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Echinacoside and *Cistanchetubulosa* (Schenk) R. Wight ameliorate bisphenol A-induced testicular and sperm damage in rats through gonad axis regulated steroidogenic enzymes

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Tel. & Fax: + 86 29 8709 1239; E-mail: zhang.xy@nwsuaf.edu.comAbstract

Ethnopharmacological relevance

Male infertility has been increasing over the last decades and being a pressing health problem nowadays. *Cistanche tubulosa* (CT) is a traditional Chinese medicine used to boost male sexual function. Echinacoside (ECH) is one of the major compounds exist in CT and might be a potential agent to protect testis and sperm injury.

Aim of the study

To investigate the mechanisms behind the possible protective effects of CT and ECH against testicular and sperm toxicity.

Materials and methods

CT was identified by 5.8s gene sequencing. The major compositions (echinacoside and acteoside) of CT were quantified by HPLC method. The adult male Sprague-Dawley rats were exposed to BPA, CT or ECH for 42 consecutive days. The sperm parameters were observed by dark-field microscope; serum hormone levels (FSH, LH and testosterone) were tested by radio immunosorbent; LDH-x activity were evaluated using commercial kits; the expressions of the key steroidogenic enzymes were evaluated by qRT-PCR, heat map, immunofluorescence and western blot.

Results

The CT and ECH treatments against BPA-induced testicular and sperm toxicity showed that CT and ECH have reversed BPA-induced abnormality in sperm characteristics, testicular structure and normalized serum testosterone. This was concomitant with the increased expression of LDH-x as well as the key steroidogenic enzymes including StAR, CYP11A1, 3β-HSD, 17β-HSD and CYP17A1, suggesting that CT and ECH enhanced testosterone biosynthesis.

Conclusions

CT and ECH attenuated poor sperm quality and testicular toxicity in rats through up-regulation steroidogenesis enzymes and ECH is the active compound of CT as a potential natural reproductive agent.

Key words: *Cistanche tubulosa*; Bisphenol A (BPA); Echinacoside; Sperm; Testis

Abbreviations

ACT, Acteoside; BPA, bisphenol A; CT, *Cistanche tubulosa* (Schenk) R. wight; CYP 11A1, cytochrome P450 side chain cleavage; ECH, echinacoside; FSH, follicle stimulating hormone ; HPG, hypothalamus-pituitary-gonad; ITS, internal transcribed spacer; LH, luteinizing hormone; PhGs, polyphenols and phenylethanoid glycosides; StAR, steroidogenic acute regulatory protein; T, testosterone; VE, Vitamin E

1. Introduction

Male infertility is one of the major health issues as it is estimated that 4.2% of male are infertile in the world (Hotaling et al., 2015). Exogenous endocrine disruptors and their impact on male infertility have gained considerable attentions. The estrogen-mimic Bisphenol A (BPA) is used as a model agent for endocrine disruption (Khan et al., 2015). BPA has been mass-produced and used in the manufacturing of epoxy resins, dental sealants, polycarbonate plastics, receipt paper and other commonly used products (Qian et al., 2014). Continuous exposure to BPA could lead to impairing functioning in sexual development (Rahman et al., 2015), reproduction (Liu et al., 2013) and behavior (Roen et al., 2015). BPA affects the testosterone production by inhibiting the steroidogenic acute regulatory protein (StAR), and

cytochrome P450 side chain cleavage (CYP 11A1) (Peretz et al., 2013 and Xi et al., 2011). Artificially synthesized hormones are the major medicines to cure/protect sperm and testis injury; however, such exogenous hormones are not recommended with high dose or long time (Saito et al., 2000; Meachem et al., 1997 and 1998), so finding some natural products to ameliorate or cure sperm and testis toxicity are necessary.

