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Antioxidant activity of selenium on bisphenol-induced apoptosis and testicular toxicity of rats

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Bisphenol A (BPA) is an industrial chemical widely used to make polycarbonate plastics for packaging and epoxy resins. This study sought to examine how selenium (Se) affects BPA toxicity in terms of albino rats' histological structure, antioxidant enzymes, sexual hormones, and reproductive organs (seminiferous tubule (coiled tubule) diameter, epithelial height and sperm count). Adult male rats were divided into four experimental groups: Group 1: The control; Group 2: Orally administered sodium selenite (10 µg/kg bw/day) for 4 weeks; Group 3: Orally administered BPA (10 µg/kg bw/day) for 4 weeks; Group 4: Treated daily with BPA (10 µg/kg bw/day) followed by Se (orally administered, 10 µg/kg bw/day) for 5 times per 4 weeks. BPA exposure caused changes in the testicular histological structure, which included apoptosis, and led to changes in several biochemical markers: Malondialdehyde, catalase, superoxide dismutase, and glutathione peroxidase. Additional changes occurred in the plasma testosterone, luteinizing hormone, and follicle-stimulating hormone levels, body and reproductive organ weights, and sperm counts. However, these BPA-induced effects were significantly ameliorated in rats treated with BPA-plus-Se. This protective effect of Se is attributable to its ability to remove potentially damaging oxidizing agents in living organisms. The results confirmed that Se countered the oxidant effects and increased the BPA-induced stress response in rats. Hence, Se promotes the healthy growth and development of mammals by protecting them from oxidative stress.

Key words: Bisphenol A, selenium, testis, histology, oxidative stress, rats.

INTRODUCTION

Bisphenol A (BPA: 2, 2-bis (hydroxyphenyl) propane) is an environmental contaminant that comes from the industrial manufacturing of polycarbonate plastics. BPA is widely used in the lining of food and beverage packaging to protect the perishable contents from contamination and to extend their shelf life (Vandenberg et al., 2009; Rochester et al., 2013). However, BPA may be released by food containers, plastic bottles, and beverage containers; moreover, it can leach from production sites and landfills (vom Saal et al., 2007). The toxicity and side effects of BPA have been investigated through experimental animal studies, and high BPA concentrations are now associated with a variety of adverse human health issues (Wang et al., 2013). For example, in mice, BPA reportedly affects the male reproduction system, by causing noticeable structural changes in the histological architecture of the testes (Aikawa et al., 2004; Zang et al., 2016). Even a low-dose exposure to BPA may lead to a reduction in spermatogenesis of male rats (Akingbemi et al., 2004;

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Jin et al., 2013), while other research suggested that BPA can adversely affect the quality and quantity of sperm and is responsible for abnormal male fertility (Akingbemi et al., 2004; Liu et al., 2013; Li et al., 2016).

BPA has been shown to cause a reduction in the activity of several key antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; and glutathione peroxidase, GPx) in rat livers. Furthermore, recent work showed that BPA significantly reduced both SOD and CAT system activity as well as oxidised glutathione (H_2O_2) generation and lipid peroxidation (Eid et al., 2015). More recently, the plasma testosterone levels and testis weight were found decreased by BPA in association with morphological alterations and changes in sperm count and motility (Zang et al., 2016).

Antioxidant molecules play a vital role in maintaining the health and defense mechanisms of biological tissues and organs. The human body has several mechanisms to counteract oxidative/nitrosative stress via the production of antioxidants (Kurutas, 2016). In particular, Selenium (Se) is an essential trace element for humans and animals (Tuormaa et al., 2000; Saito et al., 2003) that can prevent the formation of reactive metabolites induced by many toxicants (Atif et al., 2008; Djeffal et al., 2015). Selenium is well known for its effective antioxidant influence against unfavorable residues and chemicals; furthermore, Se decreases the risk organ injury, such as intestines, heart, lungs, and kidneys (Parízek, 1990; Benstoem et al., 2015) and it exhibits protective effects against cadmium-induced testicular toxicity (Li et al., 2010). Recently, Dkhil et al. (2016) and Ullah et al., (2016) reported that Se protects testicular tissue from oxidative injury and has beneficial effects on the reproduction of experimental animals.

Therefore, in this context, this study's objective was to investigate the effects of Se as an antioxidant agent against bisphenol A-induced toxicity in the testes of albino rats, with respect to their histological structure, germ cell apoptosis, antioxidant enzyme activities, sexual hormone levels and reproductive organs.

MATERIALS AND METHODS

Chemicals

Bisphenol A (BPA) (2,2 Bis-4- hydroxyl phenyl propane) was obtained from Sigma Chemicals Co. (Sigma, St. Louis, USA). BPA was suspended in water and orally administered to the treated animals at a dose of 10 µg/kg bw/day for 4 weeks (Takahashi and Oishi, 2003). Selenium (Se) was used in the form of sodium selenite (Na_2 SeO_3). This compound was obtained from British Drug Houses Ltd. (Laboratory Chemicals Division, UK) and dissolved in distilled water for administration to the treated animals at a dose of 10 µg/kg bw/day for 4 weeks.

Animals

All experiments were performed according to the local ethics committee of Umm Al-Qura University. Eighty adult male albino rats, each weighing 240 to 280 g, were caged and randomly assigned to four treatment groups (20 rats each). For 2 weeks, these groups were fed rodent food and water *ad libitum* and maintained in a controlled environment (21 ± 24˚C, 40-60% relative humidity, 12-h light-dark cycles). The animals were divided as follows:

Group 1: Served as the control. This group was used as a positive control to compare the anti-oxidative damage induced by BPA.
Group 2: Received sodium selenite (10 µg/kg bw/day; administered orally for 4 weeks).
Group 3: Received BPA (10 µg/kg bw/day; administered orally for 4 weeks).
Group 4: Received BPA (10 µg/kg bw/day) followed by Se (10 µg/kg bw/day). Both administered orally for 4 weeks.

