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Original Research Article

Role of Bisphenol-A Induced Oxidative Stress in Spermatogenesis of Wistar Albino Rats

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ABSTRACT:
Bisphenol-A is a known endocrine disruptor and reproductive toxicant. It has been associated with epigenetic modification, autophagy, oxidative stress and apoptosis. Many studies have indicated interferences in spermatogenesis due to Bisphenol-A exposure. Since oxidative stress plays important role in regulation of various stages of spermatogenesis, therefore, excessive generation of Reactive Oxygen Species can also have effects on spermatogenesis that may reach beyond hormonal regulations. Three doses of Bisphenol-A (10, 50, and 100 mg/kg body weight) were investigated for its effect on testicular antioxidant status (Catalase, Glutathione, Glutathione S Transferase, , Glutathione Peroxidase and Glutathione disulphide). Stereological estimation of germ cells (spermatogonia, spermatocytes, spermatids) and Sertoli cells was carried out to spermatogenic interferences in the histological architecture. Cause and effect analysis was tested to elucidate association between oxidative stress and numeric spermatogenesis. This study indicated severe decline (30-70%) in the activities of major antioxidant enzymes in response to Bisphenol-A exposure. Higher doses (50 and 100 mg Bisphenol-A) induced maximum damage in histological architecture of testis. Seminiferous tubules indicated loss of germ cells and consequently sperms in the lumen. The histological evaluation went in accordance with stereological evaluation of germ cells. A pattern in disorientation of germ cells was intercepted through morphometric assessment that corresponded to activity of antioxidants. Bisphenol-A induced oxidative stress plays important role in regulation of spermatogenesis. There was a clear parallel between number of germ cell and Sertoli cell decline and loss of activity of major antioxidants. BPA induced oxidative stress had differential effect on germ cells, where spermatogonia and spermatids appeared to have low toleration than spermatocytes.

Keywords: Antioxidants, Bisphenol-A, Spermatogenesis, Stereological evaluation.

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INTRODUCTION

The most recent surveys on production and consumption of Bisphenol-A indicate an increase in compound annual growth rate (Global Industry Analysts, Inc., 2015; Industry Experts, 2016; Statista, 2020). Most surveys predict that value-wise the rate of growth may reach USD 22.5 billion by the year 2022, amounting to an expected consumption of 10.6 million metric tons (Almeida et al., 2018). This trend in itself defines the mammoth global production of polycarbonates, which is the primary driver of BPA demand.

There are continuous warnings and declarations on tolerable daily intake of BPA through various regulatory agencies and research studies (Vandenberg et al., 2007; Kang et al., 2006; EFSA, 2006; Chapin et al., 2008; Vandenberg et al., 2013; FAO/WHO, 2011; Shelby, 2008; Geens et al., 2012). Despite rapid first-pass metabolism in liver (Thayer et al., 2015; Volkel et al., 2002) and effective detoxification system, detectable levels of free and/or conjugated BPA can be found in population (Calafat et al., 2008). Controlling BPA exposure through ingestion is not sufficient, as it is present in cosmetics, thermal paper, air and dust (Vandenberg et al., 2007; EFSA, 2015; Dodson et al., 2012). Thus, BPA can be absorbed through alternative means, such as, inhalation and transdermal. Notably, ingestion through transdermal route can bypass the liver metabolism, adding more unconjugated BPA into the bloodstream (Pottenger et al., 2000; Negishi et al., 2004; Tominaga et al., 2006; Lv et al., 2017; Lee et al., 2018).

BPA is an endocrine disrupting chemical thus causes adverse activity in both male and female reproductive systems. There are various studies reported that BPA causes combinatorial effects on central and peripheral functions related male reproduction (Akingbemi et al., 2004; Nanjappa et al., 2012). BPA can interfere with hypothalamic-pituitary-gonadal (HPG) axis, resulting abnormal spermatogenesis. Spermatogenesis is the key function in testicular cells that determines the fertility in male. It carries out highly complex mitotic cell division, meiosis and spermiogenesis. These processes require multiple levels of regulation at both endocrine and paracrine mechanisms. It is widely reported that BPA causes oxidative damages in testicular tissues (Olukole et al., 2020), however, there is considerable variability in the adversity that occur before and after spermiogenesis. Previous studies claim that spermatogonia are extremely tolerant to oxidative insults when compared with spermatozoa, which is rather more susceptible (Celino et al., 2011). A study by Kaur et al. (2018) explained that following administration of BPA in mice, lumen of seminiferous tubules was devoid of spermatozoa and significant decrease in germ cell count were found. Damages evident in the testicular tissues due to BPA induced oxidative stress, indicate an unspecified pattern. In this study, we aim to investigate spermatogenesis in BPA administered Wistar albino rats at stereological level to evaluate oxidative damages and compare with the histological observations.