Cistanche tubulosa (Schenk) R. wight (CT) is a phanerogamic and perennial plant of the genus *Cistanche Hoffmy*. et Link (*Cistanche*), family *Orobanchaceae*. CT is mainly distributed in semi-arid areas of western China, Mongolia and India. *Cistanche* has been used as a tonic agent to cure reproductive dysfunction and to boost male sexual function by protecting sperm and increasing the production of testosterone(Wang et al., 2015). According to the Compendium of Materia Medica (*Ben Cao Gang Mu, by Li Shizhen, the 16th century*), *Cistanche*has been documented with semen protection effect. The modern pharmacological investigations have shown that, the major compositions of *Cistanche* with a tonifying effect on brain can stimulate the hypothalamus-pituitary-gonad (HPG) axis (Wang et al., 2015). The plant is therefore called “Desert ginseng” in China owing to its excellent medical functions and nourishing effect (He et al., 1996).

As one of the main phenylethanoid glycoside, echinacoside (ECH) is considered to be biological activities ingredients of CT (Li et al., 2016). It has been reported that ECH possesses a wide variety of health benefits including anti-inflammatory (Sharma et al., 2010), antioxidant (Hu et al., 2000), neuroprotection (Zhao et al., 2016)and

rescuing osteoporosis (Yang et al., 2013). The present study aimed to evaluate the protective effect of ECH and CT in a BPA induced testicular and sperm injury rat model and to investigate the possible underlying mechanisms.

2. Materials and methods

2.1 Plant materials

The fresh succulent stem of CT was collected during flowering period from the desert area in South of Xinjiang Autonomous Region by Prof. Jian-Jun Hu (Tarim University, Xinjiang, China) and stored at 10-14 °C until further use. Then, the CT were washed thoroughly, cut into ~10 mm thick and dried. Finally, the dried slices were ground in a knife grinder and the powder was stored at 4°C.

2.2 Identification of Cistanche tubulosa and Cistanche tubulosa preparation

The dried succulent stem of CT sample was assessed from its morphological characteristic by a local botanist (Prof. Jianjun Hu, Tarim University). The fresh sample was further identified by DNA sequencing. Briefly, total genomic DNA was isolated from sample CT. The internal transcribed spacer (ITS) region containing the 5.8s rDNA fragment was amplified using primer sequences of ITS1/ITS2 5’CGTAACAAGGTTTCCGTAGAA3’and 5’TTATTGATATGCTTAAACTCAGCGGG 3’ (Invitrogen, USA) using a method described by Schneeweiss (Schneeweiss et al., 2004) and (Xiong et al., 2013). The result was compared with the CT 5.8s rDNA sequence on GenBank.

A total of 50g of dried CT was extracted twice with 250mL of ethanol (70%) in a refluxed condenser for 2h each. The obtained extracts were combined and evaporated

invacuum drying chamber (15.6g) and stored at 4 °C for further studies.

2.3 HPLC analysis phenylethanoid glycoside

In accordance with the Chinese Pharmacopoeia (2010), the quality of CT was tested by using acteoside (ACT) and echinacoside (ECH) as quality standards. Briefly, 0.1mg CT powder was added to 40μL methanol. The calibration standards were prepared by adding known volumes of ACT and ECT (5, 10, 20, 40, 80 and 160 μg/mL). Then, 20 μL of the supernatant was injected into the HPLC System (D2000 Elite series, Hitachi, Japan) which included a Symmetry C18 column (4.6 mm × 250 mm, 5 μm; Waters) and a Guard column (3.9 mm × 20mm, 5μm; Waters). The mobile phase was a gradient prepared from 0.1% phosphoric acid containing 0.04% trimethylamine (component A) and methanol (component B). The linear gradient was as follows: 70%-90% A over 0-2 min, 60%-70% A over 2-6 min, 55%-60% B over 6-8 min and then returned to 90% A at 8 min immediately. The flow rate was 0.8 mL min^{-1}. The detector was operated at 332 nm. Peak area was evaluated as the analytical measurement.

2.4 Animals and experimental protocol

Adult male Sprague-Dawley rats weighing 190g-200g were purchased from the animal center of the Fourth Military Medical University, Xi'an, China. Animals were allowed to freely access to water and food under controlled room temperature (22 °C ± 2 °C) and humidity (55% ± 5%) conditions with an automatic 12h light/dark cycle). All animals procedures were performed in accordance with the university guidelines for care and use of laboratory animals.