Histological study

From each group of animals, a subset (n = 5 rats) was sacrificed at the experiment's end (that is, after 4 weeks of the treatment) and their testes excised for histological observations. The collected testes were fixed in Bouan's solution for 24 h and washed five times with alcohol. The testes were then dehydrated in ascending grades of ethyl alcohol, cleared in xylene, and infused with paraplast for later partitioning. Serial sections 5-µm thick were cut and later colored with hematoxylin and eosin stains.

Morphometrics (body and reproductive organ weights and sperm counts)

Rat body weights were recorded at 0, 2 and 4 weeks during treatment in all four groups. At each time point, five rats from each group were sacrificed. The weights of their reproductive organs, namely testes, epididymis, seminal vesicle, and prostate gland, were recorded. To determine their sperm count, the epididymis was removed and dissected in 10 mL of 0.1 M phosphate buffer previously incubated at 37°C. The sperm numbers per volume were tallied using a hematocytometer.

Germ cell preparation for flow cytometry

Germ cell isolation

Testicular tissues of all groups (n = 3 tissues from 3 rats per group) were prepared and used for flow cytometry, as described by Urriola-Munoz et al. (2014). Briefly, the testes from the control rats and those rats treated with Se, BPA, and BPA-plus-Se were removed, decapsulated, and placed in Phosphate-buffered saline (PBS) containing 0.1 mg/mL of collagenase (Sigma). The tubules were washed several times in a PBS. Tubule cell isolation was performed via disaggregation in the presence of 0.1 mg/mL DNase (Sigma), to reduce viscosity resulting from any DNA released from damaged cells during the harvesting, by using a 21G needle applied to different segments of the seminiferous tubules (previously isolated in PBS). Then, the solutions were passed through a mesh with a 200-µm pore diameter, and filtered again using a 70-µm pore diameter. A small drop of the filtrate was observed under a microscope to check the number and integrity of released germ cells before fixing in ice-cold 70% ethanol (Sigma) at 4°C overnight. The suspensions were centrifuged at 1500 rpm for 10 min and re-suspended in a cell cycle buffer (PBS) containing 0.1% of sodium citrate, 0.3% of Triton X-100 (Sigma), 50 mg/mL of propidium iodide (PI), (Sigma), and 50 µg/mL of RNase A (Invitrogen, Carlsbad, USA). The cells were immediately used for analysis by flow cytometry.

Flow cytometric analysis of cell cycle apoptosis

Germ cell apoptosis was analyzed using a fluorescence-activated cell sorting (FACS) flow cytometer (Becton Dickinson, Sunnyvale, USA) equipped with a 15 mW air-cooled 488 nm argon-ion laser. The FL2 (PI) signals were detected through a 585/42 nm band-pass filter. A total of 20 000 events were recorded in list mode and analyzed in Cell Quest Pro software (Becton Dickinson, New jersey, USA). The cell population was gated; assuming the linear forward scatter (FSC) and side scatter (SSC) properties. Fluorescence excitation at 512 nm, with a relatively large stokes shift, emits at a maximum wavelength of 617 nm. Apoptosis was indicated by the percentage of cells in the M1, sub G_1 phase of the cell cycle.

Biochemical assays

The testes of all groups were rapidly isolated at two time points (2 and 4 weeks) during treatment (n = 5 from each group) and washed with ice-cold isotonic saline (0.9%), then stored at -80°C until they were homogenized in a 50 mM phosphate buffer (pH 7.4) using an electronic homogenizer to prepare the 10% w/v homogenate. The homogenate was then divided into aliquots by volume. The level of lipid peroxidation measured as malondialdehyde (MDA) and the activity of CAT, SOD, and GPx were determined using kits bought from Randox Laboratories Ltd (Antrim, UK).

Analysis of glutathione peroxidase (GPx) activity

GPx activity was estimated with a GPx detection kit as described by manufacturer's instructions. The reduction in absorbance was measured spectrophotometrically (S2000 UV model; WPA, Cambridge, UK) against a blank at 340 nm. One unit (U) of GPx was defined as the µmol of oxidized hydrogen peroxide (NADPH) per min per mg of tissue protein. The GPx activity level was expressed as milliunits per mg of protein (that is, mU mg^{-1} protein).

Analysis of superoxide dismutase (SOD) activity

SOD activity was evaluated with a SOD detection kit as described by manufacturer's instructions. SOD activity was then measured by the degree of inhibition of this reaction. One unit (U) of SOD caused a 50% inhibition of the rate of reduction of INT under the conditions of the assay. The SOD activity level was measured spectrophotometrically at 505 nm, and expressed as U mg^{-1} protein.

Analysis of catalase (CAT) activity

Tissue CAT activity was evaluated as described by Aebi (1984). The reaction was initiated by adding hydrogen peroxide to the reaction mixture and the enzyme's activity level was quantified spectrophotometrically as the rate at which tissue catalase decompensated the hydrogen peroxide; this was done, by monitoring the decrease in absorbance at 240 nm against a blank containing a phosphate buffer instead of the substrate (S2000 UV model). The log A1/A2 value for a measured interval was used to define a unit given the first-order reaction of the enzyme. One unit of CAT is the amount of enzyme that decomposes 1.0 nM of hydrogen peroxide per minute at pH 7.0 and 25°C.

Analysis of lipid peroxidation

Lipid peroxidation MDA level was measured by the content of MDA in the testis. Tissue-level MDA was determined by using the thiobarbituric acid reactive substance assay, as described by Buege and Aust (1978). The absorbance of the clear supernatant was determined spectrophotometrically (S2000 UV model) at 535 nm, and the MDA concentration calculated using 1.56×10^5 mol^{-1} cm^{-1} as molar absorbance coefficient. MDA results were expressed as nmol per gram of wet tissue.