MATERIALS AND METHODS

Test material

Bisphenol A [2,2-bis (4-hydroxyphenyl) propane or $C_{15}H_{16}O_2$] (99% purity) were purchased from Sigma Aldrich (MO, USA). The above was dissolved in olive oil for appropriate doses based on designed groups.

Animals model and ethical approval

Wistar albino rats (*Rattus norvegicus*) weighing around 150-200 g of 3 months old were used in this study. Animals were maintained in polypropylene cages (size 43×27×15 cm) with appropriate ratio of light and dark. Rats were provided with pellet diet and water *ad libitum*. Experiments strictly carried out under guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, 2010). Guidelines of Indian National Science Academy (INSA), New Delhi for Care and use of Animals were followed and approval for experiments was procured from Institutional Animal Ethical Committee (IAEC).

Experimental design

Healthy animals with proven fertility were divided into four groups of 10 animals each. These groups were consisted of a placebo control and three test groups of variable doses of BPA. Olive oil was used as a vehicle to administer BPA, which was dissolved in a 1:1 ratio just before administration. Groups were

labelled as, Group I-placebo control, Group II-oral administration of 10 mg BPA/kg body weight, Group III-50 mg BPA/kg body weight, and Group IV-100 mg BPA/kg body weight. All groups were investigated in parallel with each other for 6 continuous weeks.

Evaluation of oxidative stress in testicular tissues

Catalase (CAT)

The catalase activity was determined according to the method explained by Aebi (1974). In brief, rate of hydrogen peroxide decomposition was measure by estimating utilization of catalase. In reaction, 475 µl of phosphate buffer (pH 7.0) was mixed with 25 µl of H_2O and 250 µl of H_2O_2 and allowed to settle in as control. On the other side, 475 µl of phosphate buffer (pH 7.0) was mixed with 25 µl of liver tissue homogenate and 250 µl of H_2O_2 was allowed to inhibit stress. Both reactions were measured for rate of decomposition of hydrogen peroxide at 240 nm on a spectrophotometer. Activity of enzyme was noted in U/mg protein, where, U (unit) stand for µmol H_2O_2 decomposed/min.

Glutathione peroxidase (GPx)

Activity of glutathione peroxidase was measured in liver tissue homogenate according to the methods explained by Wood (1970). Supernatants were collected following ultracentrifugation of tissue homogenate at 14000 X g for 25 minutes. Briefly, 700 µl of phosphate buffer (pH 7.0) was taken in a 1 ml cuvette. To this 25 µl of glutathione reductase, 25 µl of sodium azide, 50 µl of NADPH and 50 µl of sample was added. This mixture was mixed nicely before bringing temperature of the solution to 25 ^{o}C. Once the solution is equilibrated to the said temperature hydrogen peroxide was added to the solution and absorbance was noted for 5 minutes at 1 minute interval for change of NADPH into NADP. Absorbance were noted and activity of enzyme was expressed in NADPH oxidized/min/mg protein.

Glutathione (GSH)

Glutathione content was measured fluorometrically by the method of Hissin and Hilf (1976). A 250 mg testicular cell pellet was suspended in a medium of 25% metaphosphoric acid and potassium phosphate buffer (pH 8.0), sonicated for 10 min. Then it was centrifuged at 30,000 x g for 30 min. The supernatant kept at 0°C, until used. Level of GSH was determined with a fluorescence spectrophotometer at 420 nm (excitation at 350 nm) after incubating 0.2 ml of supernatant with 1.7 ml potassium phosphate-EDTA buffer (pH 8.0) and 0.1 ml of fluorescence reagent o-phthaldialdehyde (1 mg/ml) for 15 min.