After one week acclimation, the rats were randomly distributed into seven groups (N=7) of six animals each (n=6). All the treatment groups were oral gavage for 6 weeks as per following experimental design. Normal group (corn oil 10ml/kg bw/d), model group: BPA group (treated with BPA 200mg/kg/ bw/d, corn oil), positive control group: BPA-VE group (treated with BPA, Vitamine E (VE) 300mg/kg bw/d) and experimental group: BPA-ECH group (treated with BPA, ECH 6mg/kg), BPA-CT group (treated with BPA, CT 200mg/kg), ECH group (6mg/kg bw/d) and CT group (200mg/kg bw/d)

The doses of BPA and CT were selected according to the previous reports (Wang et al., 2015) and (Nakamura et al., 2010), and the doses of ECH were selected according to the content of the extract of CT. After 6 weeks treatment, the rats were anesthetized with diethyl ether to collect blood samples from carotid artery for hormone levels analyses. The rats were then dissected to separate the testis and cauda epididymides. Then, the collected samples were measured, the cauda epididymides were put in normal saline with 5% BSA, the left testis were fixed in Bouin's fixative and the right testis were frozen in liquid nitrogen and stored at -80 °C for further investigations.

2.5 Sperm test

Sperm suspension preparation: Cauda epididymides were chopped into 4mL of normal saline with 5% BSA and incubated for 5 min at 37 °C to allow their contents to spread into medium.

Daily sperm production and sperm number: As per the method described by Yokoi

(2003), the diluted sperm suspension (10µL) was transferred to each counting chamber of a hemocytometer and was allowed to stand for 5 min; then counted under a light microscope (Nikon, Insturments Inc., Japan) at ×200 magnification (Yokoi et al., 2003). Daily sperm production (DSP) was monitored using the formula (Robb et al., 1978): DSP= *X*/6.1, where *X* is the number of spermatids at stage 19. To calculate sperm transit time through the epididymis, the numbers of sperm in each of these regions was divided by DSP.

Determination of sperm viability, motility and morphology: To evaluate sperm viability, 20µL of sperm suspension was carefully mixed with an equal volume of eosin-nigrosin stain. Morphological analysis was classified into three general categories: normal morphology, head abnormalities (isolated form or without characteristic curvature, i.e., no tail attached) and tail abnormalities (rolled into a spiral and isolated, i.e., no head attached) (Fernandes et al., 2007) sperm motility was recorded under an inverted microscope equipped with a dark-field condenser, and assessed by visual estimation and classified as: mobile or immobile (Amin, 2008). Testicular LDH-x enzyme activity was assessed in accordance to the method prescribed in LDH-x activity kit (Nanjing Jiancheng, Nanjing, China). Briefly, the samples were incubated with 1mL of a specific substrate for 3 min at 37°C. The change in absorbance was measured at 340 nm by a spectrophotometer (Analytik Jena. Ltd. Co., Germany).

2.6 Histopathological examination

After fixation, the tissues were embedded in 60°C paraffin to have 5µm serial

sections (Amin et al., 2008). The staining was done by H&E and the slides were examined under light microscope with photo micrographic attachment.

2.7 Determination of serum hormone level

Serum level of testosterone, LH and FSH were measured by agency (Beijing Sino-UK institute of biological technology, Beijing, China).

2.8 Determination of testicular androgenic gene expression by real time PCR

Total RNA was isolated from frozen testicular tissues using RNA Simple Total RNA kit (Tiangen, Beijing, China). Real-time polymerase chain reaction (quantitative RT-PCR) was carried out for the amplification of cDNA using 2×SYBR Green I PCR Master Mix (Vazyme, Nanjing, China) (Jiang et al., 2016). The following PCR primer pairs were used for the study: CYP11A1, F: 5'-GCAGCGACTCTCTTCTCCTGCG-3', R: 5'-GCCATCACCTCTTGGTTTAGGACAATT-3' (NM_017286); CYP17A1, F: 5'-GCCACGGGCGACAGAA-3', R: 5'-GCCTTTGTTGGGAAAAATCG-3' (NM_012753); HSD3β1/2, F: 5'-GACAGGAGCAGGAGGGTTTGTGG-3', R: 5'-CTCCTTCTAACATTGTCACCTTGGCCT-3' (NM_001007719, NM_001042619); HSD17ß3, F:5'-AGTGTGTGAGGTTCTCCCGGTACCT-3', R:5'-TACAACATTGAGTCCATGTCTGGCCAG-3' (NM_054007); StAR, F: 5'-AGCCAGCAGGAGAATGGAGAT-3', R: 5'-CACCTCCAGTCGGAACACCTT-3' (NM_031558).

