The effect of hormonal levels and oxidative stress on bisphenol A and soy isoflavone reproductive toxicity in murine offspring

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Abstract. Previous studies have suggested that human exposure to bisphenol A (BPA) and soy isoflavones (SIFs) can occur during pregnancy. The combination of these chemicals is hypothesized to have a toxic impact on the fetus. While BPA is an industrial chemical used widely in the manufacture of polycarbonate plastics and epoxy resins, SIFs are naturally occurring estrogen-like phytoestrogens. To determine the impact of the combination of BPA and SIFs on fetal development, the body weight, organ weight, anogenital distance and histopathological changes in the testes of F1 offspring were assessed in mice. Hormonal effects were determined by measuring serum levels of estrogen receptor (ESR), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (T). Additionally, mitochondrial DNA copy numbers, and the serum levels of malondialdehyde and superoxide dismutase, were determined to evaluate alterations in oxidative stress and potential toxicity. Exposure to BPA increased the body weight of the pups and reduced the ratio of anogenital distance to body weight, as well as testes weight. Moreover, BPA exposure also induced testicular lesions. The seminiferous tubules of testis were denatured in varying degrees and the lumen wall structure was disordered. The levels of ESR in all offspring and the T levels in male offspring significantly increased, compared with controls. Co-exposure to BPA and SIFs exacerbated these changes in body weight, testicular lesions and hormonal levels, relative to BPA exposure alone. Additionally, oxidative damage was only induced by high-dose BPA. Collectively, these findings suggested that BPA and SIFs could have synergistic effect on the reproductive system, which could be mediated by the regulation of ESR expression and testosterone release.

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Introduction

Bisphenol A (BPA) is an industrial chemical used widely in the manufacture of polycarbonate plastics and epoxy resins, which are used in the production of food containers and medical devices and are becoming the largest source of human exposure to plastic (1). BPA exposure is low but consistent across countries (2). BPA has been detected in amniotic fluid, neonatal blood, placenta, cord blood and human breast milk, demonstrating that this chemical might be passed on from mother to fetus (3). The *in vivo* and *in vitro* studies have demonstrated that BPA has estrogen-like properties leading to reproductive and developmental toxicity (4,5). Exposure to BPA during development is concerning (6,7), yet the effect of BPA exposure during pregnancy on reproductive health remains to be determined.

Soy isoflavones (SIFs) are naturally occurring estrogen-like phytoestrogens that are abundant in various soy-based foods and food supplements, such as soymilk, tofu, tempeh and soy-based infant formula. Previous studies have suggested that SIFs can interact with estrogen receptors (8,9) and, similarly to BPA, trigger estrogen-dependent downstream effects. Thus, the fetus can be simultaneously exposed to SIFs and BPA during pregnancy. However, whether concomitant exposure to BPA and SIFs can induce an additive or synergistic effect leading to exacerbated toxicity is largely unknown.

The adverse effects of BPA are predominantly related to its estrogenic activity, which may be involved in regulating gonadotropin-releasing hormone and steroid receptor transcription (10,11). Moreover, BPA has other effects such as induction of inflammatory cytokines (12,13) and oxidative stress (14,15), which are independent of its estrogenic activity. Increasing evidence suggests that the induction of oxidative damage in male reproductive tissues represents another common

response to exposure to environmental toxicants (16-18). Imbalances in redox systems induce oxidative damage, which in turn can negatively influence the reproductive process. For instance, mitochondrial dysfunction is detectable in clinically proven infertile men, and exposure to environmental toxicants is a major factor in this context (19-21). However, the relationship between BPA exposure, oxidative stress and reproductive toxicity is still unclear.

The aim of the present study was to evaluate the possible toxic effects of BPA and the synergistic actions of BPA and SIFs exposure on the reproductive systems of murine F1 offspring. Accordingly, organ weights were recorded, and the anogenital distance (AGD) was measured. Histopathological examination of testes was also carried out. In addition, hormonal status and oxidative stress in the F1 offspring were examined. The present findings may provide insight into the mechanisms through which BPA and SIFs might induce reproductive toxicity.

Materials and methods

Chemicals. BPA and diethylstilbestrol (DES) were purchased at >99% purity from Sigma-Aldrich (Merck KGaA). BPA and DES were first dissolved in 100% ethanol, then diluted in corn oil as previously described (22,23), with a final ethanol concentration in corn oil <1%. DES was used as a positive control to confirm responsiveness of animals to estrogenic compounds. SIFs (>99%) were purchased from Zhengzhou Linuo Biotechnology Co., Ltd., and SIFs were prepared for suspension with corn oil.

