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RESEARCH ARTICLE

Ameliorative effect of ginseng extract on phthalate and bisphenol A reprotoxicity during pregnancy in rats

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Abstract

Phthalates (such as DEHP) and bisphenol A (BPA) are widely used chemicals in plastics manufacturing and exert public health concerns as endocrine disrupters. This study was designed to investigate the deleterious effect of DEHP and BPA on endocrine profile of pregnant female rats and the combined treatment with ginseng extract (*Panax ginseng*). Seventy-two pregnant rats were divided into six groups (control, ginseng, DEHP, BPA, Gin + DEHP, and Gin + BPA), 12 females per each group. The drugs were supplemented from pregnancy day 0 until day 20. Determination of serum sex hormones (testosterone, progesterone, and estradiol) were determined on days 4, 10, and 20 of pregnancy. mRNA transcripts of *STAR, HSD17B3, CYP17, AKT1*, and *PTEN* were relatively quantified against ACTB in the ovary and placenta of days 10 and 20 pregnant females by relative quantitative polymerase-chain reaction (RQ-PCR). DEHP and BPA significantly decreased the endocrine profile of testosterone, progesterone, and estradiol of days 10 and 20 of pregnant females. Combined administration of these chemicals along with ginseng extracts has returned the hormones to normal levels when compared with the control group. The ovarian and placental *CYP17* and *HSD17B3* mRNA transcripts showed variable expression pattern in both tissues and they were significantly affected by DEHP and BPA administration, concomitantly correlating to *STAR, AKT1*, *PTEN,* progesterone, and testosterone levels on pregnancy days 10 and 20. The results confirm the reprotoxicity of DEHP and BPA as endocrine disruptors and indicate that ginseng could be used to alleviate the toxic effects of these chemicals.

Keywords Bisphenol A · Phthalates · DEHP · Pregnancy · Ginseng · Rats

Islam M. Saadeldin and Mohamed A. Hussein contributed equally to this work.

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Abbreviations

AKT1	Alpha serine/threonine-protein kinase
BPA	4,4′-Isopropylidenediphenol or bisphenol A
CYP17	Cytochrome P450 17alpha hydroxylase/17,20 lyase
DEHP	Bis(2-ethylhexyl) benzene-1,2-dicarboxylate or bis(2-ethylhexyl) phthalate
Gin	Ginseng
HSD17B3	17β-Hydroxysteroid dehydrogenase 3
PTEN	Phosphatase and tensin homolog
STAR	Steroidogenic acute regulatory protein

Introduction

Plastics are used in a wide variety of consumer products. Some plastics are made of toxic plasticizers to render flexibility. Chemicals such as bisphenol A and phthalates (DEHP), widely used in the food packaging industry and baby bottles, have been identified in human fluids and are considered high production volume man-made chemicals (Konieczna et al. 2015; Woodruff et al. 2011).

BPA and DEHP are synthetic additives used to harden polycarbonate plastics and epoxy resins present in many consumer products; furthermore, it can be released from industrial products by various physical or chemical processes and be absorbed through the human skin or inhaled as dust (Brotons et al. 1995). Extensive reports of phthalates and BPA are available showing these additives as endocrine disrupting agents and causing reproductive system disorders in children and adults (Diamanti-Kandarakis et al. 2009; Fenichel et al. 2013). The risk of occupational and non-occupational exposure to these chemicals has attracted much attention, also because of its potential threat to public health with special attention to pregnant females and conceptuses.

Ginseng is a highly valued traditional herb in the Far East and has gained popularity worldwide over the past two decades. The major active components of ginseng are ginsenosides, a diverse group of steroidal saponins, which demonstrate the ability to target a myriad of tissues, producing diverse pharmacological responses (Attele et al. 1999; Choi et al. 2013; Gillis 1997; Li et al. 2014). Several studies have described the beneficial effects of ginseng and its constituents; however, few have reported the beneficial effects of ginseng against DEHP and BPA toxicity (El-Drieny et al. 2009; Yang et al. 2014).

The current study aims to investigate the deleterious effects of BPA and DEHP on the endocrine profile of pregnant female rats and to examine the effects when combined with ginseng treatment. Serum sex hormone (testosterone, progesterone, and estradiol) levels were determined on days 4, 10, and 20 of pregnancy. The mRNA transcripts of steroidogenesis (*STAR, HSD17B3, CYP17*) and some regulatory pathways including *AKT1* and *PTEN* were relatively quantified in the ovary and placenta of pregnant female rats.

Materials and methods

Experimental animals

We used 72 adult female albino rats with average weight of 150–200 g. The animals were obtained from the central animal house, Faculty of Veterinary Medicine, Zagazig University, Egypt. They were housed in separate stainless steel cages, and water and food were available ad libitum. They were maintained at a temperature of 25 °C and 12 h light/12 h dark cycle throughout the course of the experiment. Food debris, feces, and urine were removed daily to prevent food and water contamination. Females in the estrous phase were monitored through vaginal smear test, which was characterized by presence of irregular cornified cells. Two females in estrous were kept with one adult male (~ 250 g) overnight, after which another vaginal smear was performed to examine the presence of spermatozoa, which indicates pregnancy day 0.

Drug administration and aqueous extraction of ginseng

Bisphenol A (BPA) [IUPAC name: 4,4′-isopropylidenediphenol; chemical formula: $C_{15}H_{16}O_2$; and molecular weight, 228.29 g/mol (Fig. 1a)] was administered at 150 mg/kg/day (Schonfelder et al. 2002). This dose was expected to result in adult systemic toxicity in rat during gestation period (Stump et al. 2010). Phthalates (DEHP) [IUPAC name: bis(2-ethylhexyl) benzene-1,2-dicarboxylate; chemical formula: $C_{24}H_{38}O_4$; molecular weight: 390.56 g/mol (Fig. 1b)] were administered at 100 mg/kg/day (Koch et al. 2006). This dose was used to induce reproductive toxicity in rats (Christiansen et al. 2010). Rats were given daily supplementation (200 mg/kg) of an aqueous ginseng (*Panax ginseng*) extract (Kopalli et al. 2015). Briefly, *Panax*