2.9 Immunofluorescence staining

The tissue sections were incubated with appropriated antibody (anti-CYP11A1 1:400; anti-CYP17A1 1:400; Bioss, Beijing, China) at 4°C overnight, then washed

thrice using PBST and incubated with secondary antibody (Alexa 568-conjugated goat-anti-rabbit, 1:500; Bioss, Beijing, China) for 1h at room temperature. After washing with PBST, the tissue sections were counterstained with nuclear stain kit (Hoechst 33342) for 10min and covered with anti-fade mounting medium (Beyotime, Beijing, China). Fluorescence images were captured using a digital camera (Leica, EVOS f1, Germany).

2.10 Western blot

Protein extracts were subjected to SDS-polyacrylamide gel electrophoresis under reducing conditions on 12% gels. Separated proteins were then transferred to PVDF membranes. Anti-CYP11A1 and anti-CYP17A1 antibodies (1:1000; Bioss, Beijing, China) in TBST at 4°C for 2h, followed by ahorseradish peroxidase (HRP) conjugated anti-rabbit IgG antibody (1:3000; Sangene, Tianjin, China). The optical density (OD) of each band was determined by Quantity One (Bio-Rad, California, USA) and the relative abundances of CYP11A1 and CYP17A1 protein levels were expressed as ratios of OD of these proteins to that of GAPDH.

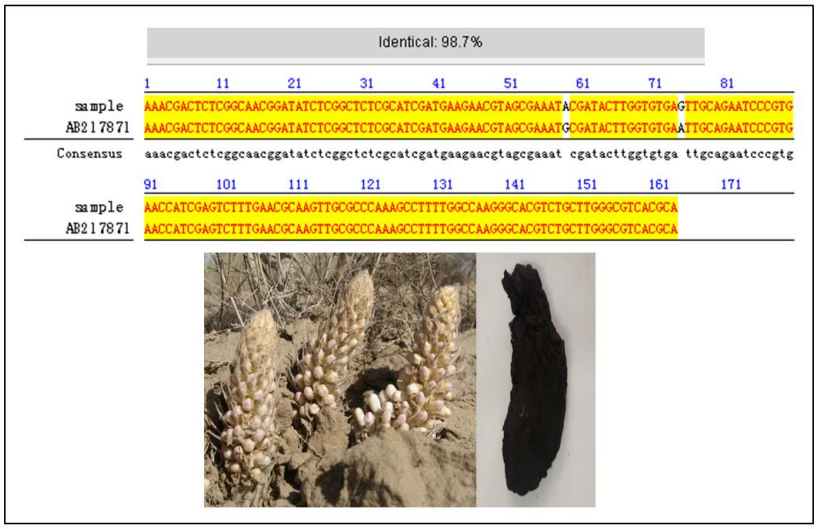
2.11 Statistical analysis

The data were analyzed using SAS software (version 9.1, SAS Institute Inc., Cary, NC, USA). A one way ANOVA was employed for comparison among the groups. Tukey's comparison tests of significant differences among groups were determined. The results were expressed as mean ± standard deviation (SD) using Graph Pad Prism software v.5 (San Diego, CA, USA).

3. Results

3.1 Identification of Cistanche sample

The sequence alignment indicated that amplified sequence of *Cistanche* sample was the same with *Cistanche tubulosa* (Schenk) R. wight gene for 5.8S rDNA sequence (Gen-Bank accession number AB217871) (Supplement. Fig.1). On the basis of 5.8s rDNA gene sequence and morphological characteristics, the investigated sample was identified as *Cistanche tubulosa*.