Glutathione disulfide (GSSG)

Glutathione disulphide was measured fluorometrically by the method of Hissin and Hilf (1976). Supernatant from testicular pellet was collected similarly as explained earlier for GSH activity estimation. The supernatants were assayed on the same day. The GSSG level was estimated, by incubating 0.5 ml of the supernatant with 0.2 ml of 40 m*M N*-ethylmaleimide (NEM) for 30 min at room temperature. After adding 4.3 ml NaOH (0.1 M), 0.2 ml of mixture was incubated with 1.7 ml 0.1 M NaOH and 0.1 ml OPT solution, 1 mg/ml, for 15 min. Fluorescence was determined similarly to that of GSH assay. The result of assays was calculated against a standard calibration curve for GSH and GSSG.

Glutathione S transferase (GST)

GST activity was measured by the method of Habig et al., (1974). The reaction mixture containing 1 ml of buffer, 0.1 ml of 1-chloro-2, 4-dinitrobenzene (CDNB), 0.1 ml of homogenate and 1.7 ml of distilled water was incubated at 37°C for 5 min. The reaction was then started by the addition of 1 ml of glutathione. The increase in absorbance was followed for 3 minutes at 340 nm. The reaction mixture without the enzyme was used as blank.

Stereological analysis of testis

Optical dissector method was used to determine the number of germ cells in testicular tissues (Zhengwei et al., 1998; Wreford, 1995). One cell was assumed through confirmation of one nuclear number. Sections were analysed using a 40X lens on an axioscopic microscope equipped with a high definition camera. Cells were counted manually with the help of Image J software (National Institute of Health (NIH), USA) for enhanced imaging and clear nuclear counting. Microscopic fields for counting were selected

using a systematic uniform random sampling scheme (Gundersen and Jensen, 1987). Thirty frames of 100 μm², corresponding to 3000 μm² were evaluated per animal.

The numerical density (N_V) of each cell type was calculated by dividing the number of cells counted by the volume of all dissectors:

$$Nv = \frac{\text{Number of cells counted}}{\text{Area of frame} \times \text{Number of frames} \times \text{Depth of section}}$$

The number of cells (Nc) per testis was calculated as:

$$Nc = Nv \times \text{Testis weight}$$

The germ cells were grouped into spermatogonia, spermatocytes, round and elongated spermatids. Number of Sertoli cell were determined as described earlier for the germ cells.

Histological evaluation of testis

A portion of testis was fixed in 4% paraformaldehyde for overnight. Further, dehydrated in ethanol, cleared by Xylene and embedded in paraffin wax. A 5 μm thin section were stained with Harris's haematoxylin and eosin. Following staining specimens were observed under light microscope at various magnifications.

Statistical analysis

The mean values were compared using respective standard error of mean (SEM) followed by statistical comparison between control and test groups for evaluation of significant changes in values by one way analysis of variance (ANOVA) test along with Tukey's multiple comparisons (MINITAB, Pennsylvania, US). Significant variation will be assessed based on confidence intervals (CIs) of 95%, 99%, and 99.99%, regarded as significant, highly significant and extremely significant, respectively. Radar plot (MS-EXCEL, Microsoft, SV, US) was used to reveal skewness in variations of numeric data between parameters. Linearity between variables of stereological evaluation was examined against sham control. For comparative analysis of numeric variation between parameters were assessed for strength of association and potential linkage through Pearson's correlation test (MS-EXCEL, Microsoft, SV, US).

RESULTS

Status of antioxidants in the testicular tissues

Antioxidants activities in testicular tissues revealed a constant decline with respect to the administered doses of BPA. Results indicated significant to extremely significant decline in activity of CAT, GPx, GSH, and GST, in response to the investigated BPA doses. Highest dose of BPA (Group IV) led to lowest activity of these antioxidants. When compared with control, administration of 100 mg BPA (Group IV) induced 55%, 67%, 70%, and 72% decline in the activity of CAT, GPx, GSH, and GST, respectively. Whereas, Group III showed 40-62% and Group II 30-32% decline in the activities of same antioxidants (Figure 1). Level of GSSG however, largely remained unresponsive to BPA induced toxicity. Level of GSSG in Group I was recorded as 80.08±1.66 mM/g tissue, whereas, in groups II-IV it was estimated as 76.60±1.38, 84.12±1.95, and 83.13±1.81 mM/g tissue, respectively. Although Group II indicated decline in level of GSSG, nonetheless, group III and IV indicated insignificant but higher levels than control (Figure 1).

