. Level of testosterone, FSH and LH were least affected by lower doses of BPF. Based on observations of histological micrograph, spermatogenesis was affirmatively affected by the doses exceeding 100 µg/kg body weight/day, however, no strong evidence of reproductive toxicity was observed. Thus, though lipid peroxidation was high in testicular tissues of exposed rats, the change in response of antioxidants indicated manageable toleration. It also reflected that BPF induced oxidative stress at the investigated doses exert limited adversities on testicular function or structure. Stereological evaluation revealed trend of depletion in spermatogonial cell count by doses over 500 µg/ kg body weight/day, which indicate potential fertility inadequacy in long-term exposure.

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CONFLICT OF INTEREST

There is no conflict of interest.

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