S. Fig. 1 The 5.8s rDNA gene sequence and morphological characteristics of CT

3.2 Quantitative determination of the echinacoside and acteoside content of CT

The contents of ACT and ECH in CT were quantitated by using HPLC (Fig. 1). The retention times for ECH and ACT were 5.3 min and 8.3 min, respectively at a maximum UV absorbance of 330 nm. The standard curves of the ECH and ACT concentrations were Y=16421X-50378 (R^2 =0.997) and Y=22391X-18749 (R^2 =0.999), respectively, where Y was the peak area and X was the concentration of ACT or ECH. The ECH and ACT contents of CT extract were 42mg/g and 23mg/g, respectively.

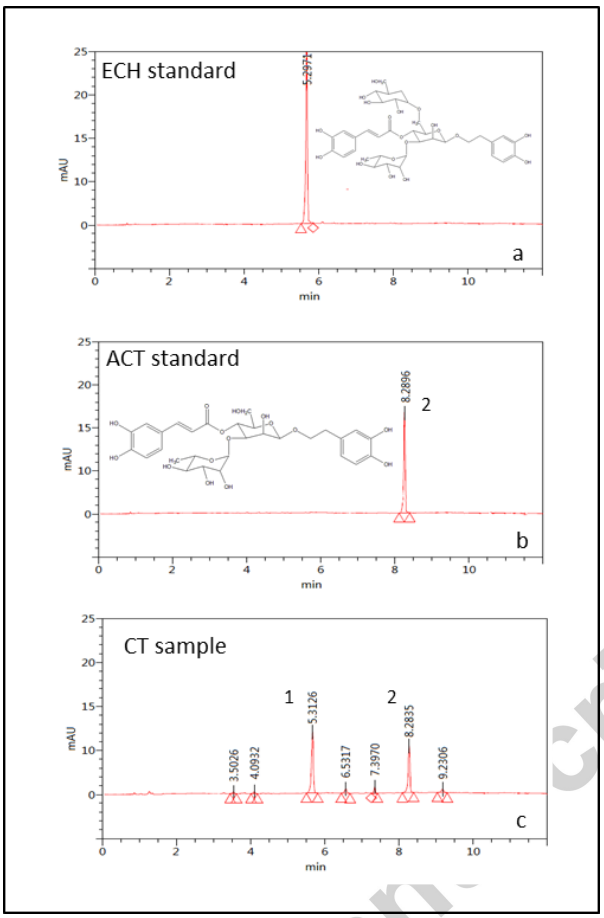


Fig. 1 The HPLC profile of CT

Notes: peak 1, ECH; peak 2, ACT.

3.3 Sperm quality parameters

BPA treatment decreased the sperm number and daily sperm production (DSP) in the testis by 26.15% ($p<0.05$) and 26.16% ($p<0.05$) respectively (Table 1). The exposure to BPA has led to increase of abnormal spermatozoa (Fig. 2). ECH and CT both were significantly reduced the percentage of abnormal spermatozoa. CT and ECH treatments considerably altered the BPA induced significant reduction of mobile spermatozoa (Fig. 2) ($p<0.05$).

Table 1 Sperm number, daily sperm production and sperm transit time through cauda epididymal.

Sperm parameters	Control	BPA	BPA + CT	BPA + ECH	BPA + VE	CT	ECH
Sperm number in testis ($\times 10^6$)	106.33 ±4.18^{b}	78.52 ± 9.41^{c}	86.42 ± 7.75 ^{bc}	94.85 ± 6.25^{b}	88.42 ± 6.95 ^{bc}	100.08 ± 9.05 ^{b}	126.05 ± 10.06^{a}
DSP ($\times 10^6$/ testis/ day)	27.43 ± 0.69 ^{b}	22.87 ± 1.54 ^{c}	24.02 ± 1.22 ^{b}	25.55 ± 1.03 ^{b}	23.06 ± 1.32 ^{b}	28.85 ± 2.51 ^{ab}	30.66 ± 1.65 ^{a}
Sperm number in Cauda epididymal ($\times 10^6$)	74.84 ± 10.17 ^{b}	51.87 ± 8.46 ^{c}	55.86 ± 5.06 ^{bc}	64.31 ± 4.42 ^{b}	54.82 ± 4.56 ^{bc}	75.22 ± 5.21 ^{ab}	81.50 ± 4.08 ^{a}
Transit time in Cauda epididymal (days)	2.71 ± 0.59 ^{a}	2.23 ± 0.66 ^{c}	2.31 ± 0.59 ^{bc}	2.51 ± 0.28 ^{b}	2.36 ± 0.59 ^{bc}	2.60 ± 0.48 ^{ab}	2.65 ± 0.20 ^{a}

Notes: $^{a, b, c}$ Different letters represent groups that differ statistically ($p < 0.05$), n=6.