Hormonal assays

Five blood samples from each group were collected (one sample/rat) at 2 and 4 weeks from the treatment (that is, n = 5 rats from each group). A 1-ml blood sample from each animal was collected from the dorsal pedal vein, and the blood plasma was separated by centrifuging at 2000 g for 10 min in a refrigerated centrifuge (Eppendorf 5804R, Hamburg, Germany) and used immediately for the testicular hormone analysis. Plasma testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were assayed at two time points (2 and 4 weeks), using an enzyme immunoassay kits (Alfa Scientific Designs, California, USA), and their levels analyzed on an absorbance microplate reader (ELx808, USA). This assay is based on the competitive binding technique.

Statistical analysis

Data are expressed here as mean ± SEM. The significance of differences among the four group means was evaluated by multiple two-way ANOVAs, in SPSS v19 for Windows; multiple comparisons between group means were then done using the Tukey-Kramer test. Differences at $P < 0.01$ were considered as highly statistically significant, while those at $P < 0.05$ as statistically significant.

RESULTS

Histological observations

Figure 1A shows a section of the rat testis from the control group. The histological arrangement appeared healthy, with normal functional and structural seminiferous tubules. Each tubule contained multiple spermatogenic stages that included spermatogonia, primary and secondary spermatocytes, spermatids, spermatozoa, and Sertoli cells. The histological examination of the Se treated group (Figure 1B) revealed no histological or structural differences from the control group. By contrast, the testes section of BPA-treated rats (Figure 2A) showed clear alterations in their seminiferous tubules and interstitial tissue; noteworthy is the reduced spermatogenic cell size with an abnormal arrangement. The interstitial tissue appeared degenerated with signs of intertubular hemorrhaging. The blood vessels of the BPA-treated section were also enlarged and congested with interstitial edema (Figure 2B). Necrotic germ cells with pyknotic nuclei were exfoliated in the center of the tubule lumens of the section treated with BPA (Figure 2C). Figure 2D shows the degeneration of seminiferous tubules and the inhibition of spermatogenesis. However, the histological structure of testes of rats given BPA-plus-Se showed fewer pathological changes, and displayed intact histological structures, similar to the healthy

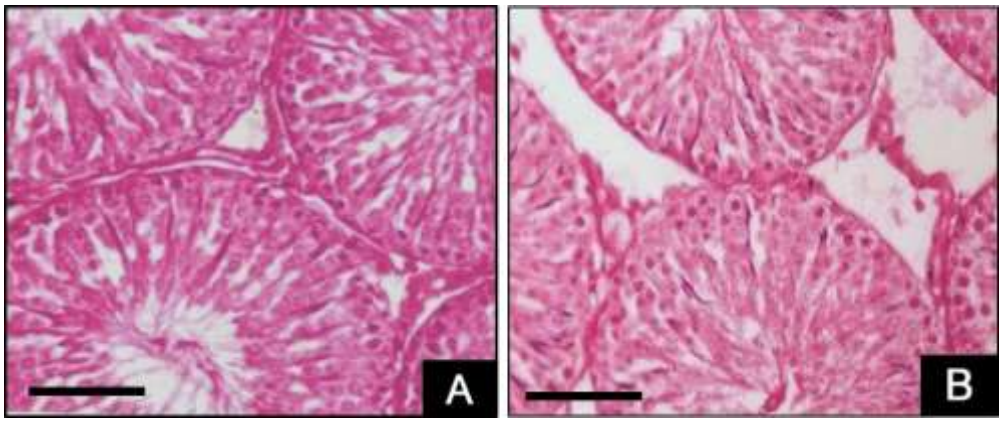

Figure 1. Photomicrograph sections of rat testes treated for 4 weeks. **(A)** Testis of control rat showing normal histological structure and healthy arrangement of seminiferous tubules. **(B)** Section of rat testis treated with Se showing normal Seminiferous tubules with various stages of sperm development.