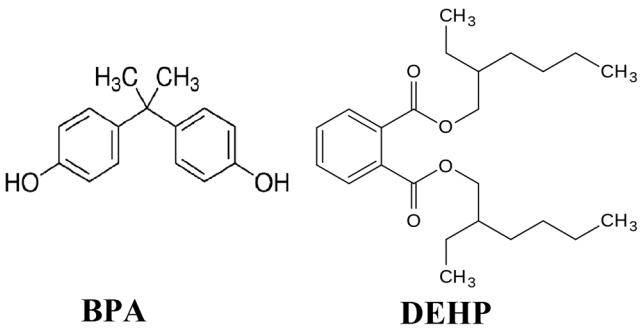

Fig. 1 Structure of 4,4′-isopropylidenediphenol (bisphenol A, BPA) and bis(2-ethylhexyl) phthalate (DEHP)

ginseng roots powder was kindly supplied via Pharco Pharmaceuticals Co., Alexandria, Egypt. Ginseng powder was soaked for 5 h in water at 40 °C (three separate times). The solution was then filtered under vacuum at 45 °C. The three pools were then combined and concentrated again under vacuum at 45 °C to obtain 60% dry basis.

Experimental design

Pregnant rats were randomly divided into six groups of 12 females [control, ginseng (Gin), phthalates (DEHP), bisphenol A (BPA), Gin + DEHP, and Gin + BPA]. The drugs were supplemented from pregnancy day 0 until day 20 orally using stomach gavage. Mortality and the general conditions of animals were observed daily throughout the study period.

Sampling

Serum samples were collected on days 4, 10, and 20 of pregnancy (12 h after the last doses were administered). In brief, whole blood samples were withdrawn from the animals by puncturing the retro-orbital venous plexus using sterilized glass capillary tube. The samples were then placed in a slanted position until clotting and centrifuged at 3000 rpm for 10 min to obtain the serum, which was frozen at − 20 °C until measurement of sex hormones levels. For tissue samples, rats on pregnancy days 10 and 20 were killed by cervical decapitation, the ovaries and placentae were dissected immediately, wrapped in sterilized aluminum foil, and immediately dipped in liquid nitrogen until use in PCR.

Measuring serum sex hormone levels

Enzyme-linked immunosorbent assay (ELISA) for the measurement of sex steroid hormones was performed using ELISA kits purchased from Diagnostics Research Group (Mountainside, NJ), and was measured by (Union Medical Pharmaceutical Technology, Tianjin, China). The analytical sensitivity of each kit was 0.1 ng/ml for progesterone, 0.083 ng/ml for testosterone, and 6.3 pg/ml for estrogen. All samples were run in duplicates, and all intra- and interassay coefficients of variability were less than 10%. Some samples were diluted to match the dynamic range of each ELISA kit. Mean values for each sample were used in this analysis.

Tissue sampling, RNA extraction, and complementary DNA synthesis

Total RNA was extracted using E.Z.N.A spin column RNA extraction kit (easy-BLUE total RNA extraction kit; Cat. No. 17061; iNtRON Biotech, Seoul, South Korea). First-strand complementary DNA (cDNA) synthesis was performed using one step kit (premix) RT-PCR kit (Cat. No. 25101; iNtRON Biotech).

Relative quantitative PCR

PCR amplification was performed using 2X PCR master mix (SensiFAST cDNA Synthesis kit; Cat.No.65053; BIOLINE). PCR was carried out in a 50-μL volume consisting of 0.1 μg cDNA, 25 μL 2X master mix, variable amounts of forward and reverse primers according to the specific gene, and volume made up to 50 μl using nuclease-free water. Supplemental Table 1 shows specific PCR conditions for the primers used. Oligonucleotide primers were designed with Eugene version 2.2 software (Ambion). A primer pair for β-actin (Ambion, Austin, TX) was used as an internal control for PCR analysis; β-actin was chosen as the reference gene because its expression did not differ across treatment groups. All reactions were performed in a Stratagene (Applied Biosystems) in which the samples underwent a 10-min initial denaturation step to release DNA polymerase, followed by number of cycles (Table 1). Expression data were generated using the mathematical standard comparative (ΔΔCt) method. The ΔCt was calculated by subtracting the β-actin Ct value from the gene of interest Ct value. The ΔΔCt was calculated as the difference between the ΔCt between the treatment groups and the control reference group. The relative fold change of expression was then equaled to $2^{(-\Delta\Delta Ct)}$ for each sample (Livak and Schmittgen 2001). PCR products were separated on a 1.5% agarose gel in triacetate EDTA buffer with 0.5 μg/ml ethidium bromide to confirm the presence of the PCR band.

Statistical analysis

All data were expressed as means ± SEM. Statistical significance ($P < 0.05$) was evaluated by one-way analysis of variance (ANOVA) using SPSS 18.0 (SPSS 2009) followed by Tukey post hoc comparison. Pearson's linear correlation coefficients were calculated to determine the correlation (R) between the means of different mRNA transcript expressions in the ovary and placenta of pregnant rats, with R values $> \pm 0.7$, strong positive/negative linear relationship; $R > \pm 0.5$, moderate positive/negative linear relationship; or $R < \pm 0.5$, weak positive/negative linear relationship (Petrie and Watson 2013).

Availability of data and material All data generated or analyzed during this study are included in this article. Any inquiries should be sent directed to the corresponding author.

Results

Effect of ginseng, BPA, and DEHP on serum sex hormone levels

On pregnancy day 4, ginseng administration significantly increased testosterone (0.39 ± 0.01 ng/mL, Fig. 2) and

Table 1 Sequences of the primers used in relative quantitative PCR

Gene name	Forward 5′ >> > 3′	Reverse 5′ >> > 3′	Size (bp)	Accession no.
ACTB	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC	97	V01217.1
STAR	ACCACATCTACCTGCACGCCAT	CCTCTCGTTGTCCTTGGCTGAA	81	NM_031558.3
HSD17B3	AGAGTGTCATCCACTGCAAC	AGTACAGGCTATACAGAGGC	144	NM_054007.1
CYP17	AGTGATCATCGGCCACTATC	GAGCTACCAGCATCTGCAAA	141	M31681.1
AKT1	TTTGTCATGGAGTACGCCAATG	CACAATCTCCGCACCGTAGAA	102	NM_033230.2
PTEN	GACGACAATCATGTTGCAGCA	GCCTTTAAAAACTTGCCCCG	101	NM_031606.1

progesterone (62.03 ± 2.4 ng/mL, Fig. 3) levels. Gin + BPA significantly increased testosterone levels (0.45 ± 0.02 ng/mL, Fig. 2).