Counts of germ cells and Sertoli cells

Total estimated count of spermatogonia, spermatids, spermatocytes and Sertoli cells in 1 cm³ (or 1 g tissue) of testis of placebo control (Group I) was 1.43±0.01, 0.88±0.03, 1.61±0.02, and 1.07±0.02 mil. Groups II-IV showed extremely significant decline in all investigated cells (Figure 2). According to the results a dose dependent reduction in number of spermatogonia, spermatids and Sertoli cells was evident. However, numbers of spermatocytes were unexpectedly increased in Group II by almost 41%. Similarly, no alteration in numbers of spermatocytes was witnessed in Group III and was thus, remained within control range. However, number of spermatocytes in Group IV was significantly ($p<0.001$) lower than control. Therefore, an overall decline in numbers of germ cell and Sertoli cells was only observed in Group IV (Figure 2).

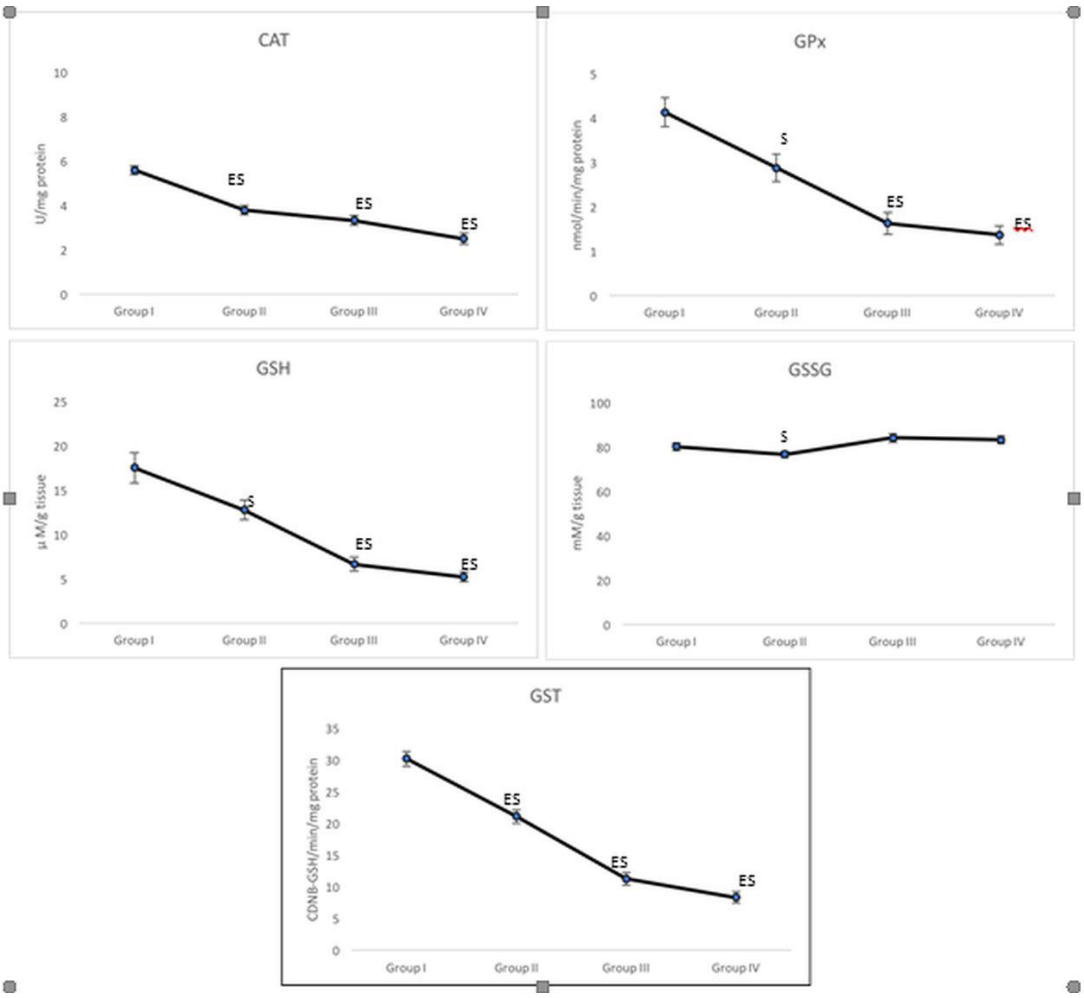

Figure 1: Effect of BPA on testicular antioxidants. Catalase (CAT), Glutathione peroxidase (GPx), Reduced glutathione (GSH), Glutathione disulphide (GSSG), and Glutathione-S-transferase. Values are represented as mean±SEM after 6 weeks of treatment. Significance level was compared against Group I; S=significant ($p<0.05$); ES=extremely significant ($p<0.001$)