Animals and experimental design. A total of 30 female and 10 male Kunming mice (4-5 weeks old, weighing 22-29 g) for each time-point were obtained from the Laboratory Animal Center of Guilin Medical University. The mice were housed in polycarbonate cages with sawdust bedding at a controlled temperature (23±1˚C) and 50-60% humidity under a 12-h light/dark cycle. Food and tap water were available *ad libitum*. Animals were acclimated to the laboratory environment for 7 days before the start of the experiment. All animal experiments in the present study were approved by The Animal Ethics Committee of the Guilin Medical University (approval no. GLMC201803066).

Female mice were randomly divided into 6 groups per time-point and were placed in cages with male mice in a 2:1 ratio overnight. Mating was confirmed by the presence of a vaginal plug. The day the vaginal plug was observed was considered to be gestation day (GD) 1. On GD 1, the females with vaginal plugs were removed from males, weighed and individually caged. On GD 9 until the birth of pups, the females were treated by gavage daily with: i) A dose of 2, 20 or 200 mg/kg BPA alone (BPA2, BPA20 and BPA200 groups); ii) combination of 20 mg/kg BPA and 300 mg/kg SIFs (BPA20 + SIF300 group); iii) 0.25 mg/kg DES (DES group), which was the positive control; and iv) corn oil (control group). The concentrations of BPA, SIFs and DES given by gavage were based on previous studies (12,23). The gestational time, pup numbers and the sex ratios of the pups were recorded (~30 pups per time-point). The pups were weighed on postnatal day (PND) 0, 14 and 26. The AGD, defined as the distance between the anus and the genital tubercle, was measured on PND 0, 7, 14, 21 and 26 using calipers. The ratio of AGD to body weight was also calculated. The offspring were euthanized on PND 7 or PND 26 and ~0.5-1.0 ml blood samples were collected by cardiac puncture under anesthesia with 40-50 μl diethyl ether per mouse. Vital parameters and disappearance of corneal and pain reflexes were monitored to ensure the animals were fully anesthetized. Following blood collection, mice were sacrificed by CO_2 inhalation at 10-30% chamber volume/min. Death was confirmed by cessation of the heartbeat and breathing. The thymus, liver, spleen, heart, lung, kidney, brain, testes and uterus from the offspring were carefully dissected free of adhering fat and mesentery, then weighed.

Histological analysis of testes tissue. The tissue blocks, which were 1x2x0.2 cm in size were put into 10% formaldehyde solution at room temperature. The tissue blocks were dehydrated with gradient alcohol (70, 80, 95 and 100% ethanol) and washed with xylene. Paraffin sections at a thickness of 5 μm were mounted on a slide and treated with xylene dewaxing twice (10 min each time) and gradient alcohol rehydration (volume fraction of alcohol was 100, 95, 80, 70 and 0%). The paraffin sections were kept in hematoxylin for 5-10 min at room temperature, then the nuclei were stained. The paraffin-embedded sections were put into a mixed solution of hydrochloric acid and alcohol (70% hydrochloric acid volume fraction) for 30 sec, then the paraffin-embedded section was hydrated (the non-specific staining was differentiated to make the chromatin in the nucleus more clear). At the same time, the paraffin-embedded section was immediately put into the water, washed with water for blueing for 10-15 min, and then eosin staining was performed on the paraffin-embedded section (to stain the cytoplasm) at room temperature. Then, the sections were dehydrated with gradient alcohol, washed with xylene and sealed with resin adhesive. Histological examination was carried out under a Nikon Eclipse Ti-S fluorescence microscope in a bright-field with light (magnification, x100 and x400; Nikon Corporation).

Serum estrogen receptor and hormone analysis. Blood samples were kept at 2-8˚C for 12 h, then centrifuged at 1,000 x g for 15 min at 4˚C for serum collection. The serum estrogen receptor (ESR; cat. no. ml260315), follicle-stimulating hormone (FSH; cat. no. ml263000-3), luteinizing hormone (LH; cat. no. ml063366-1) and testosterone (T; cat. no. ml001948-1) levels were measured using ELISA kits (Shanghai Meilian Biotechnology Co., Ltd.) according to the manufacturer's instructions. Specifically, 40 μl dilution buffer and 10 μl serum were added to antibody-coated 96-well microplates and incubated at 37˚C for 30 min. After washing, horseradish peroxidase-labeled secondary antibody was added to each well. The presence of enzyme complexes was detected by the addition of TMB reagent. The measurable protein ranges for ESR, FSH, LH and T were 10-320 ng/l, 0.5-16 U/l, 70-2400 pg/ml and 8-240 nmol/l, respectively.

Measurement of mitochondrial DNA (mtDNA) copy number. DNA was extracted from whole blood using a commercial kit (Tiangen Biotech Co., Ltd.). The relative mtDNA copy number was measured using quantitative PCR with the SYBR-Green Real-time PCR Master Mix (Toyobo Life Science). Relative mtDNA levels were normalized to actin. The primers used were as follows: Actin forward, 5'-AGCCATGTACGTAGC

Table I. Offspring number in the F1 generation.