Similarly, on pregnancy day 10, ginseng significantly elevated testosterone levels either individually or combined with DEHP or BPA on pregnancy day 10 (Fig. 2). BPA on the other hand was found to significantly reduce progesterone levels (Fig. 3).

On pregnancy day 20, DEHP and BPA significantly reduced testosterone (0.92 ± 0.04 and 0.37 ± 0.03 ng/mL, respectively) and progesterone levels compared to the control and ginseng-treated groups.

Estradiol level showed no change among different treatments, except on day 20 after BPA treatment, which significantly reduced estradiol levels (Fig. 4).

Effect of ginseng, BPA, and phthalates on mRNA transcripts of steroidogenesis

Different treatments showed variable expression pattern of steroidogenesis mRNA transcripts (Fig. 5) in the ovary or placenta when compared with the control negative group. We set the values of control negative group as arbitrary units (onefold) and were compared with the different treatments. On day 10 of pregnancy, at the ovarian level, (1) STAR transcript expression was significantly reduced by DEHP, Gin + DEHP, and Gin + BPA, while it showed significant increase with BPA treatment (~ 1.8-fold increase, $P < 0.05$); (2) *HSD* transcript expression showed significant reduction after Gin, DEHP, Gin + DEHP, and Gin + BPA; and (3) *CYP17* mRNA transcript was significantly reduced in the phthalate-treated groups, while Gin+BPA significantly increased its expression (~ 3-fold increase, $P < 0.05$). In the placenta, (1) *STAR* showed significant reduction after Gin and Gin + Phth, while it showed a significant increase after DEHP or BPA treatments (1.8- and 1.5-fold, respectively, $P < 0.05$); (2) *HSD* was significantly reduced by Gin and Gin + Phth; and (3) *CYP17* showed significant reduction by either phthalate or BPA treatments; however, Gin significantly alleviated and countered this decline, resulting in a 2.3- and 3.3-fold increase ($P < 0.05$).

Moreover, on pregnancy day 20 (Fig. 6), *STAR* expression showed significant reduction in the treated groups either in the ovary or placenta. Ovarian *HSD* expression significantly increased in ginseng-treated groups (eightfold increase, $P < 0.05$), while its placental expression showed significant reduction in Phth, BPA, and Gin + BPA groups. Phthalates significantly reduced *CYP17* expression in the ovary and placenta,

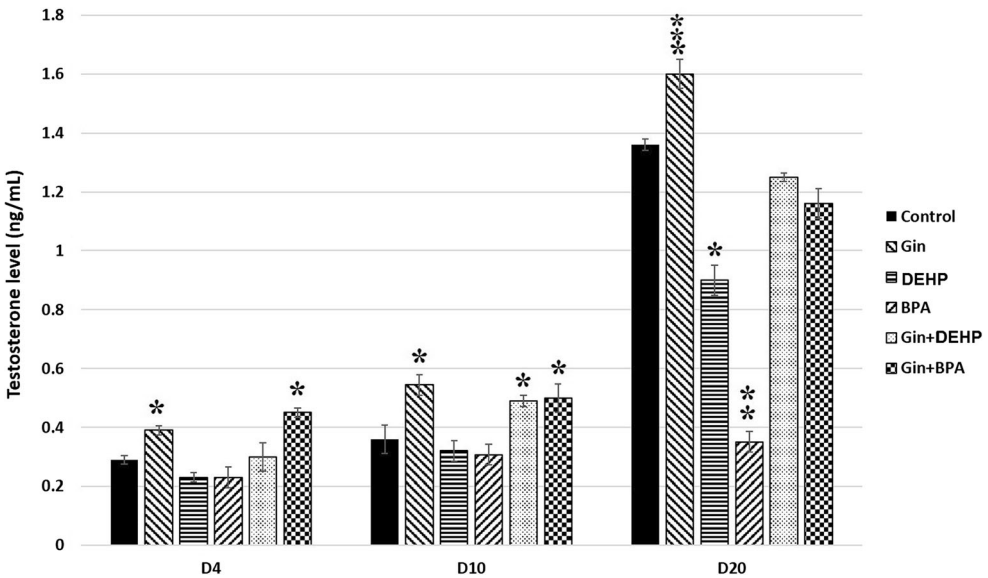

Fig. 2 Effect of ginseng, phthalates, and bisphenol A on serum testosterone level of pregnant rats. Columns carrying asterisks (*, **, and ***) are considered significantly different ($P < 0.05$) from other treatments (without asterisks) on the same day of sampling (days 4, 10, and 20). D4, D10, and D20 are days 4, 10, and 20 of pregnancy; Gin, ginseng; DEHP, bis(2-ethylhexyl) phthalate; BPA, bisphenol A