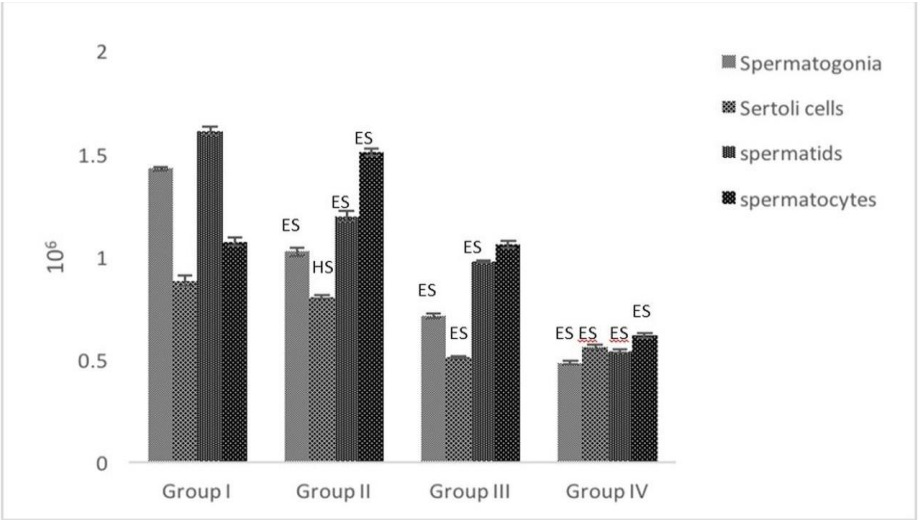

Figure 2: Number of cells per testis in BPA treated groups and placebo control group. Values are represented as mean±SEM after 6 weeks of treatment. Significance level was compared against Group I; S=significant ($p<0.05$); HS=Highly Significant; ES=extremely significant ($p<0.001$)

Histology of testis in comparison with volumetric assessment of germ cells

Histopathological evaluation of testicular tissues of placebo control indicated round and oval seminiferous tubules containing germ cells of various stages covering complete spermatogenesis. Basal lamina was thick showing clear association with spermatogoniaand Sertoli cells. Elongated spermatids were visible and lumen was filled with spermatozoa. Figure 3 shows the histological evaluation of investigated groups, in parallel with volumetric assessment of densities of germ cells and Sertoli cells, estimated through stereology. A close to rhombus shape was observed in the radar plot, drawn based on numeric volume of germ cells and Sertoli cells. However, histological architecture of Group II indicated irregular smooth muscles in between lumens, causing thinner and disoriented basal lamina. Lumen of seminiferous tubules were partially filled and hardly round or oval in shape. Disorientation and lack of Sertoli cells was evident in the photomicrograph. This assumption was also confirmed in the numeric histometry, which clearly showed uneven distribution in the radar plot for Sertoli cells and spermatocytes comparing to control. Histopathology of Group III indicated partially filled lumen and disorientation in the stages if germ cell maturation (Figure 3).

Spermatogonia were not resting at the basal lamina and appeared to have fallen in the lumen. Numerical evaluation of germ cells counts also indicated similar response as of the Group II, however, reduction in number of spermatogonia was observed besides further declination of Sertoli cells. Histological appearances of Group IV indicated severe adversities in the testicular architecture. Basal lamina was abnormally thin and at many occasions complete desertion of basal lamina was evident. Appearances of perivascular edema was evident between seminiferous tubules, spermatogonia were less and largely disoriented. Lack of Sertoli cells witnessed and germ cell appeared to have fallen into lumen. Number of germ cells of all stages were significantly reduced and lumen contained less number of spermatozoa. Volumetric densities enumerated numerically also indicated significant decline in number of germ cells and Sertoli cells. Although number of cells were extremely low in Group IV, unlike other low dose groups (II and III), count of germ cells and Sertoli cells indicated a balance. Nonetheless, the distribution of these cells was not comparable to control as the shape signified more of a square than a rhombus (Figure 3).