Group	Gestation time, days	P-value	Pups/litter, n					
			Total	P-value	Females	P-value	Males	P-value
Control	19.8±0.6	N/A	12.3±2.1	N/A	6.0±1.0	N/A	6.3±1.1	N/A
BPA2	20.2±0.6	0.103	13.3±0.5	0.203	6.8±0.9	0.363	6.3±0.9	1.000
BPA20	19.9±0.5	0.356	13.6±2.2	0.102	7.8±1.5	0.105	6.2±1.1	0.363
BPA200	19.8±0.5	0.766	9.1±1.7[a]	0.011	4.3±1.5	0.075	4.7±0.6	0.107
BPA20 + SIF300	20.3±0.5	0.103	10.1±1.1[b]	0.006	4.8±0.8	0.238	4.8±0.8	0.107
DES	19.8±0.3	0.360	10.3±2.1[a]	0.018	4.0±1.0	0.049	6.3±1.2	1.000

Data are presented as the mean ± SD. n≥3 pregnant mice per dose group. [a]P<0.05, [b]P<0.01 vs. control. N/A, not applicable; BPA, bisphenol A; SIFs, soy isoflavone; DES, diethylstilbestrol.

CATCCA-3' and reverse, 5'-TCTCCGGAGTCCATCACC ATG-3'; mitochondrial DNA-ND1 forward, 5'-CCATTTGCA GACGCCATAAA-3' and reverse, 5'-GAGTGATAGGGTAGG TGCAATAA-3'. The thermocycling conditions consisted of an initial denaturation step at 55˚C for 10 min, followed by 40 cycles at 95˚C for 30 sec, 55˚C for 30 sec, then 72˚C for 60 sec. Relative mtDNA levels were calculated using the $2^{-\Delta\Delta Cq}$ method (24), where $\Delta Cq=Cq_{actin}-Cq_{ND1}$.

Measurement of serum malondialdehyde (MDA) levels and superoxide dismutase (SOD) activity. Serum MDA levels were measured using an MDA determination kit (cat. no. A003-1-2; Nanjing Jiancheng Bioengineering Institute) based on the thiobarbituric acid detection method for lipid peroxides. MDA in the serum reacts with thiobarbituric acid, producing a color change, with maximum absorbance detectable at a wavelength of 532 nm.

Inhibition of hydroxylamine oxidation by the xanthine-xanthine oxidase system was assessed by measuring serum SOD levels (25). This was carried out using a SOD assay kit (cat. no. A001-1-2; Nanjing Jiancheng Bioengineering Institute). Briefly, the reaction was initiated by incubating serum with hypoxanthine, hydroxylamine and xanthine oxidase at 37˚C for 40 min. The reaction was terminated by adding 16% (v/v) acetic acid solution containing sulfanilic acid and naphthyl ethylenediamine, and the absorbance was measured at 550 nm to determine SOD activity.

Statistical analysis. Statistical analysis was conducted using Prism 5.0 software (GraphPad Software, Inc.). All data are presented as the mean ± SEM or medians (n=5). Differences between groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Reproductive toxicity of F0 female mice. All pregnant mice underwent normal parturition. The reproductive and fetal findings are presented in Table I. No significant differences were found in gestation length between groups.

Moreover, on PND 0, sex was determined by examining external genitalia and was confirmed by autopsy at the end of the experiment (Table I). The sex distribution did not differ between the offspring in the control group and any BPA or DES-treated group or BPA + SIF-treated group. However, the number of live pups per litter in BPA200, BPA20 + SIF300 and DES-treated groups were significantly reduced, compared with the control. Besides, there were fewer females pups in the BPA20 + SIF300 group compared with BPA20 alone (P<0.05).

Total body weight and relative organ weight in F1 offspring. All body weights were recorded on PND 0, 14 and 26 (Fig. 1). On PND 0, the body weight of all offspring in the BPA and DES-treated groups were significantly greater than controls, except for the BPA200 group. On PND 14, male offspring had significantly larger weights in the BPA2, BPA20 + SIF300 and DES groups, compared with the control. The offspring exposed to the low dose of BPA appeared heavier than those exposed to the higher dose of BPA. By contrast, female offspring weight was significantly higher in all treated groups, compared with the control. On PND 26, all offspring weighed more in the all treated groups, compared with the control group, except for the female offspring in the BPA200 group. A general trend observed was that at higher BPA doses body weight was lower. In addition, pups in the BPA20 + SIF300 group weighed more than pups in the BPA20 group at each time-point.