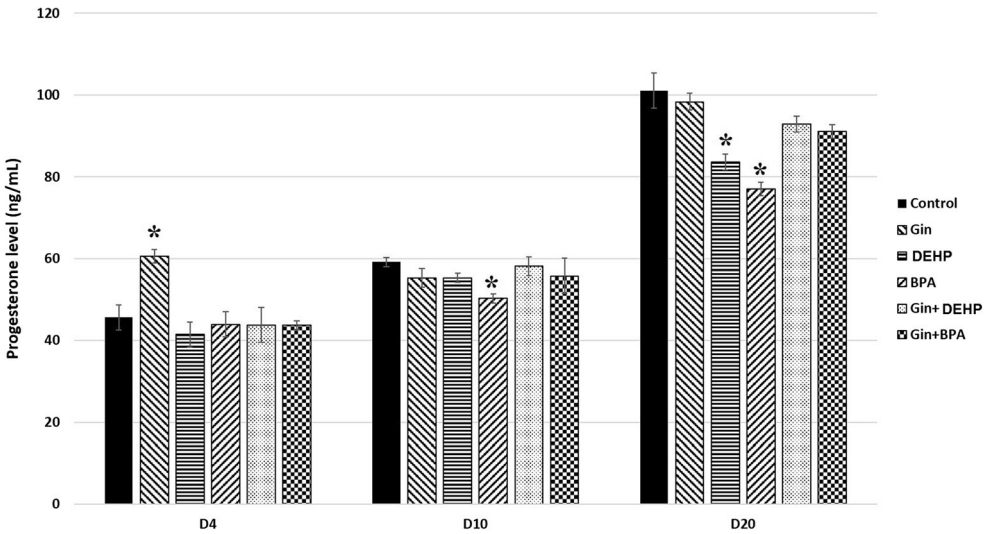

Fig. 3 Effect of ginseng, phthalates, and bisphenol A on serum progesterone level of pregnant rats. Columns with asterisk (*) considered significantly different ($P < 0.05$) from other treatments (without asterisks) on the same day of sampling (days 4, 10, and 20). D4, D10, and D20 are days 4, 10, and 20 of pregnancy; Gin, ginseng; DEHP, bis(2-ethylhexyl) phthalate; BPA, bisphenol A

an effect that was alleviated by combined ginseng treatment (three and twofold increase, respectively, $P < 0.05$).

Effect of ginseng, BPA, and phthalates on mRNA transcripts of *AKT1* and *PTEN*

On pregnancy day 10, ovarian *AKT1* showed significant decrease after DEHP and BPA treatments, which significantly declined further after combined ginseng treatments (Fig. 7). Similarly, placental *AKT1* significantly decreased in Gin + Phth and Gin + BPA groups when compared with phthalates and BPA individually (Fig. 7). Ovarian *PTEN* was significantly increased by BPA while ginseng treatment significantly reduced its expression (Fig. 8). Similarly, placental *PTEN* was elevated by phthalate and BPA treatments, but was significantly reduced with combined ginseng treatment (Fig. 8).

On pregnancy day 20, Gin and Gin + Phth significantly reduced ovarian *AKT1* expression (Fig. 7). While all treatments significantly reduced placental *AKT1* expression, the Gin + Phth group showed slight mitigation of *AKT1* expression (Fig. 7). Ovarian *PTEN* expression showed significant reduction in all treated groups except for phthalates (Fig. 8), while its placental expression showed significant reduction in all treated groups and showed slight alleviation in Gin + Phth treatment (Fig. 8).

Correlation coefficient of expression of different mRNA transcripts and serum sex steroids

On pregnancy day 10, the ovarian STAR showed strong positive correlation with *PTEN* ($R = 0.7$) and *HSD* ($R = 0.8$), and moderate positive correlation with *AKT* ($R = 0.6$), (Supplemental Table 2). *AKT* showed strong correlation with *PTEN* and moderate positive correlation with *HSD*. In addition, *PTEN* showed strong positive correlation with *HSD* (Supplemental Table 2). *PTEN* showed negative correlation with all sex steroid levels. In addition, *AKT* and *HSD* showed negative correlation with testosterone level.

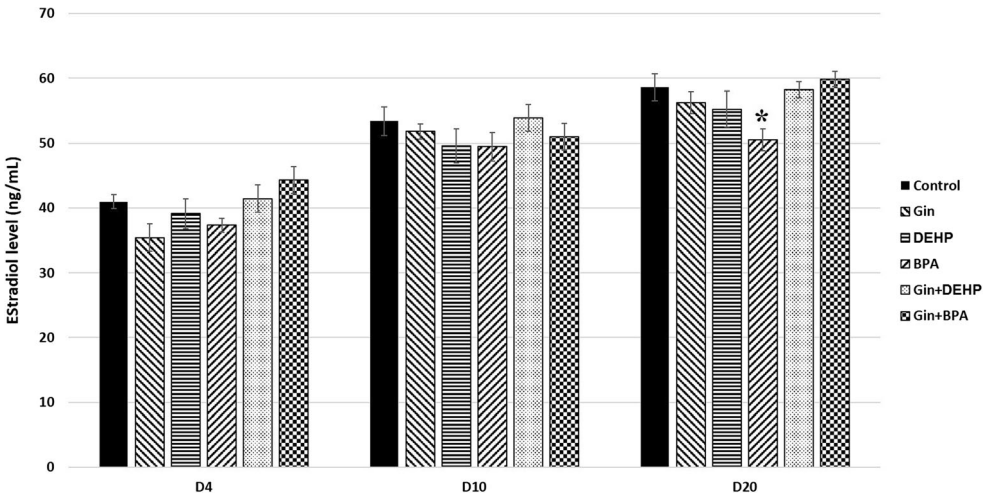

Fig. 4 Effect of ginseng, phthalates, and bisphenol A on serum estradiol level of pregnant rats. Columns carrying asterisks (*) considered significantly different ($P < 0.05$) from other treatments (without asterisks) on the same day of sampling (days 4, 10, and 20). D4, D10, and D20 are days 4, 10, and 20 of pregnancy; Gin, ginseng; DEHP, bis(2-ethylhexyl) phthalate; BPA, bisphenol A