Cause and effects analysis

Pearson's correlation test indicated strong associations between spermatogonia, spermatids, and Sertoli cells and CAT, GSH, GST and GPx was observed (Table 1). However, alterations in the number of spermatocytes were only weakly associated with CAT, GSH, GST and GPx. Analysis showed strongest associations (>0.980) between spermatogonia and GSH, GST, and GPx, whereas, weakest association (<0.200) was observed between spermatocytes and CAT. Level of GSSG exclusively indicated negatively moderate association with all investigated germ cells and Sertoli cells.

Table 1: Cause and effect analysis between oxidative stress parameters and BPA led group-wise variations in number of germ cells.

	CAT	GSH	GST	GPx	GSSG
Spermatogonia	0.974	0.981	0.985	0.977	-0.611
Sertoli cells	0.642	0.838	0.819	0.823	-0.887
Spermatids	0.934	0.895	0.903	0.887	-0.502
Spermatocytes	0.166	0.301	0.280	0.237	-0.661

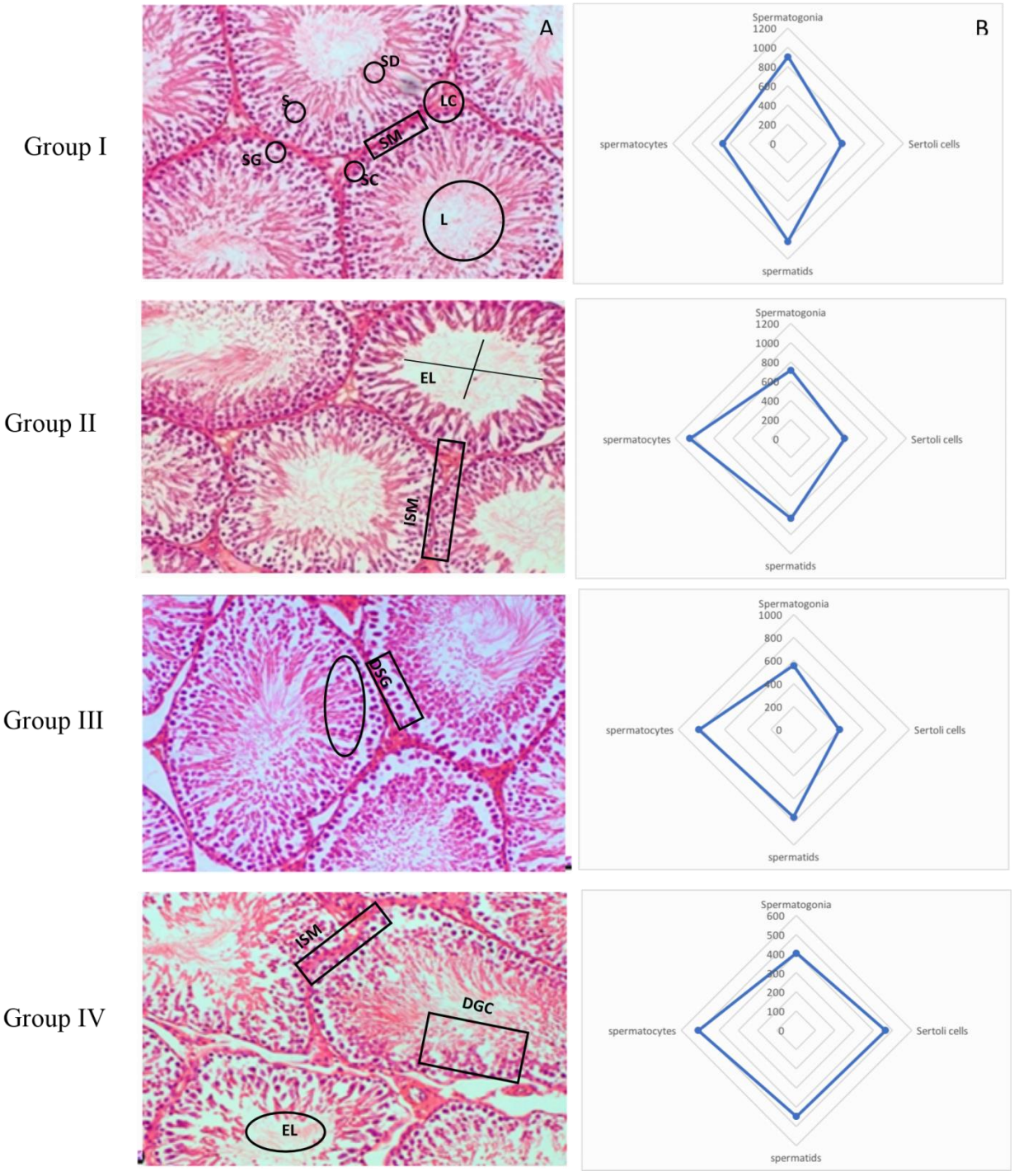

Figure 3: (A) Testicular histology of BPA administered groups alongside with (B) morphometric assessment of spermatogonia, spermatocytes, spermatids, and Sertoli cells. Radar plot is representing volumetric count of germ cells and Sertoli cells. SG=Spermatogonia; S=Spermatocytes; SD=Spermatids; SC=Sertoli Cells; SM=Smooth Muscles; LC=Leydig Cells; L=Lumen; EL=Emptied Lumen; ISM=Irregular smooth muscle; DSG=Disoriented Spermatogonia; DGC=Disoriented Germ Cell

DISCUSSION

Spermatogenesis includes both endocrine and paracrine mode of regulation, involving follicle stimulating hormone, luteinizing hormone, testosterone produced by Leydig cells, and gonadotropins (de Kretser et al., 1998; Huleihel and Lunenfeld, 2004). Although these hormones are important for successful spermatogenesis, other factors such as; cytokines and growth factors have been acknowledged for functions such as stem cell renewal (mitotic division) and complex transition of meiotic cell divisions (Nishimura and L'Hernault, 2017). Germ cells lack receptors for FSH and/or testosterone, therefore, Sertoli cells supports signal transduction between hormones and germ cells (de Kretser et al., 1998). A complex metamorphosis of spermatocytes to spermatids and spermatids to spermatozoa is also carried out during later stage of spermatogenesis. The success of this diverse dynamic process is dependent on precise coordination of various genes and proteins.

Spermatogenesis vitally requires adequate balance between ROS generation and antioxidant depletion to achieve functional maturation of spermatozoa. Notably, oxidation of the sulfhydryl groups in numbers of proteins is vital for maturation of germ cells (Maiorino and Ursini, 2002). Excessive endogenous and/or exogenous ROS generation can have detrimental effects on germ cells and its maturation (Aitken and Clarkson, 1987). Previous studies have reported that BPA induced toxicity has severe adversities on spermatogenesis (Jin et al., 2013; Santiago et al., 2021; Hu et al., 2017). Nevertheless, most of these adverse effects were associated with hormonal alterations. This study revealed that there is crosslink between activities of antioxidants and number of germ cells.