The relative weights of the testes and uterus were determined on PND 7 and 26 (Fig. 2). On PND 7, the relative weight of the testes or uterus did not differ across groups, both in male and female offspring. However, on PND 26, the relative weight of testes declined in a dose-dependent manner in male offspring following treatment with BPA. Moreover, the relative weight of the testes was significantly lower for mice in the BPA20 + SIF300 group, compared with mice in the BPA20 group. On PND 26, the relative uterus weight of the female offspring was elevated only in the DES-treated group.

Furthermore, the relative weights of the thymus, liver, spleen, heart, lung, kidney and brain were also obtained both for male and female mice (Tables SI and SII). The relative brain weight decreased after treatment with BPA and DES. Lastly, in all offspring on PND 26, the thymus and lung weight was higher in the BPA and DES treatment groups.

AGD in F1 offspring mice. AGD was measured on PND 0, 7, 14, 21 and 26. Overall, exposure to BPA and DES resulted

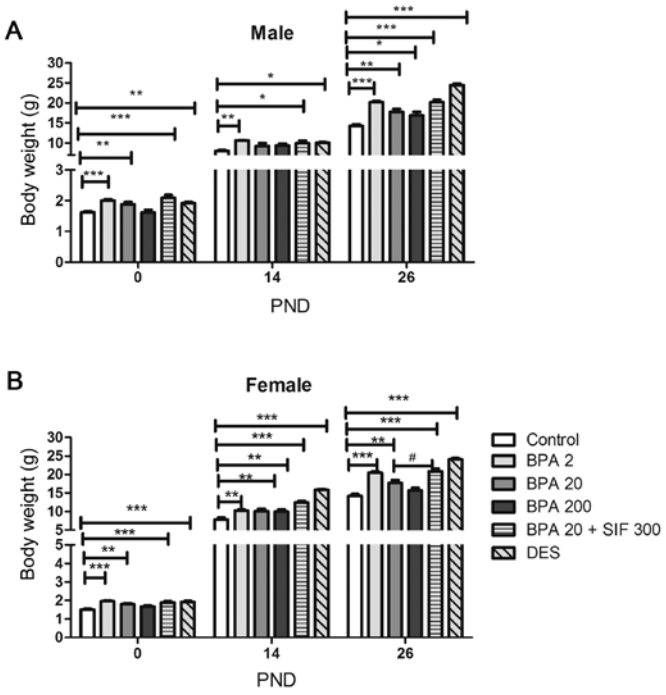

Figure 1. Combined effects of BPA and SIFs on the body weight of F1 offspring on PND 0, 14 and 26. (A) Male offspring. (B) Female offspring. Data are presented as the mean ± SEM. *P<0.05, **P<0.01, ***P<0.001 vs. control; $^{\#}$P<0.05 vs. BPA20. BPA, bisphenol A; SIFs, soy isoflavone; DES, diethylstilbestrol; PND, postnatal day.

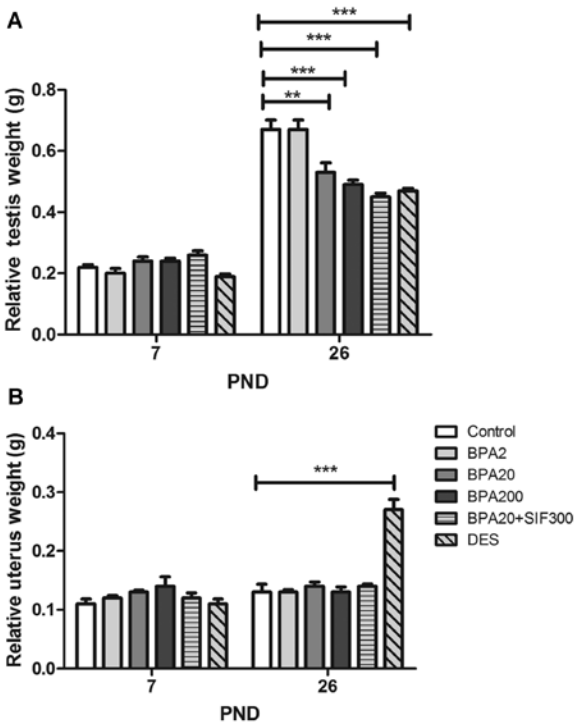

Figure 2. Combined effects of BPA and SIFs on relative testes and uterus weight of F1 offspring on PND 7 and 26. (A) Relative testes weight. (B) Relative uterus weight. Data are presented as the mean ± SEM. **P<0.01, ***P<0.001 vs. control; BPA, bisphenol A; SIFs, soy isoflavone; DES, diethylstilbestrol; PND, postnatal day.

in a significant increase in AGD at each time-point, both in male (Fig. S1) and female offspring (Fig. S2). From PND 14 to 26, the effect of BPA was dose-dependent, as increasing BPA dose during pregnancy was associated with longer AGD at these timepoints. However, there was a significant difference in male of PND 7 (P<0.05; Fig. S1).