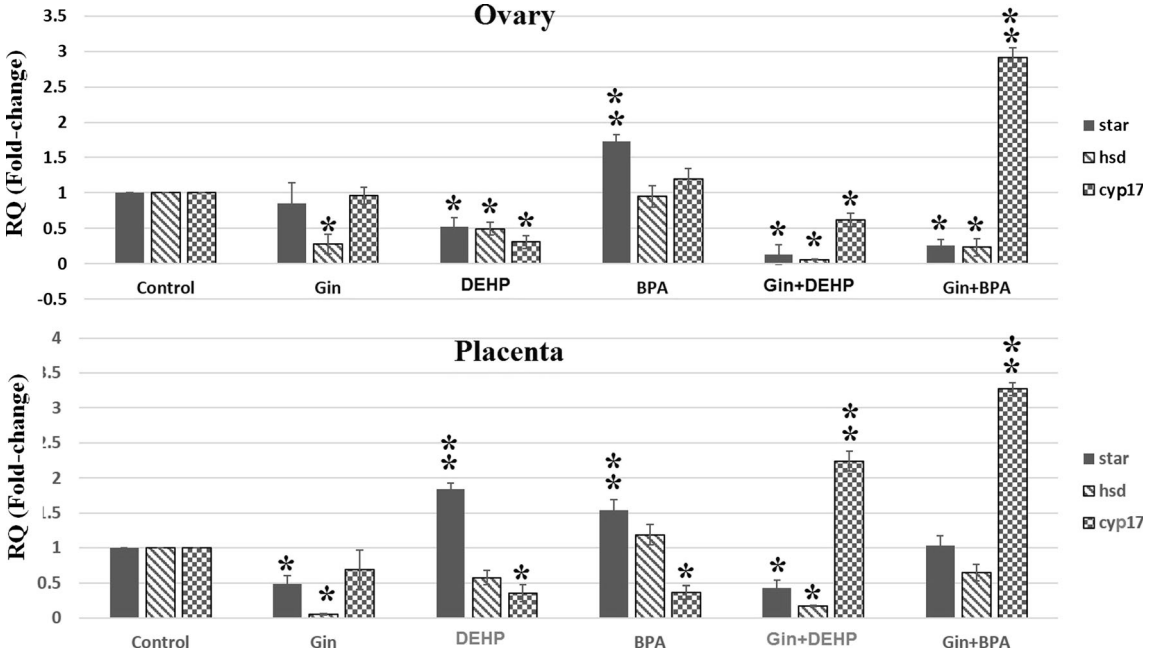

Fig. 5 Effect of ginseng, phthalates, and bisphenol A on relative quantification (RQ or fold changes) of mRNA of STAR, HSD17B3, and CYP17 in the ovary and placenta of day 10 pregnant rats. Columns carrying asterisks (* and **) are considered significantly lower or higher, respectively ($P < 0.05$), than the arbitrary control group (onefold). *Star*, STAR; *hsd*, HSD17B3; *cyp17*, CYP17; Gin, ginseng; DEHP, bis(2-ethylhexyl) phthalate; BPA, bisphenol A

Moreover, *STAR* showed negative correlation with both testosterone and progesterone levels. On the other hand, progesterone and estrogen showed strong positive correlation (Supplemental Table 2). However, in the placenta, *STAR* showed strong positive correlation with *AKT* and *HSD*, and moderate positive correlation with *PTEN*. *AKT* showed strong positive correlation with *PTEN* and *HSD*, but showed strong negative correlation with *CYP17*. *PTEN* and *HSD* showed moderate positive correlation with *CYP17* (Supplemental Table 3). Similar to the ovarian

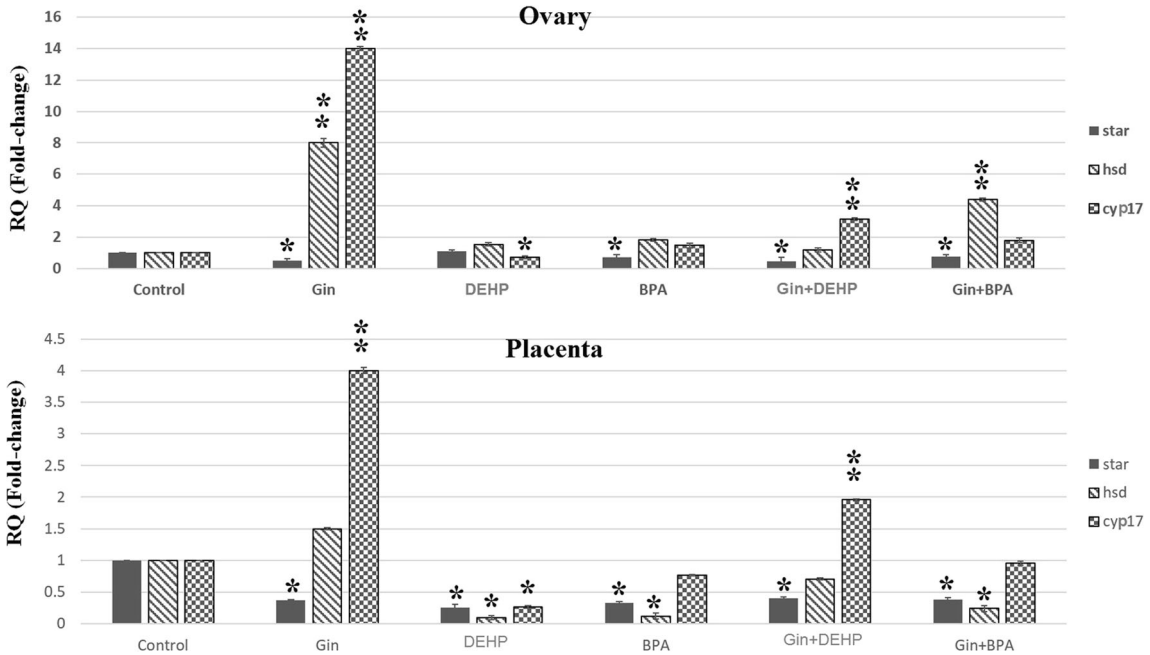

Fig. 6 Effect of ginseng, phthalates, and bisphenol A on relative quantification (RQ or fold changes) of mRNA of STAR, HSD17B3, and CYP17 in the ovary and placenta of day 20 pregnant rats. Columns carrying asterisks (* and **) are considered significantly lower or higher, respectively ($P < 0.05$), than the arbitrary control group (onefold). *Star*, STAR; *hsd*, HSD17B3; *cyp17*, CYP17; Gin, ginseng; DEHP, bis(2-ethylhexyl) phthalate; BPA, bisphenol A