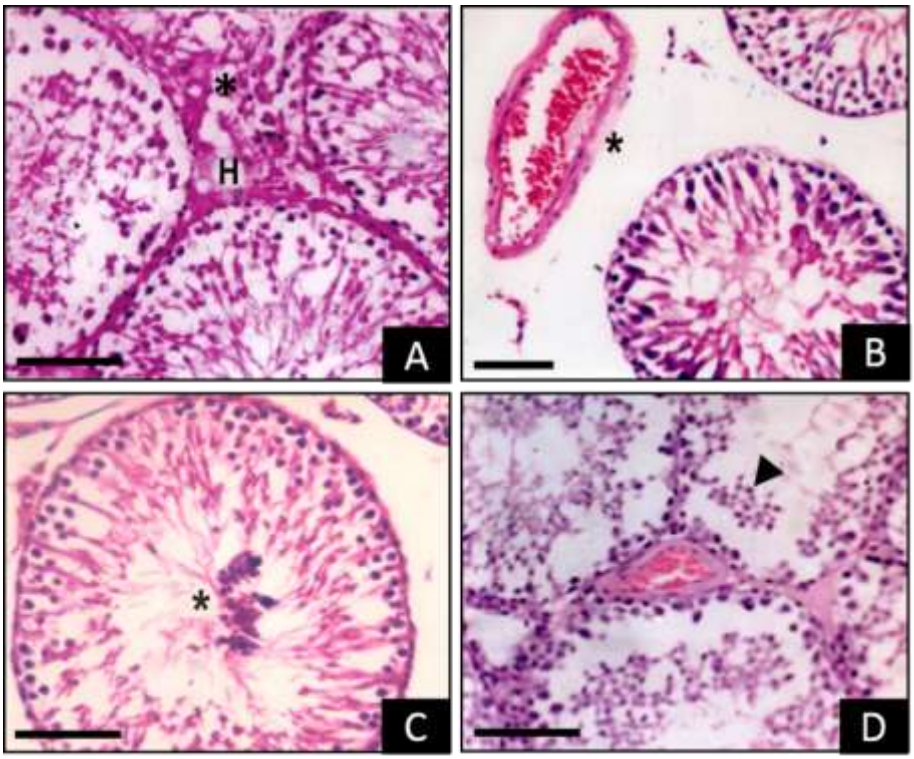

Figure 2. Photomicrograph sections of rat testes treated with BPA for 4 weeks. **(A)** Seminiferous tubules of rat testis showing degenerated interstitial tissue (*) and haemorrhage (H); **(B)** Section of rat testis showing oedema and congested blood vessel and disorganized structure; **(C)** Section of rat testes **s**howing degenerated germ cells (*) exfoliated in the centre of seminiferous tubules; **(D)** Section of rat testis showing degeneration of seminiferous tubules and inhibition of spermatogenesis (arrow-head).

arrangement observed in both the control and Se sections. Most of the seminiferous tubules apparently contained normal spermatogenic cells, with normal interstitial tissue (Figure 3A). Figure 3B shows the normal seminiferous tubules with numerous active germ cells, spermatocytes, spermatids, and Leydig cells (Figure 3C).

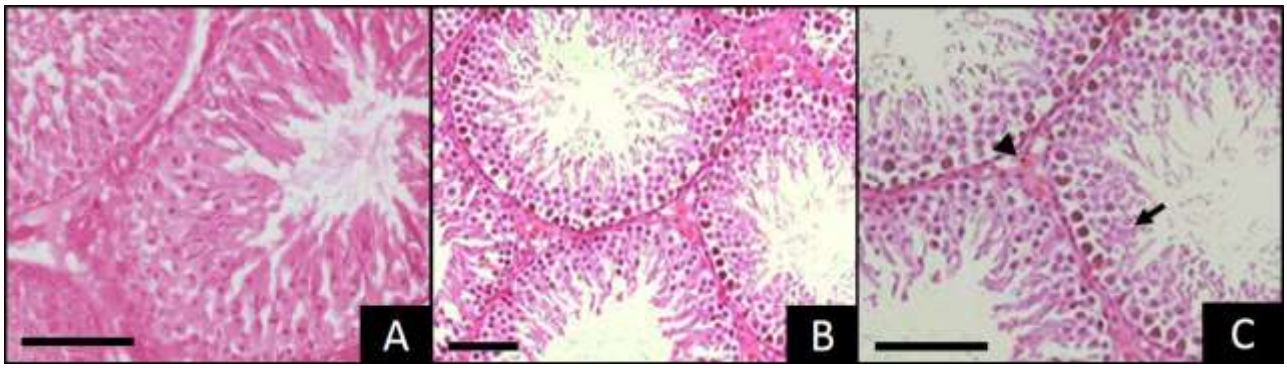

Figure 3. Photomicrograph sections of rat testes treated with BPA plus Se for 4 weeks. **(A)** Section of rat testis showing a significant improvement of the germ cells and regeneration of spermatogenesis, and normal seminiferous tubule formation; **(B)** Section of rat testis showing normal seminiferous tubules with numerous active germ cells; **(C)** Magnified photomicrograph of rat testis showing normal seminiferous tubules (arrow), spermatocytes, spermatids and leydig cells (arrow-heads).

Table 1. Effect of BPA and selenium on seminiferous tubules (coiled tubules of the testis) diameter, epithelial height and sperm count of male rats.

Time (weeks)	Seminiferous tubules (µm)	Epithelial height (µm)	Sperm count (×10^5/Epididymis)
Control			
2	252 ± 4.8	110 ± 5.3	21 × 10^5 ± 3.1
4	260 ± 5.6	115 ± 6.7	22.2 × 10^5 ± 3.7
		BPA	
2	160 ± 4.3**	70.5 ± 5.2**	9.2 × 10^5 ± 3.8**
4	130 ± 6.8**	53.4 ± 6.9**	5.5 × 10^5 ± 3.4**
		Selenium	
2	262 ± 6.4	111 ± 4.6	20 × 10^5 ± 3.2
4	267 ± 8.5	108 ± 5.7	22.7 × 10^5 ± 2.9
		BPA + Selenium	
2	230 ± 5.4*$^{++}$	90.1 ± 7.2*$^{+}$	17.4 × 10^5 ± 2.7*$^{++}$
4	220 ± 5.3*$^{++}$	84.5 ± 7.8*$^{++}$	15.3 × 10^5 ± 4.6**$^{++}$

The results are expressed as mean ± SEM, n = 5 rats/group. ***,**:** Significant in comparison with the control group at P <0.05 and P <0.01, respectively; **+,++:** Significant in comparison with the BPA group at P <0.05 and P <0.01, respectively

Morphometrics

A morphometric investigation was done to obtain quantitative information on the seminiferous tubules, epithelial height, and sperm count of all four experimental groups. Changes occurred in the seminiferous tubule dimensions in those rats given BPA for 4 weeks: they had a significantly decreased diameter of the seminiferous tubules (coiled tubules) at 2 and 4 weeks relative to the control and Se-treated group. However, the group treated with BPA-plus-Se for 2 and 4 weeks showed an increase in the diameter of the seminiferous tubules when compared that of the BPA-treated group (Table 1). Hence, treating the rats with both BPA and Se caused their seminiferous tubule diameter to enlarge.

With respect to the epithelial height per unit length, at 2 weeks it was higher in the control and Se groups. Compared with these, treating rats with BPA for 2 and 4 weeks, led to decreases in epithelial height, whereas rats in the group treated with BPA-plus-Se for 2 and 4 weeks showed increases in epithelial height over the BPA-treated group (Table 1). The epididymal sperm count was remarkably reduced in the BPA-treated rats: Compared with control, a significant reduction was already evident at 2 and 4 weeks. By contrast, those rats receiving BPA and Se showed higher sperm counts at 2 weeks and 4 weeks (Table 1).