However, the AGD to body weight ratio displayed a different trend (Fig. 3). On PND 26, this ratio decreased in male offspring in the BPA2, BPA20 + SIF300 and DES groups, compared with the control group. Similarly, the AGD to body weight ratio in female offspring was lower in the BPA2 and DES-treated groups on PND 14 and 26. Co-exposure to BPA and SIFs appeared to result in a reduction in AGD to total body weight ratio, compared with BPA20, although this was not statistically significant.

Histological analysis of testes tissue. On PND 7, histological examination of the testes in the control group indicated growing seminiferous tubules, with the interstitial space being relatively large and predominantly filled with mesenchymal cells. By contrast, in the DES group, growth of the tubules was defective and the interstitial mesenchymal tissue was loosely organized (Fig. 4A). Upon exposure to BPA and SIFs, structural disturbance of testes was also observed. On PND 26, the architecture of the seminiferous tubules in the control group offspring was normal, with regularly arranged rows and complete set of germinal epithelia (Fig. 4B). Furthermore, while the diameter of the tubules increased, the interstitial space appeared to decrease. However, in the DES group, there was tubular degeneration and loss of cellular architecture in spermatogenic series. Sloughing of seminiferous epithelium

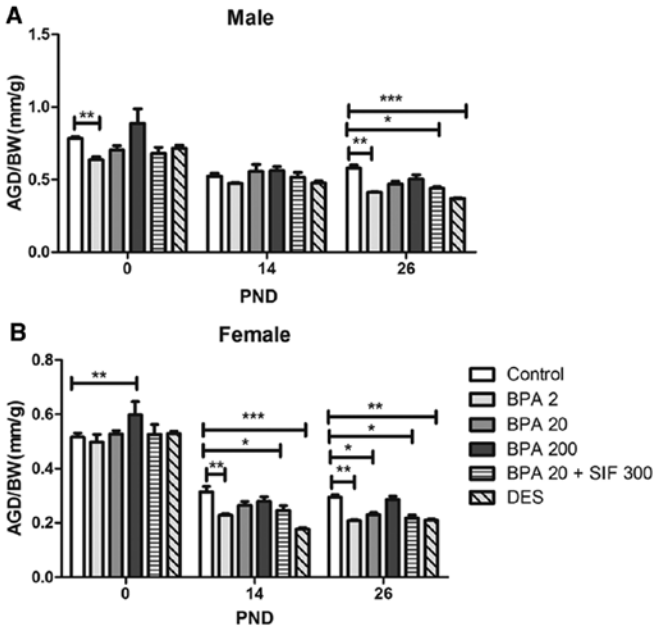

Figure 3. Combined effects of BPA and SIFs on the AGD/BW ratio in F1 offspring on PND 0, 14 and 26. (A) Male offspring. (B) Female offspring. Data are presented as the mean ± SEM. *P<0.05, **P<0.01, ***P<0.001 vs. control. BPA, bisphenol A; SIFs, soy isoflavone; DES, diethylstilbestrol; PND, postnatal day; AGD, anogenital distance; BW, body weight.

and spermatogenic cells into the lumen of the seminiferous tubules was also observed in the DES-exposed group. Testicular lesions in male offspring progressed with increasing doses of BPA. Moreover, a higher degree of damage was observed in