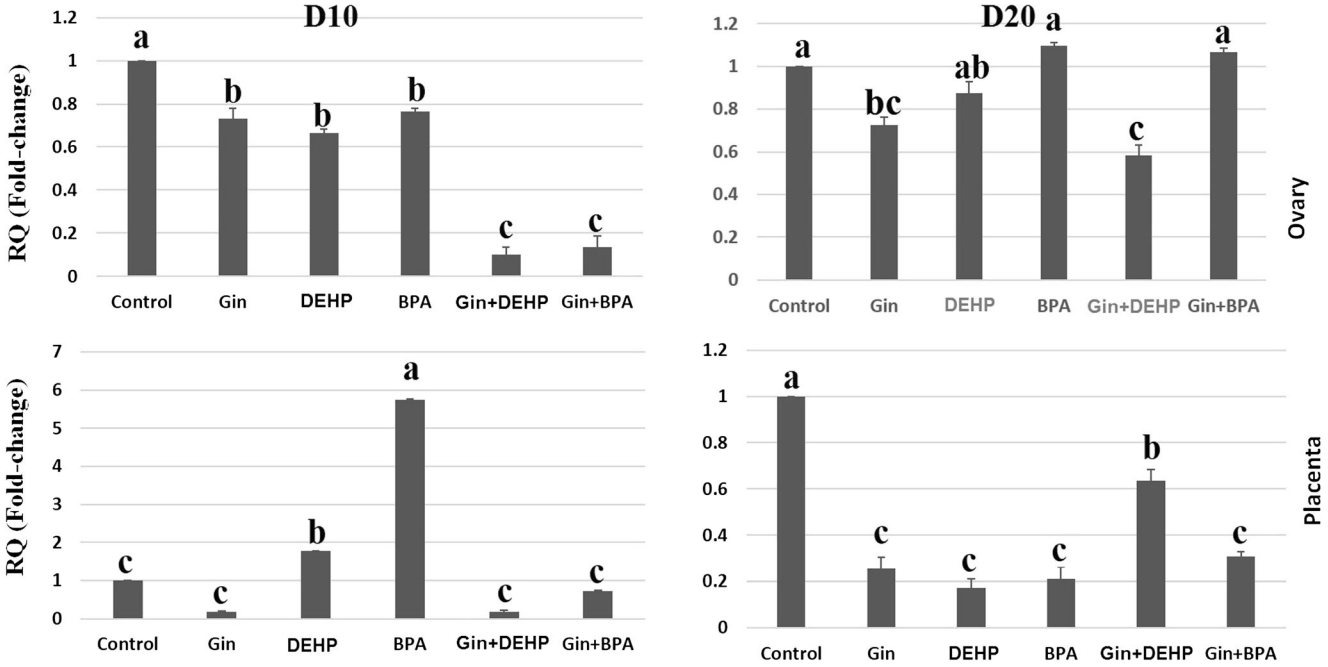

Fig. 7 Effect of ginseng, phthalates, and bisphenol A on relative quantification (RQ or fold changes) of mRNA of *AKT1* in the ovary and placenta of day 10 and day 20 pregnant rats. Columns carrying different superscript letters (a, b, and c) are considered significantly different ($P < 0.05$) from the arbitrary control group (onefold). Gin, ginseng; DEHP, bis(2-ethylhexyl) phthalate; BPA, bisphenol A

expression, *STAR*, *AKT*, and *PTEN* showed negative correlation with all sex steroids. In addition, *HSD* showed strong negative correlation with testosterone levels. However, *CYB17* showed positive correlation with testosterone levels. Moreover, progesterone and estrogen showed strong positive correlation.