The weights of the testes, epididymis, vas deference, seminal vesicle, and prostate gland were significantly reduced (P < 0.01) in rats treated with BPA at two time

Table 2. Effect of BPA and selenium on weight (g) of gonadal organs of male rats.

Time (weeks)	Testes	Vas deferens	Epididymis	Seminal Vesicle	Prostate gland
Control					
2	1.37 ± 0 .06	0.10 ± 0.003	0.28 ± 0.01	0.41 ± 0.04	0.27 ± 0.02
4	1.2 5 ± 0.09	0.08 ± 0.002	0.28 ± 0.01	0.45 ± 0.005	0.31 ± 0.006
			BPA		
Time	**Testes**	**Vas deferens**	**Epididymis**	**Seminal Vesicle**	**Prostate gland**
2	0.65 ± 0.02**	0.07 ± 0.004**	0.16±0.004**	0.21±0.004**	0.16±0.005**
4	0.37 ± 0.005**	0.05 ± 0.004**	0.11±0.004**	0.10±0.003**	0.10±0.006**
			BPA and Selenium		
Time	**Testes**	**Vas deferens**	**Epididymis**	**Seminal Vesicle**	**Prostate gland**
2	1.16 ± 0.07^{++}	0.09 ±0.003^{++}	0.29 ± 0.02^{++}	0.38 ± 0.002^{++}	0.25 ± 0.005^{++}
4	1.10±0.06^{++}**	0.06±0.002^{+}*	0.21±0.004**$^{++}$	0.30±0.001**$^{++}$	0.20±0.007^{++}**

The results are expressed as mean ± SEM; n = 5 rats/group.***,**:** Significant in comparison with the control group at P <0.05 and P <0.01, respectively **+,++ :** Significant in comparison with the BPA group at P <0.05 and P <0.01, respectively.

Table 3. Effect of BPA and selenium on body weight (g) of male rats relative to time Zero.

Time	Animal group			
	Control	**Selenium**	**BPA**	**BPA + Selenium**
Zero	250.70 ± 10.80	257.30 ± 7.50	260.10 ± 8.60	265.10 ± 8.30
2 weeks	274.35 ± 5.30	270.28 ± 7.40	263.55 ± 10.40	289.50 ± 9.65
Change %	9.43	5.04	1.32	9.20
4 weeks	312.30 ± 5.43	314.44 ± 10.50	284.64 ± 11.20	305.80 ± 8.75
Change %	24.57	22.20	9.43	15.3

The results are expressed as mean ± SEM; n = 5 rats/group.

points (2 and 4 weeks) relative to the control group. However, compared with the BPA-treated rats, those given BPA-plus-Se showed a remarkable increase (P < 0.01) in the weight of their gonadal organs (Table 2). In addition, the body weight gains of rats treated with BPA were lower than those of the control group (Table 3).

Flow cytometry of apoptosis

Cell cycle analysis was evaluated via the flow cytometry of PI-stained germ cells. No significant changes were detected in the control and Se-treated rats in all phases of the cell cycle. There was a significant increase (P < 0.05) in the percentage of germ cell apoptosis (M1, sub G_1) in the testes of rats treated with BPA for 2 and 4 weeks, but a decrease in other phases of the cell cycle (M_2, M3, M4) in comparison with the control and Se groups. At 2 and 4 weeks, the respective mean percentage of apoptosis in BPA-plus-Se group was 1.7- and 1.8-fold that of the BPA group (Figures 4 and 5).

Biochemical results

Changes in the lipid peroxidation marker and antioxidant enzymes

Compared with the control, those rats in the BPA-treated group exhibited a significant increase in their MDA level (Table 4), as well as a significant decrease in the antioxidant enzyme activity of CAT, SOD, and GPx. However, rats treated with BPA-plus-Se had relatively greater SOD, CAT, and GPx activity levels but these were still less than those of the control group (Table 4), whereas, the level of MDA was significantly decreased.