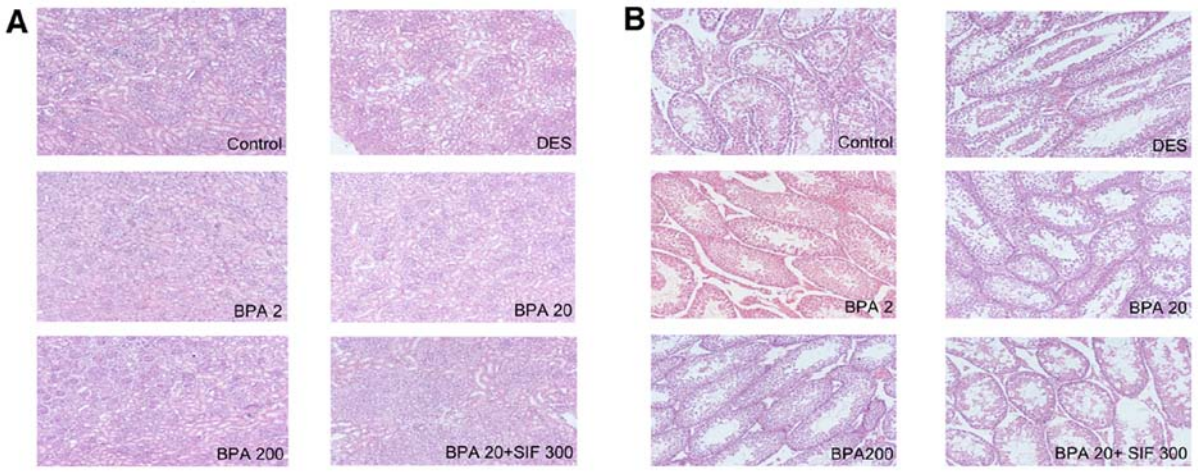

Figure 4. Effects of BPA and SIFs on the testicular histology of male offspring. Testicular histology in the indicated groups on PND (A) 7 and (B) 26. Magnification, x100. BPA, bisphenol A; SIFs, soy isoflavone; DES, diethylstilbestrol; PND, postnatal day.

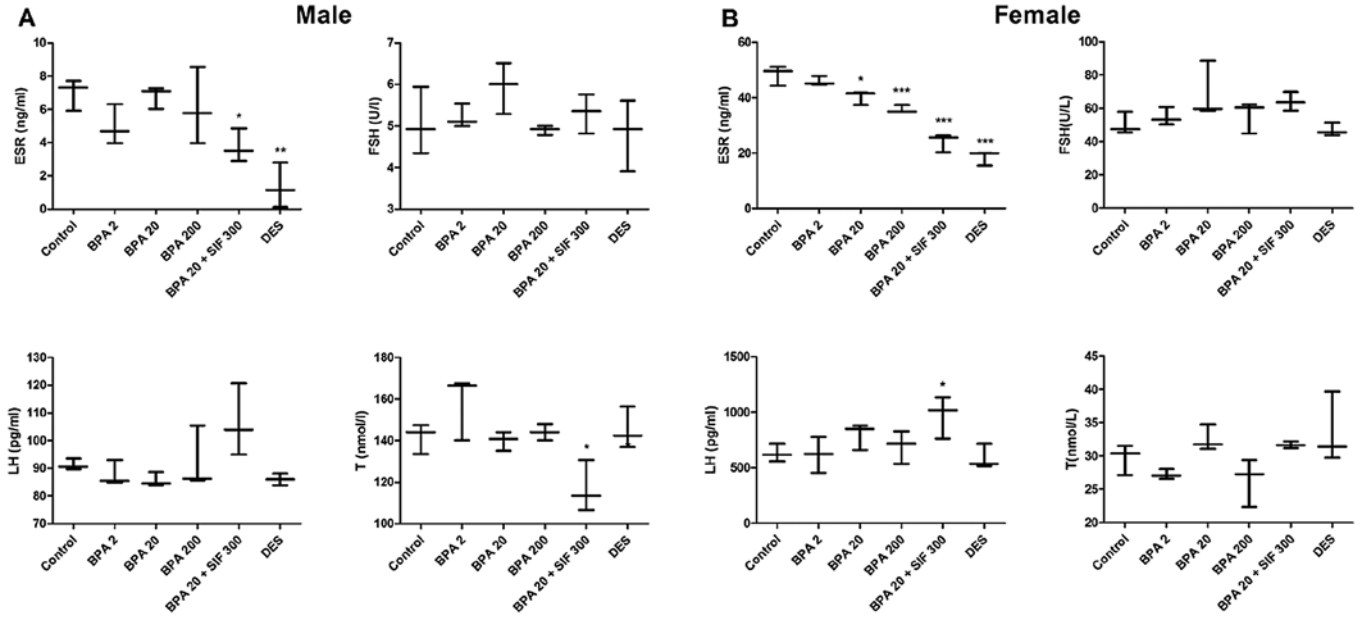

Figure 5. Effects of BPA and SIFs on serum levels of ESR, FSH, LH and T. Serum levels of the indicated groups in (A) male and (B) female pups on PND 26. Data are presented as the medians. *P<0.05, **P<0.01, ***P<0.001 vs. control. BPA, bisphenol A; SIFs, soy isoflavone; DES, diethylstilbestrol; PND, postnatal day; ESR, estrogen receptor; FSH, follicle-stimulating hormone; LH, luteinizing hormone; T, testosterone.

the BPA20 + SIF300 offspring compared with groups exposed to BPA only.

Serum ESR and hormonal analysis. Serum ESR, FSH, LH and T levels were determined on PND 26. In male offspring, ESR levels significantly decreased in the BPA20 + SIF300 and DES groups, compared with the control group. Moreover, co-exposure to BPA and SIFs significantly decreased serum T levels, compared with BPA20. No significant differences were noted in FSH and LH levels across the groups (Fig. 5A).