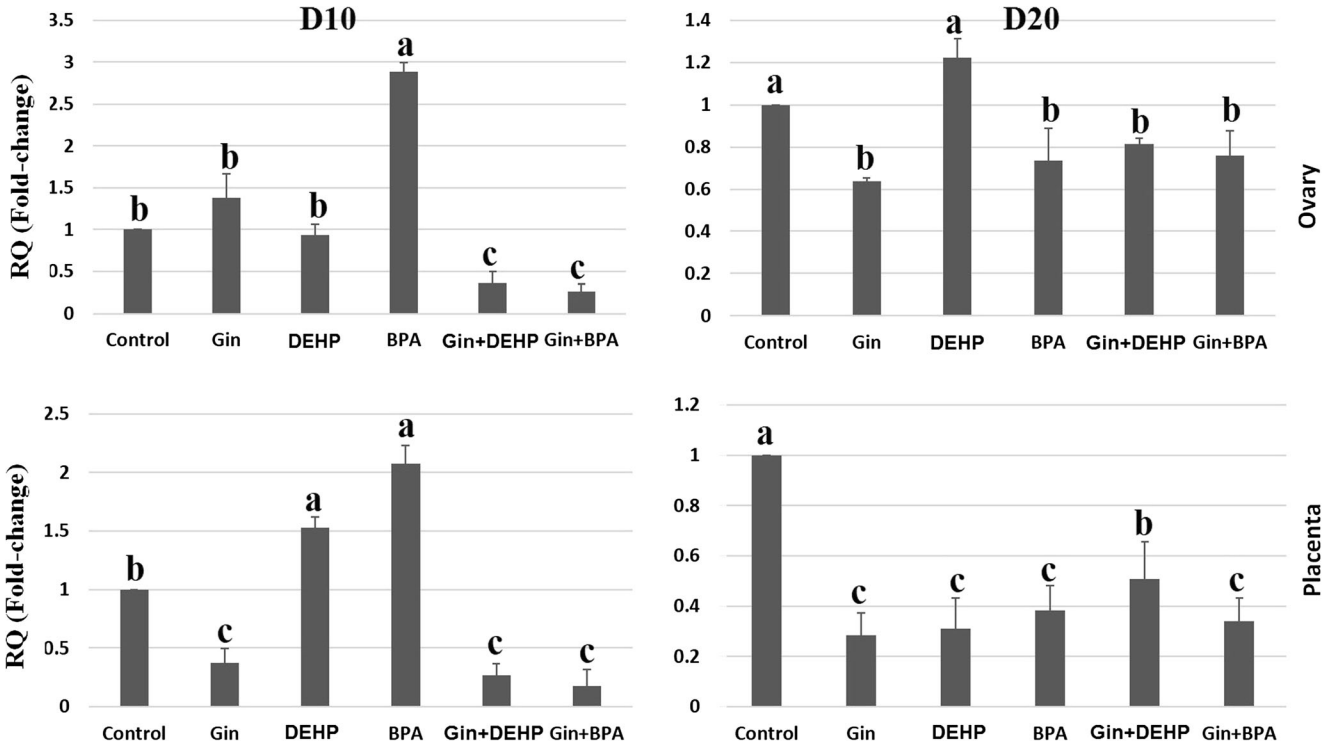

Fig. 8 Effect of ginseng, phthalates, and bisphenol A on relative quantification (RQ or fold changes) of mRNA of *PTEN* in the ovary and placenta of day 10 and day 20 pregnant rats. Columns carrying different superscript letters (a, b, and c) are considered significantly different ($P < 0.05$) from the arbitrary control group (onefold). Gin, ginseng; DEHP, bis(2-ethylhexyl) phthalate; BPA, bisphenol A

On pregnancy day 20, ovarian *PTEN* showed strong positive correlation with *AKT* and moderate positive correlation with *STAR*, while *AKT* showed moderate negative correlation with *HSD* and *CYP17*. Moreover, *PTEN* was negatively correlated with *CYP17*. *HSD* showed strong positive correlation with *CYP17* (Supplemental Table 4). Testosterone showed positive correlation with *AKT* but with positive correlation with *CYB17*. Placental *STAR* showed strong positive correlation with *AKT* and *PTEN*, which showed strong positive correlation with each other. Further, *HSD* showed strong positive correlation with *CYP17* (Supplemental Table 5). Progesterone showed positive correlation with *HSD* and *CYB17*. While, estrogen showed moderate positive correlation with *AKT* expression. Sex steroids showed interactive concomitant strong positive correlation in this late stage of gestation.

Discussion

Chemicals such as DEHP and BPA are components of a wide variety of plastic consumer products and are suspected endocrine disrupters although with low toxicity levels (Larsson et al. 2014; Liu et al. 2014). Consequently, the risk of non-occupational exposure to these chemicals attracted much attention, because of its potential threat to public health with special attention to pregnant females and conceptuses. Our results show that DEHP and BPA altered the levels of sex steroids (testosterone, progesterone, and estrogen), and the mRNA of steroidogenesis enzymes during the stages of pregnancy in rats. Interestingly, ginseng extract alleviated some of these endocrine alterations when administered together with these toxicants. Notably, there are differential effects of both DEHP and BPA on the major organs responsible for steroidogenesis, which are the ovaries and placentae. These in utero hazardous effects of phthalates and BPA, including the testicular and ovarian steroidogenesis of the conceptuses, can cause problems with the reproductive life of future generations (Gray 2006; Shultz et al. 2001; Susiarjo et al. 2007; Zhang et al. 2011).

Unlike the human placenta, which plays an important role in producing progesterone and estrogen, the rat placenta does not produce estrogen, as rats do not express aromatase (CYP191a), and only secretes progesterone and testosterone (Gibori and Sridaran 1981; Matt and MacDonald 1984). Indeed, the rat placenta is the main source of testosterone, and this testosterone serves as the substrate for estradiol synthesis in the corpus luteum (Durkee et al. 1992; Jackson and Albrecht 1985). Therefore, the rat placenta indirectly sustains the ovarian biosynthesis of E2, although it does not produce it. Therefore, the present study was designed to explore the potential effects of BSA or DEHP to disturb sex steroids synthesis pathway by rat placenta and ovary, in addition to the circulating progesterone, testosterone, and estradiol levels.

STAR is a transporter protein that mediates cholesterol transfer within the mitochondria to the cholesterol side chain cleavage enzyme system, which is the rate-limiting step in the production of steroid hormones (Kallen et al. 1998). CYP17A1 is required for the production of androgenic and estrogenic sex steroids by converting 17α-hydroxypregnenolone to dehydroepiandrosterone (DHEA) (DeVore and Scott 2012). 17β-Hydroxysteroid dehydrogenase 3 (HSD17B3) is an enzyme involved in transforming androstenedione into testosterone (Strauss 2014). Hence, the effect on these cascade of enzymes and other steroidogenic enzymes chain will affect the final output of the steroid-producing cells.

Testosterone and progesterone levels showed slight reduction associated with DEHP and BPA treatments on pregnancy days 4 and 10, and this reduction became significant by the end of pregnancy when compared to co-treatment with ginseng and control groups. Feng et al. (2016) showed the toxic effect of triclosan, a chemical compound with properties similar to BPA, on reducing testosterone and progesterone levels, which might be attributed mainly to targeting the alteration in steroid hormone metabolism in the placenta of pregnant rats. Salvati et al. (1996) showed that ginseng treatment significantly increased testosterone levels throughout pregnancy and that *Panax ginseng* extract increased the plasma total and free testosterone.

At a molecular level, DEHP showed concomitant decrease in the ovarian transcript levels for key steroidogenic enzyme including *STAR* and *HSD* in pregnant mice (Guo et al. 2015). Also, DEHP inhibited steroidogenesis in rat ovarian granulosa cells (Svechnikova et al. 2007). Similarly, another type of phthalate, di(*n*-butyl) phthalate (DBP) showed downregulation of the mRNA transcripts of *STAR* and other steroidogenesis enzymes in rat testes (Barlow 2003) and this indicated similar effects of different phthalates on steroidogenesis on different genders.

Interestingly, previous studies also showed dose-dependent and paradoxical effects of endocrine disrupting chemicals, including DEHP and BPA, on steroidogenesis enzyme mRNA expressions (Guo et al. 2015; Peretz et al. 2014).

Additionally, mono(2-ethylhexyl) phthalate (MEHP) downregulated the steroidogenesis enzymes (including STAR, CYP17, and HSD17) in cultured mouse whole ovaries and antral follicles (Hannon et al. 2015). Hannon and Flaws (2015) extensively reviewed the toxic effects of phthalates on the ovary and steroidogenesis. Similarly, BPA showed hazardous impacts on female reproduction and steroidogenesis, as reviewed in (Peretz et al. 2014).