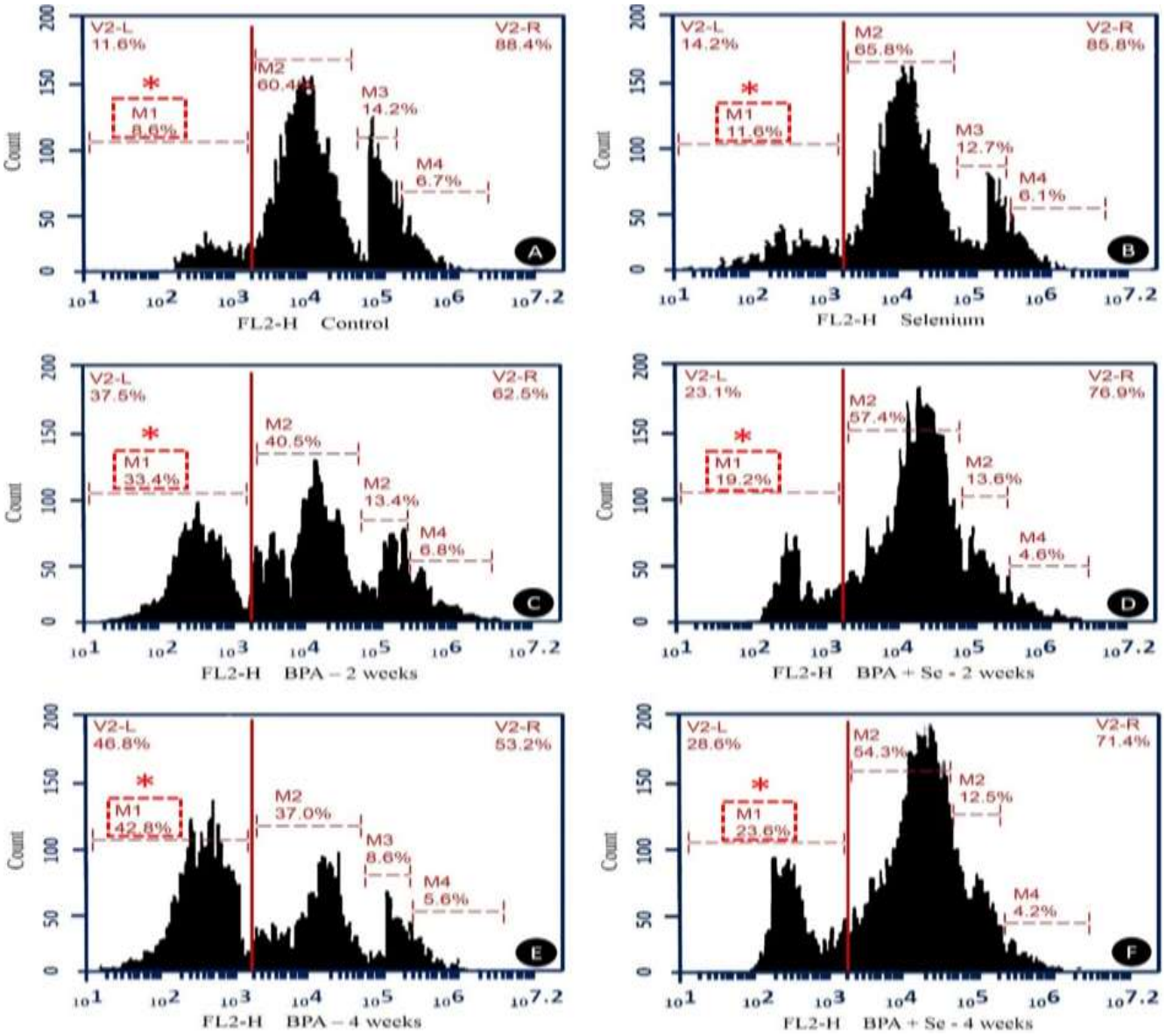

Figure 4. Effect of selenium (Se) against BPA oxidative stress. Flow cytometric of DNA cycle analysis, location of apoptotic cells (Ap) on DNA content histograms. The presence of apoptotic cells in individual experimental groups stained with **Propidium iodide (PI).** (M1=apoptosis % (*), M2=G0/1, M3=S % and M4=G2/M). **(A)** Control group, **(B)** Group treated with selenium, **(C)** Group treated with BPA for 2 weeks, **(D)** Group treated with BPA and Se for 2 weeks, **(E)** Group treated with BPA for 4 weeks, **(F)** Group treated with BPA and Se for 4 weeks.

Changes in plasma testosterone, LH and FSH

Treating rats with BPA significantly decreased their plasma testosterone and LH levels, but significantly increased their FSH level (Table 5). In comparison, treatment with BPA-plus-Se caused a significant increase in both the plasma testosterone and LH while reducing FSH hormones (that is, the rat response to added Se in the presence of BPA). The rats in the control and Se-treated groups showed similar responses at both time points (Table 5).

DISCUSSION

Bisphenol A (BPA) is being increasingly studied due to its widespread use and accumulation in various human body fluids and tissues. This investigation demonstrates that BPA can induce histopathological alterations and inhibit spermatogenesis in the testes of albino rats. Administration of BPA significantly induced germ cell apoptosis and decreased the weights of testes and other reproductive organs in male rats. This result may be due to the inhibition of spermatogenesis, and decreased

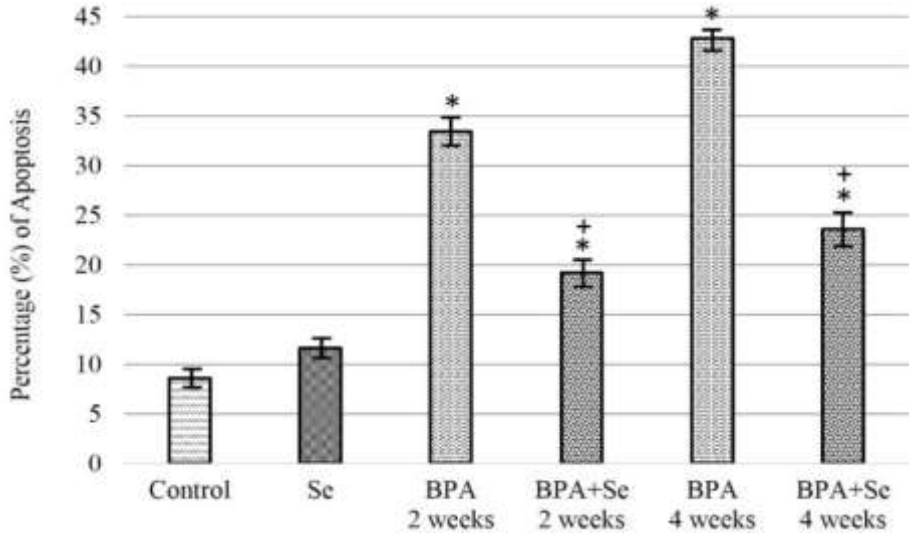

Figure 5. Changes in germ cells apoptosis activity among different animal groups. The group treated with BPA showed a significant increase in apoptotic activity compared with control and Se groups. The group treated with BPA and Se showed a positive effect, leading to a reduction in the apoptotic level compared to the BPA treated group. ***,**:** Significant in comparison with the control group, p<0.05, p<0.01; **+,++:** Significant in comparison with the BPA group, p<0.05, p<0.01.

Table 4. Effect of BPA and selenium on malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) level of male at 2 and 4 weeks of treatment.