In female offspring, ESR levels followed a dose-dependent decline in the offspring from BPA-treated mice and were lowest in the DES group, compared with the control. Female offspring from the BPA20 + SIF300 groups displayed lower levels of ESR, compared with BPA20. In addition, a significant increase in LH levels was only observed in female offspring from mice exposed to BPA and SIFs. There was no statistically significant difference in FSH and T levels among the groups (Fig. 5B).

Oxidative stress parameters. MtDNA damage is related to increased oxidative stress and inflammation (26,27). Thus, on PND 26, the relative mtDNA copy number in whole blood was evaluated in the offspring. The mtDNA copy numbers of the ND1 gene significantly increased by 322.2% in the BPA200 group, compared with the control group (Fig. 6A).

MDA is one of the most frequently used indicators of lipid peroxidation (28). The serum levels of MDA significantly increased in the BPA200 group in comparison with the control group (Fig. 6B).

SOD is a key antioxidant enzyme that is essential for the control of free radical production (25). SOD activity in the

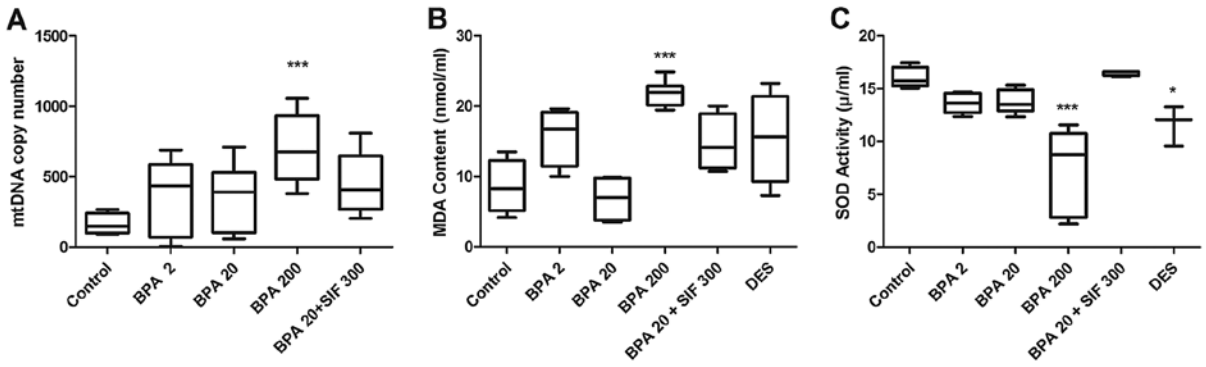

Figure 6. Effects of BPA and SIFs on oxidative stress-related parameters. (A) mtDNA copy number, (B) MDA content and (C) SOD activity of F1 offspring mice on PND 26. Data are presented as the medians. *P<0.05, ***P<0.001 vs. control. BPA, bisphenol A; SIFs, soy isoflavone; DES, diethylstilbestrol; PND, postnatal day; mtDNA, mitochondrial DNA; MDA, malondialdehyde; SOD, superoxide dismutase.

BPA200 and the DES group decreased significantly, compared with the control group (Fig. 6C).

No differences in mtDNA copy number, MDA levels or SOD activity were observed between the BPA20 and BPA20 + SIF300 groups.

Discussion

EDCs are a structurally diverse class of synthetic and natural compounds that alter endocrine and hormonal functions. Exposure to EDCs often occurs in combination with several types of diet and can result in adverse health outcomes, such as reproductive damage, developmental impairment and cancer (29-33). However, the effects of co-exposure to EDCs are poorly understood. In the present study, the combined effects of two types of EDC, BPA and SIFs, on the reproductive system were evaluated in mouse offspring that were exposed gestationally. BPA exposure increased the body weight of pups and decreased the AGD to body weight ratio, especially in low-dose exposure groups. Moreover, decreased weight and histopathological changes were identified in the testes of male offspring. These BPA and SIFs-induced adverse effects were found to be accompanied by serum hormonal alterations, which have an impact on the reproductive process. Moreover, co-exposure to BPA and SIFs aggravated these changes, compared with BPA alone. However, SIFs exposure alone was not evaluated in the present study, which represents an important limitation of the present findings.

EDCs contribute to the progression of metabolic disorders, including obesity and diabetes (34). Children are hypothesized to be more sensitive to EDCs, as they take up more calories per body surface area and have higher minute ventilation (35). Previous animal studies suggested that prenatal and/or neonatal exposure to low doses of BPA led to an increase in body weight in the offspring (36,37). The present findings were consistent with this trend. Epidemiological studies also suggested that BPA is associated with obesity in adults (38-41). However, the lowest concentration of BPA causing adverse effects has been difficult to find, which indicates an urgent need for reevaluation of BPA safety.