Notably, the effect of endocrine disrupting agents, particularly BPA, can be modulated by diet components (Muhlhauser et al. 2009); which raises the question whether supplementing natural herbs (such as ginseng) can ameliorate the side effects of these chemicals. Interestingly, our results showed that ginseng significantly alleviated the effects of DEHP and BPA on

the testosterone and progesterone levels throughout pregnancy. Yang et al. (2014) showed that ginseng efficiently protected women from the harmful effects of BPA and that ginseng is a safe chemopreventive for BPA-induced gynecological complaints. Similarly, Wang et al. (2012) suggested that ginsenosides have protective effects against BPA-induced Sertoli cell damage. El-Drieny et al. (2009) indicated that ginseng has protective effects against the hazards of DEHP on the adrenal cortex in rats.

Interestingly, on pregnancy day 10, the pattern of ovarian and placental *AKT1* transcript expression directly correlated with *STAR* expression (Supplemental Tables 2 and 3). Similarly, on pregnancy day 20, the placental *AKT1* directly correlated with *STAR* expression, while the ovarian *AKT1* showed positive relation with *STAR* (Supplemental Table 5) in the presence of DEHP administered either individually or in combination with ginseng (Figs. 5, 6, and 7). According to the correlation analysis, we might explain the decrease of progesterone level in BPA-treated rats on day 10 of pregnancy due to the negative correlation (Supplemental Tables 2 and 3) with the increased levels of ovarian and placental expression of *STAR* (Fig. 5), placental *AKT* (Fig. 7), and both ovarian and placental *PTEN* (Fig. 8). On the other hand, we might suggest that ginseng treatment resumed the level of decreased progesterone in BPA-treated group through increasing *CYB17* expression (Fig. 5) and decreasing *AKT* (Fig. 7) and *PTEN* (Fig. 8) expressions, which confirmed the negative correlation between progesterone and *AKT/PTEN* (Supplemental Tables 2 and 3).

On day 20, estradiol decrease in BPA-treated rats might be caused by the decrease in both ovarian and placental *AKT* expression (Fig. 8) and confirmed the positive correlation between the two variables (Supplemental Table 5). While, progesterone decrease on the day 20 of pregnancy in both BPA- and DEHP-treated groups is mainly caused due to the positive correlation with placental *HSD* and *CYB17* (Supplemental Tables 4 and 5; Fig. 6). Ginseng reversed this decrease through elevation of ovarian and placental expression of *HSD* and *CYB17* expression in DEHP-treated group, while through elevation of HSD only in BPA-treated group (Fig. 6).

On the other hand, BPA-treated rats showed significant reduction in testosterone levels when compared with DEHP, which showed significant decrease than other treated groups. This effect might be reduced due to the positive correlation with placental expression of *AKT* (Supplemental Table 4; Fig. 7), and the cumulative decrease in ovarian and placenta expression of *STAR*, *HSD, CYB17* (Fig. 6), and *PTEN* (Fig. 8).Similarly, on day 20, ginseng treatment showed amelioration of testosterone decrease caused by DEHP through increase of ovarian and placental *CYB17*, while ameliorated the level in BPA-treated group trough elevation of ovarian *HSD* expression (Fig. 6), which confirms the positive correlation between testosterone, *HSD* and *CYB17* (Supplemental Tables 4 and 5).

There is accumulating evidence that the PTEN/AKT signaling pathway is a critical regulator of ovarian function including granulosa cell proliferation and differentiation (Froment et al. 2005) and reviewed in Makker et al. (2014). Both PTEN and AKT were chosen because they are integral regulators of primordial follicle recruitment. Specifically, PTEN inhibits PI3K signaling and ultimately maintains primordial follicle dormancy. Additionally, AKT is a secondary messenger of PI3K signaling and ultimately promotes primordial follicle recruitment (Hannon et al. 2015; Reddy et al. 2010). Studies showed that AKT mediates stimulation of steroidogenesis, specifically STAR enzyme, in mouse Leydig cells (Lai et al. 2014), swine ovaries (Nteeba et al. 2015), and bovine granulosa cells (Mani et al. 2009). Additionally, Fukuda et al. (2009) showed that LH stimulates *CYP17* mRNA expression and androgen production in theca cells via activation of the PI3K/AKT pathway. This coincides with our results as testosterone and *AKT* showed positive correlation on day 20 of pregnancy (Supplemental Table 4).

Surprisingly, recent studies showed that ginseng can modulate PTEN/AKT pathway, as it inactivates the PI3K/AKT signaling in cultured cells (Yang et al. 2017) and also suppresses AKT pathway (Han et al. 2017). Moreover, a study showed that the compound K from *Panax ginseng* inhibited AKT signaling in tumor necrosis factor-α stimulated human dermal fibroblast (Lee et al. 2014). This might explain our results of AKT expression after administering ginseng (Fig. 7), probably as an indirect effect of ginseng on steroid synthesis. Furthermore, ginsenoside Rg1 was found to significantly reduce PTEN protein in tissues (Jin and Ma 2017). The ameliorative effects of ginseng on BPA toxicity have been studied in women (Yang et al. 2014); however, to our knowledge, this is the first study to report the potential chemoprotective effect of ginseng against DEHP. A recent study revealed that ginseng caused an increase in the levels of some enzymes responsible for steroidogenesis such as CYP17A (Cheng et al. 2016), which might explain the direct effect of ginseng on the enzymes responsible for testosterone synthesis and confirms the positive correlation that were found between *CYP17A* and testosterone in the current study.

Conclusion

We report here that ginseng extract can alleviate the reprotoxicity effects of DEHP and BPA in pregnant rats as explained by reversal of the abnormal levels of steroid hormones to normal levels, and by modulating mRNA transcripts of steroidogenesis enzymes such as STAR, HSD17B3, and CYP17B, either directly or through the action of the AKT/PTEN pathway.

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Compliance with ethical standards

Consent for publication Not applicable.

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