Time (weeks)	MDA (nmol/g^{-1} tissue)	CAT U mg^{-1} tissue	SOD U mg^{-1} tissue	GPx mU mg^{-1} tissue
Control				
2	52.5 ± 0 .05	3.6 ± 0.02	44.7 ± 0.02	108.8 ± 0.04
4	53.1 ± 0.07	3.5 ± 0.03	44.5 ± 0.03	123.6 ± 0.005
		Selenium		
2	53.4 ± 0.02	3.51 ± 0.004	44 ± 0.02	112.2 ± 0.04
4	56.7 ± 0.05	3.59 ± 0.04	45.2 ±0.04	131.8 ± 0.03
		BPA		
2	60.2 ± 0.07*	2.81 ± 0.04*	29.7 ± 0.05**	80 ± 0.003*
4	71.3 ± 0.06**$^{++}$	2.4 ± 0.005**	23.5 ± 0.03**	64 ± 0.001**
		BPA + Selenium		
2	52.5 ± 0.04^{+}	3 ± 0.04*$^{+}$	36.4 ± 0.06**$^{+}$	94 ± 0.04*$^{+}$
4	58.3 ± 0.04*$^{++}$	3.15 ± 0.03*$^{++}$	40.4 ± 0.03*$^{++}$	92.3 ± 0.06**$^{++}$

The results are expressed as mean ± SEM, *n* = 5 rats/group. *,**: Significant in comparison with the control group at *P* <0.05 and *P* <0.01, respectively; +,++: Significant in comparison with the BPA group at *P* <0.05 and *P* <0.01, respectively.

elongated spermatids and steroidogenic enzyme activity. It has been shown that administering BPA to male mice reduced their sperm count and quality, and impaired spermatogenesis by decreasing the levels of reproductive hormones (Li et al., 2010; Jin et al., 2013; Tamilselvan et al., 2013). Takahashi and Oishi (2003) and Abdel-Halim et al. (2016) reported that administering BPA decreased the number of spermatogenic cells in the seminiferous tubules within the intertubular spaces, reduced the sperm count and testes weight, and caused a marked decline in steroidogenic enzyme activity in mice. Additionally, sperm abnormalities were found to be caused by DNA strand breaks in the germ cells of testis tissue that resulted from PBA-induced apoptotic activity (Eid et al., 2015).

Table 5. Effect of BPA and Selenium on plasma testosterone, **Luteinizing hormone** (LH) and Follicle stimulating hormone (FSH) level of male at 2 and 4 weeks of treatment.

Time (weeks)	Plasma testosterone (ng/ml)	Plasma LH (mIU/ml)	Plasma FSH (mIU/ml)
Control			
2	3.3 ± 0.06	2.9 ± 0.04	3 ± 0.04
4	3.6 ± 0.05	2.86 ± 0.06	3.13 ± 0.06
		Selenium	
2	3 ± 0.02	2.8 ± 0.003	2.79 ± 0.04
4	3.3 ± 0.05	2.6 ± 0.06	2.8 ± 0.07
		BPA	
2	1.8 ± 0.07**	0.9 ± 0.05**	3.83 ± 0.05*
4	0.8 ± 0.06**	1.3 ± 0.007**	5.95 ± 0.07**
		BPA + Selenium	
2	2.8 ± 0.05*	2.7 ± 0.008*$^{++}$	3.4 ± 0.008*$^{+}$
4	2.1 ± 0.004**$^{++}$	2.1 ± 0.003**$^{++}$	4.7 ± 0.006**$^{++}$

The results are expressed as mean ± SEM, n = 5 rats/group. *,**: Significant in comparison with the control group at P <0.05 and P <0.01, respectively; +,++: Significant in comparison with the BPA group at P <0.05 and P <0.01, respectively.

Kitraki et al. (2015) found that exposure to BPA can lead to a considerable increase in the numbers of necrotic and apoptotic cells in the testes and semen of rats, and hypothesized that BPA may induce oxidative stress, which would cause germ cells to undergo apoptosis. The present study's results clearly indicated that exposure to even a low dose of BPA disrupted spermatogenesis by decreasing the activity of antioxidant enzymes and the levels of key reproductive hormones. The results also showed that BPA could significantly inhibit the seminiferous epithelium height, seminiferous tubule diameter, and sperm count.

Concerning the biochemical results, exposure to BPA led to significant changes in the activity of antioxidant enzymes in rats. Specifically, BPA caused an elevation in the lipid peroxidation marker, testicular malondialdehyde (MDA) and a reduction of the CAT, SOD, and GPx antioxidant enzymes in rat testes. These results confirmed that BPAinduced oxidative stress and changes in SOD and CAT levels in testes, reduce the epididymal sperm count, and cause abnormal reactions with radical oxygen in several biological systems (Aitken et al., 2008). As such, antioxidant enzymes can become inhibited with an increase in the lipid peroxidation products such as MDA (Obata and Kubota, 2000).

Endocrine disrupting chemicals are defined as any exogenous agents that can interfere with the synthesis, metabolism, and action of endogenous hormones (Phillips et al., 2008). Zang et al. (2016) reported that BPA affects the functioning of the reproductive system and leads to infertility in male mice. The present study's results indicated that exposing rats to BPA caused a significant abnormality in their sexual hormone levels. Nonetheless, few reports have discussed the underlying effects of BPA on sexual functions. Notably, Zhou et al. (2013) found that BPA reduced the testosterone levels in mice and inhibited their sexual behaviors. In rats, the results here indicated that plasma testosterone and LH levels were both significantly lowered, whereas the FSH level was markedly increased by BPA. Moreover, the results showed that BPA was able to reduce the body and gonadal weights of the male rats. A plausible explanation is that BPA caused cellular damage which affected the antioxidant enzymes' activity and sexual hormones of male rats.