LDH-*x* is a major energy source for sperm motility. BPA+CT and BPA+ECH treated rats enhanced the LDH-*x* activity by 32.7% and 34.6% (p<0.05), respectively, when compared to BPA treated group (Fig. 2).

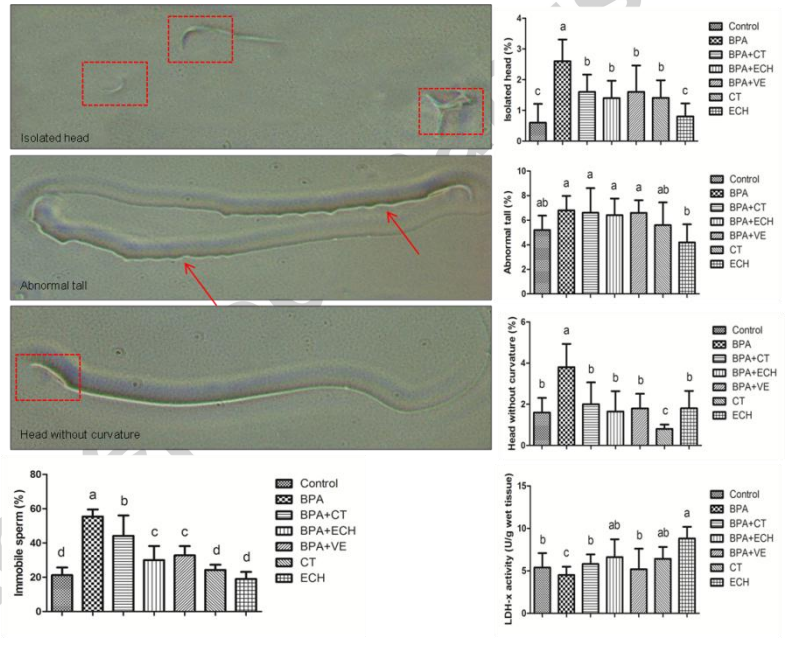


Fig. 2 Sperm morphology and motility.

Notes: A values expressed as percentage. n=5. $^{a, b, c, d}$ Different letters indicated statistical difference among groups ($p < 0.05$).

3.4 Investigations on Testicular histopathology

In photomicrographs (Fig. 3), normal histological structures were observed in

control, CT and ECH. Testicular sections of BPA (200mg/kg bw/d) treated rats showed remarkable degenerative changes, disorganization and necrosis of the histological structure of seminiferous tubules. In addition, obvious degeneration and irregular arrangement of spermatids, clotted dead and intertwined spermatids were observed. Administrations of CT and ECH significantly prevented BPA induced testicular injury, where normal histological pattern, normal spermatogenic series, and spermatids formation were observed.