Human exposure to EDCs is frequent, persistent and usually occurs in combination with other chemicals, leading to unpredictable combined effects (42). Exposure to BPA and SIFs in particular is common during pregnancy. A previous study demonstrated that SIFs displayed numerous biological properties, including antitumor activity, osteoporosis prevention and increase of cognitive function (43). However, there is growing concern regarding their safety, based largely on their estrogen-like properties. Co-exposure to BPA and SIFs has been reported to influence certain aspects of growth, weight gain and puberty, suggesting that BPA and SIFs may interact with each other, leading to these adverse outcomes. For example, in a previous study, the anxiogenic phenotype induced by BPA exposure during development could be mitigated by a soy-rich diet (44). Moreover, a soy-rich diet was demonstrated to modulate the effects of BPA on meiotic processes in periovulatory oocytes (45). Furthermore, previous studies have suggested that co-exposure of BPA and SIFs might have an additive effect on the reproductive system. For instance, Do *et al* (40) demonstrated that high isoflavone content and BPA had a synergistic effect on the induction of uterine peroxidase. Moreover, BPA and SIFs have been implicated in ESR-mediated transcriptional transactivation (46). Consistent with these previous findings, co-exposure to BPA and SIFs potentiated the reproductive toxicity of BPA in the present study. The present results also indicated that SIFs could have potential adverse effects in early life, particularly when combined with other EDCs, such as BPA. Thus, the use of EDCs requires accurate risk assessment. Moreover, understanding the underlying mechanisms through which these interactions between BPA and SIFs occur is also critical for addressing public health concerns.

The present study also suggested that the toxicity of BPA and SIFs during development, as well as their combined effects, may be mediated via the ESR. The ESR serves important roles in differentiation and maintenance of the reproductive system, as evidenced by the abnormal shape of the reproductive organs perinatally exposed to the synthetic estrogen diethylstilbestrol (47-50). The ESR regulates gene expression and proliferation in epithelial and stromal cells. Both BPA and SIFs have been reported to bind to the ESR, leading to different protein and mRNAt changes (51-55).

FSH, LH and T are essential for the development and function of the reproductive system. LH and FSH are secreted by the anterior pituitary gland in response to hypothalamic

gonadotropin-releasing hormone. In men, LH stimulates T release from Leydig cells in the testes. T is also an essential factor in normal spermatogenesis (56). Moreover, an increase in T levels during puberty promotes development of the sexual organ and enables semen production (57). In the present study, co-exposure to BPA and SIFs led to a decrease in serum ESR and T, compared with offspring exposed to BPA only, demonstrating a synergistic effect on ESR expression and T release. These observed hormonal changes could account for the smaller AGD to body weight ratio and relative testes weight following BPA and SIFs co-exposure. Histopathological examination also demonstrated spermatid damage and degeneration in a dose-dependent manner following treatment with BPA, which was exacerbated following BPA and SIFs co-exposure. Therefore, the possible synergetic effects of BPA and SIFs on the reproductive system could be attributable to changes in ESR and testosterone levels.

In addition to hormonal regulation, alterations in the redox system have also been implicated in the regulation of reproductive processes in both animals and humans (58,59). SOD enzymes participate in the removal of O_2^- and regulate intracellular O_2^- levels. In semen samples from patients with infertility with high O_2^- levels, prolonged inhibition of sperm mitochondrial function could inhibit sperm motility (60,61). Since mitochondria regulate energy metabolism and reactive oxygen species release in response to extracellular stimuli, mitochondrial constituents, including mtDNA, are particularly susceptible to oxidative damage (62). A previous study demonstrated that mtDNA copy numbers (mitochondrial genome as a whole) are critical to fertilization outcomes and can serve as an important marker of oocyte quality (63). The extent of oxidative damage can be assessed by measuring MDA levels, one of the final products of lipid peroxidation. Increased MDA levels are associated with decreased sperm motility (64). In the present study, both the mtDNA copy number and MDA levels significantly increased following gestational co-exposure to BPA200. SOD activity diminished with exposure to high-dose BPA. By contrast, there were no differences between combined BPA and SIFs exposure, and BPA alone. Thus, high doses of BPA alone can lead to dysregulation of reproductive function via oxidative damage in F1 offspring, which indicated that the changes in oxidative stress-related parameters were not due to the synergistic effect of BPA and SIFs.

In conclusion, the present study revealed that co-exposure to BPA and SIFs could have a synergic effect on the reproductive system. The interaction between BPA and SIFs could be mediated by regulation of ESR and hormone release. These results may aid in the development of precise prevention strategies and treatment of BPA exposure.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JM, YLi, YZ and PY conceived the methodology; LY, YLi and SW validated and formally analyzed the data; JM, YLu, YN and YH performed the experiments; HZ conducted the data curation. HZ and YLu prepared the original draft. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments in the present study were approved by The Animal Ethics Committee of the Guilin Medical University (approval no. GLMC201803066).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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