Many clinical and experimental trials have used antioxidant agents to protect spermatogenesis from exposure to toxicants (Aitken et al., 2008). Selenium is one of the elements classified within the group of micronutrients that play an important role in maintaining the health and performance of human and animals (Mehdi and Dufrasne, 2016). Selenium is more commonly known for its antioxidant capacity, which protects cells from free radicals (Parízek, 1990; Mistry et al., 2012). In the present study, Se was used as an antioxidant agent against the side effects and toxicity of BPA in male rats. Se was able to reduce germ cell apoptosis in the rat testes and had a positive countervailing influence against the histological, biochemical, and morphometrical damages caused by BPA. Se led to a significant increase in spermatogenesis, seminiferous epithelium height, seminiferous tubule (coiled tubules of the testis) diameter, sperm count, body and reproductive organ weights, as well as elevating antioxidant enzymes (MDA, CAT, SOD and GPx) activity and the levels of sexual hormones (plasma testosterone, LH, and FSH). During spermatogenesis cycle, germ cell apoptosis removes most abnormal spermatogenic cells

and thereby maintains a normal quantity and quality of sperm (Print and Loveland, 2000). Abnormal apoptosis of germ cells is a critical determining factor for oligozoospermia and azoospermia, which causes detrimental histological and physiological changes in testes (Tesarik et al., 1998; Saygin et al., 2015).

The obtained results revealed a marked improvement in the testicular structure in the group given BPA with Se when compared with group given only BPA. Further, inclusion of Se had a positive effect on the body and reproductive organ weights of rats and increased their sperm count. It has been reported that sodium selenite protects testicular gametogenic and spermatogenic disorders against carbimazole and prevents testicular oxidative stress by increasing the antioxidant status (Long et al., 2016). Recently, Erkekoglu et al. (2012) reported that supplementation of Se to di(2-ethylhexyl) phthalate-treated rats lowered the activity of testicular germ cell apoptosis. In another study, a notable decrease in the apoptotic index of spermatogenic cells was recorded after scrotal hyperthermia in Se-supplemented mice (Kaur and Bansal, 2015).

The results also showed that co-administration of Se led to a decrease in MDA and an increase in the activities of CAT, SOD, and GPx. These effects are likely linked to a suppression of *GPx1* gene expression, and *GPx4*, leading to a disruption in protein oxidation (Fátima et al., 2013). Ahmed et al. (2012) reported that changes in antioxidant enzymes' activities (CAT, SOD) are related to the overexpression of the selenoprotein iodothyronine deiodinase, thus leading to the high-level production of reactive oxygen species (ROS). Some studies also suggest that the stimulation of antioxidant activity by Se may be related to the effects of GH and IGF *in vitro* (Aksu et al., 2013), which would promote oxidation and diminish GPx activity when they act as somatic growth inductors. Therefore, Se and GPx, among other selenoproteins, may thus play a key role in biological development, namely by preventing concomitant oxidation.

This study's results also confirmed that Se has positive effects against BPA-induced abnormal testicular hormones and body and gonadal weights, while confirming that it provides cellular protection against oxidative stress. Selenium's positive effect against abnormalities of the gonadal hormones and sperm count as caused by cadmium has been documented (Alhazza, 2005). Said et al. (2012) reported that Se enhanced folliculogenesis in rats via ovarian granulosa cells' expansion, estradiol and FSH discharge, and GPx movement, while diminishing both lipid peroxidation and oxidative anxiety. Selenoprotein P is the most common Se-binding protein and it is important for supplying Se to organs, especially the testis and kidney (Swathy et al., 2006; Said et al., 2012). Selenoprotein synthesis is therefore highly dependent on Se, and there is a hierarchy of selenoprotein expression in mammals when Se is limited (Said et al., 2012; Xi et al., 2012). Selenoproteins include enzymes such as the glutathione peroxidases (GPxs), thioredoxin reductases, deiodinases, selenophosphate–synthetase 2, and selenoprotein H (Said et al., 2012). Furthermore, when and how Se is active in animal systems is mainly determined by such selenoproteins. Hence, it seems reasonable to posit that the action of Se against BPA toxicity in testes may be attributable to the presence of selenoprotein P (Schomburg et al., 2009).

Conclusion

The results obtained from this investigation demonstrate that selenium provides cellular protection against oxidative stress induced by BPA. Moreover, the reduced percentage of apoptosis in the BPA-plus-Se group compared with BPA-treated group represents the protective effect of Se against BPA toxicity, which led to a relatively healthier histological arrangement, antioxidant enzyme activity, sexual hormone levels, and body and reproductive organ weights. This experimental study confirms Se plays a crucial role as an antioxidant agent by ameliorating or even eliminating the negative effects and toxicity of other components, including BPA, thus suggesting Se possesses effective therapeutic countervailing properties against BPA toxicity. In summary, Se can promote the healthy growth and development of animals and protect them from oxidative stress.

ABBREVIATIONS

Bisphenol A, [BPA, 2, 2-bis (hydroxyphenyl) propane]; **Se,** Selenium; **MDA,** Malondialdehyde; **SOD,** Superoxide dismutase; **GPx,** glutathione peroxidases; **GPx1,** Glutathione Peroxidase 1; **GPx4,** Glutathione Peroxidase 4; **GSH,** superoxide dismutase; **CAT,** catalase; **LH,** luteinizing hormone; **FSH,** Follicle stimulating hormone; **EDC,** endocrine disrupting chemicals; **ROS,** reactive oxygen species; **GR,** oxidised glutathione; **H_2O_2,** hydrogen peroxide; **NADPH,** Nicotinamide adenine dinucleotide phosphate; **FZCS,** fluorescence activated cell sorting; **PBS,** Phosphate buffered saline.

ETHICS APPROVAL

Animal care and experimental procedures were performed per the Umm Al-Qura University, Faculty of Applied Sciences regulations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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