This study revealed that higher doses of BPA causes higher depletion in the activities of antioxidants. Khalaf et al (2019) also reported elevation in level of hydrogen peroxide and significant depletion in the antioxidant defence system. Among investigated antioxidants most BPA susceptible enzymes were GPx, GSH, and GST. All three susceptible enzymes are interlinked, where GPx and GST act as catalyst to recycle GSH from reduced to oxidized form (Traverso et al., 2013). Although excessive depletion of GSH does not undermines activity of other antioxidants such as CAT. However, considering excessive BPA toxicity related histological damages and interference in spermatogenesis depletion of GSH indicates its importance. This study revealed that GSSG an oxidized state of GSH was undeterred by the role of BPA in the testicular tissues. Which can be interpreted as obstruction in effective recycling of GSH, thus could be potential reason behind extremely significant decline in level of GSH in testis. During an oxidative insult, initial fall of GSH and concomitant rise of GSSG has been reported, which subsequently decline and therefore, increase in the level of GSH resumes to initial value (Koster and Slee, 1983; Spector et al., 1985; Jongkind et al., 1989). The evident status of antioxidants in testicular tissues indicated intolerable generation of ROSs through BPA toxicity. It was also evident that doses above 10 mg/kg body weight of BPA causes maximum decline in the level of antioxidants.

Stereological evaluation of spermatogonia, spermatocytes, and spermatids revealed that BPA induced toxicity critically reduced number of germ cells. This observation was clearly evident in animals administered with 100 mg BPA/kg body weight, number of germ cells were extremely lower than what was observed in placebo control. Another notable observation was the distribution of germ cells per testis. This study indicated that animals treated with 100 mg BPA/kg body weight has almost even distribution comparing to control, which is not possible under normal spermatogenesis, as the number of spermatids and spermatocytes are always higher than numbers of spermatogonia. In spite of lower number of spermatogonia number of spermatocytes and spermatids should be at least higher by 30%. Therefore, it can be assumed that 100 mg BPA can arrest spermatogenesis at mitotic stage. There are various studies that acknowledge impairment of spermatogenesis throughout species by variable doses of BPA (Gonzalez-Rojo et al., 2019; Jin et al., 2013). However, arrest of spermatogenesis has been pointed at different stages. For example, study by Gonzalez-Rojo et al. (2019) reported an increase in apoptosis