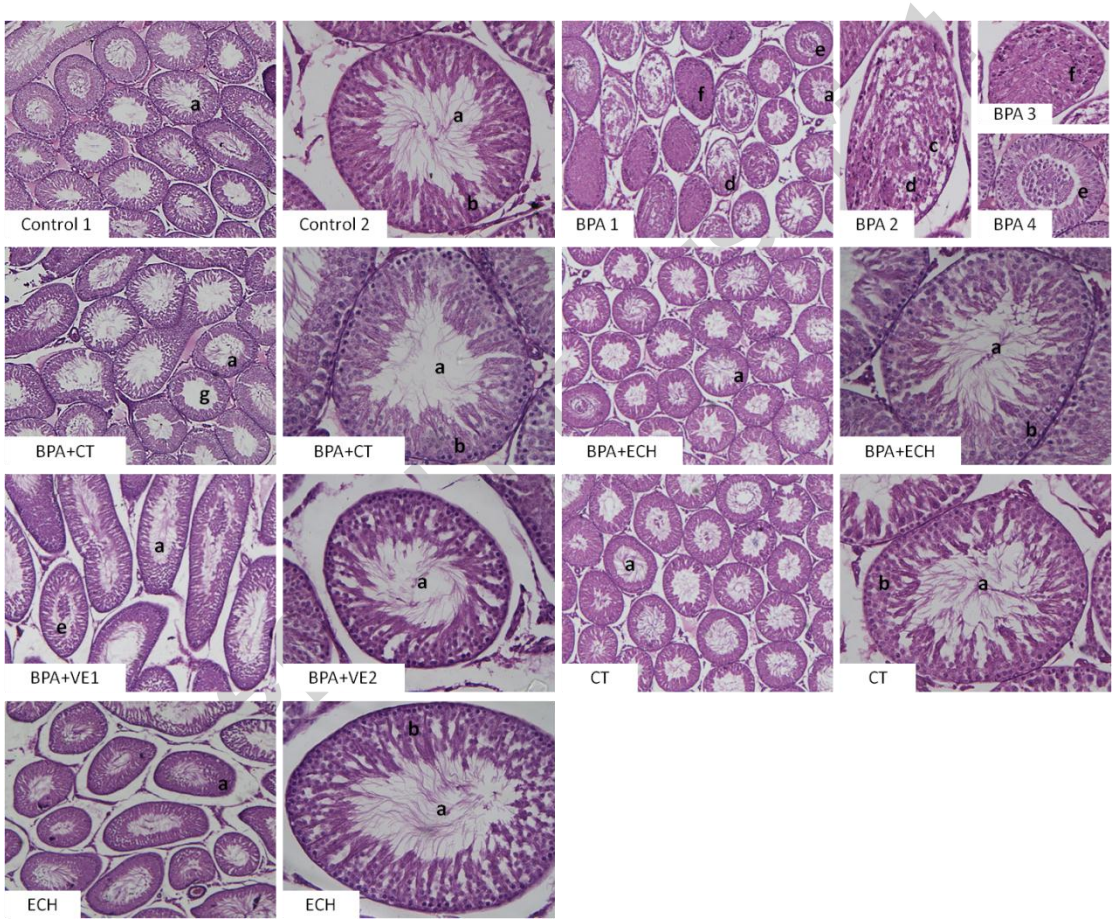


Fig. 3 Histopathology observation in the rat testis.

Notes: Magnification ×100 (Control1, BPA1, BPA+CT1, BPA+ECH1, CT1 and ECH 1), ×400 (Control2, BPA2, BPA3, BPA4, BPA+CT2, BPA+ECH2, CT2 and ECH2). Photomicrographs Control showing normal histological structures of mature active

seminiferous tubules with complete spermatogenic series (a), and intact and mature Sertoli cells (b); loss of primary, secondary, and tertiary spermatogonial cells (c); deposition of fat vacuoles (d), clotted dead spermatids (e), necrosis (f); few of sperm (g).

3.5 hormone levels

The serum levels of FSH, LH and testosterone were reduced by 23.61%, 27.68% and 77.21%, respectively, while interventions of CT and ECH significantly increased those levels in BPA group (Fig. 4).

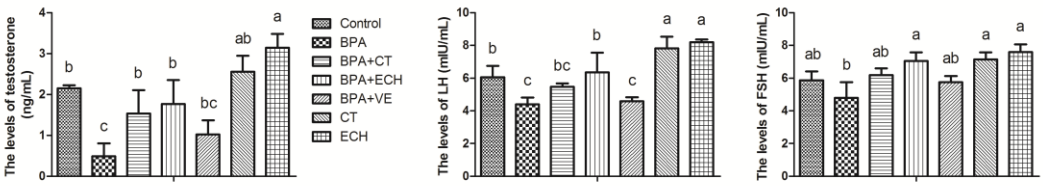


Fig. 4 Hormonal concentration changes in the rat testis.

Notes: [a, b, c] Different letters indicate statistically different groups (p<0.05), n=6.