of spermatocytes and downregulation of ccnb1 and sycp3 genes leading to meiotic arrest. This study was carried out in zebrafish with doses varying between 100-2000 µg/l, which is eventually lower than doses used in this study. Even distribution of germ cells was exclusively witnessed in maximum dose. In low and moderate doses (i.e. 10 mg and 50 mg BPA/kg body weight) impairment seem to shift through various stages. In 10 mg BPA group, extraordinary increase in spermatocytes and substantial reduction in number of spermatids indicate arrest at meiotic stage, that appeared to have continue in 50 mg BPA group.

This study signifies differential pattern in impairment of spermatogenesis based on doses of BPA. Number of Sertoli cells in 50 and 100 mg BPA groups indicated extremely significant reduction. Highly significant reduction in Sertoli cells was observed in 10 mg BPA treated groups. However, change in number of Sertoli cells between 50 mg BPA group and 100 mg BPA group were insignificant. Which was interpreted as indirect effect of BPA on Sertoli cells. A study by Ge et al. (2014) reported that variable concentration of BPA act on Sertoli cells in a biphasic manner. Authors of this study explained that micromolar BPA inhibits proliferation of Sertoli cells by increasing oxidative stress and nanomolar BPA stimulates proliferation by supporting energy metabolism. It can be speculated that no alteration in number of Sertoli cells in high dose group with respect to moderate dose is due to biphasic role of BPA. However, more study is required to understand how high dose BPA exposure perform in real-time.

Irregular spermatogenesis was visible in the histological observations of all test groups. Major histopathological alterations that was common in all BPA treated groups were thin and irregular basal lamina, loss of round or oval shape lumen of seminiferous tubules, and lumen was partially filled with sperm. Observation of this study was in accordance with Munir et al. (2017). Authors revealed in their study that administration of 25 mg BPA/kg body weight in rats led to disruption of spermatogenesis and no sperm in the lumen. Low dose group (10 mg BPA) of this study evidently showed intact resting spermatogonia in the basal lamina, also the number of spermatocytes were abundant. This observation was confirmed in the stereological estimation of volumetric density of spermatocytes and Sertoli cells. Histology of testis in moderate dose group (50 mg BPA) clearly indicated disassociation of spermatogonia from basal lamina and a distinct gap between spermatogonia and rest of the germs cells was found. Although number of spermatids were seen falling into lumen which could be confirmed through volumetric count. High dose group (100 mg BPA) indicated complete collapse of spermatogenesis. It can be speculated from this study that intensity of BPA led toxicity on spermatogenesis is dose dependent, which corresponds to activity of antioxidants investigated.

Therefore, a cause effect analysis can measure strength of association between activities of antioxidants and number of germ cells. This study noted extremely strong strength of association between antioxidants and germ cells excluding spermatocytes. It appeared that spermatocytes were least likely to have any association with BPA induced oxidative stress. A study by Celino et al. (2011) reported high tolerance of spermatogonia against oxidative stress due to abundance of superoxide dismutase. Since this study examined glutathione and catalase exclusively relevance to SOD would be inappropriate. However, they did not mention spermatocytes in their study, which also shows toleration against oxidative stress (AlmansaOrdonez et al., 2020). There could be differential consequences of induced oxidative stress by different toxic compounds. BPA has evidently shown dose dependent variation in observed adversities, where low doses have shown higher damages with respect to high dose (Vandenberg et al., 2013; Fujiwara et al., 2018; Jin et al., 2013). This study revealed spermatocytes as most tolerant to BPA induced oxidative stress, however this observation was based on investigated antioxidants.

CONCLUSION

It was concluded from the study that BPA induced oxidative stress plays important role in regulation of spermatogenesis. Reduction in number of germ cells and Sertoli cells due to BPA administration were paralleled with activity of antioxidants. BPA induced

histological adversities resembled numeric estimation of germ cells. Recycling of GSH and GSSG was significantly affected by BPA in a dose dependent manner. Spermatocytes indicated maximum toleration against oxidative insults generated by all investigated doses of BPA.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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