3.6 Testicular steroidogenic enzymes expression

BPA significantly inhibited the expressions of key steroidogenic enzymes in the testis. The relative levels of steroidogenic expression were decreased to 50% in BPA treatment. The mRNA levels of CYP17A1, 3β-HSD and 17β-HSD in BPA+CT and BPA+ECH were increased more than 2-fold compared to the BPA treatment. The expressions of CYP11A1, 17β-HSD, CYP17A1 and 3β-HSD were significantly increased in CT and ECH compared to control (Fig. 5), while the level of StAR mRNA in ECH was no significant differences (P>0.05).

The comprehensive analysis the effect of ECH and CT on the levels of

steroidogenic enzymes was using heatmap soft. The result shows that ECH significantly increases the mRNA levels of CYP11A1, 17β-HSD and CYP17A1, and CT notably increases the mRNA levels of StAR, CYP11A1, 17β-HSD and CYP17A1.

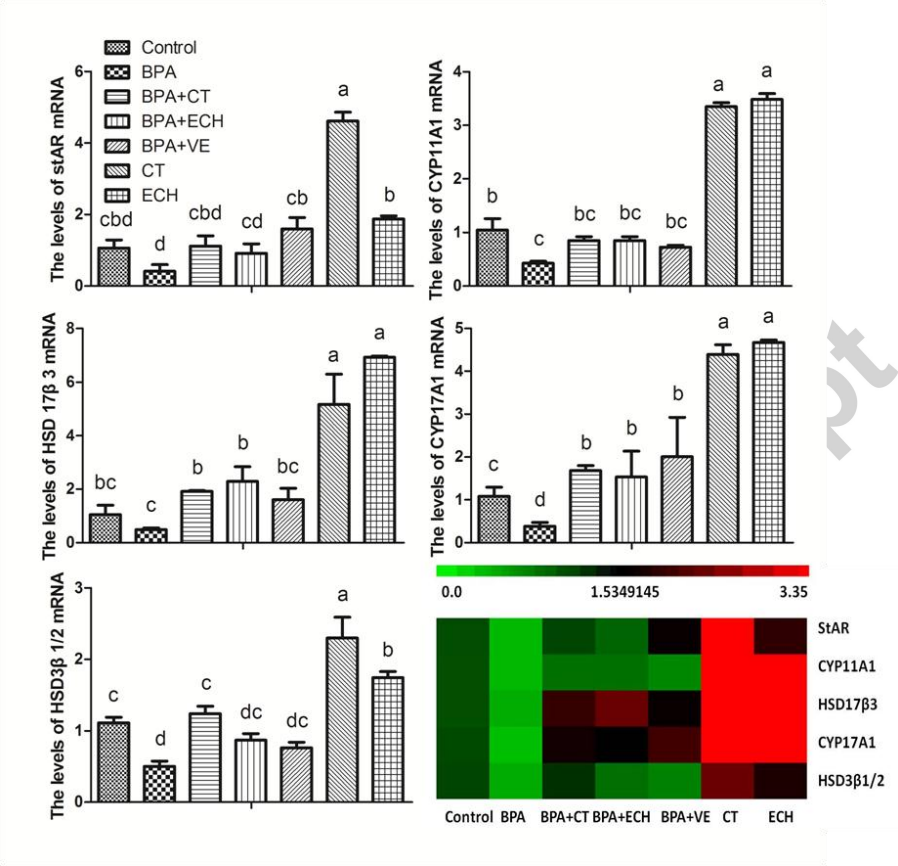


Fig. 5 The levels of steroidogenic enzyme expression.

Notes: (a, b, c, d, e): $^{a, b, c, d}$ Different letters indicate statistically different groups (p<0.05). (f): hierarchical clustering of mRNA expression of different steroidogenic enzymes. Green, green-red and red colors represent low-, medium- and high abundance, respectively, n=6.

3.7 CYP11A1 and CYP17A1 protein expressions

The expression of CYP11A1 and CYP17A1 were tested by IF and WB. The CYP11A1 and CYP17A1 were majorly expressed in leydig cells. Quantitatively, CT and ECH increased both proteins expression (Fig. 6A). The levels of CYP11A1 and

CYP17A1 were lower in BPA treatment than control, whereas, they were increased after exposure to CT and ECH (Fig. 6B).

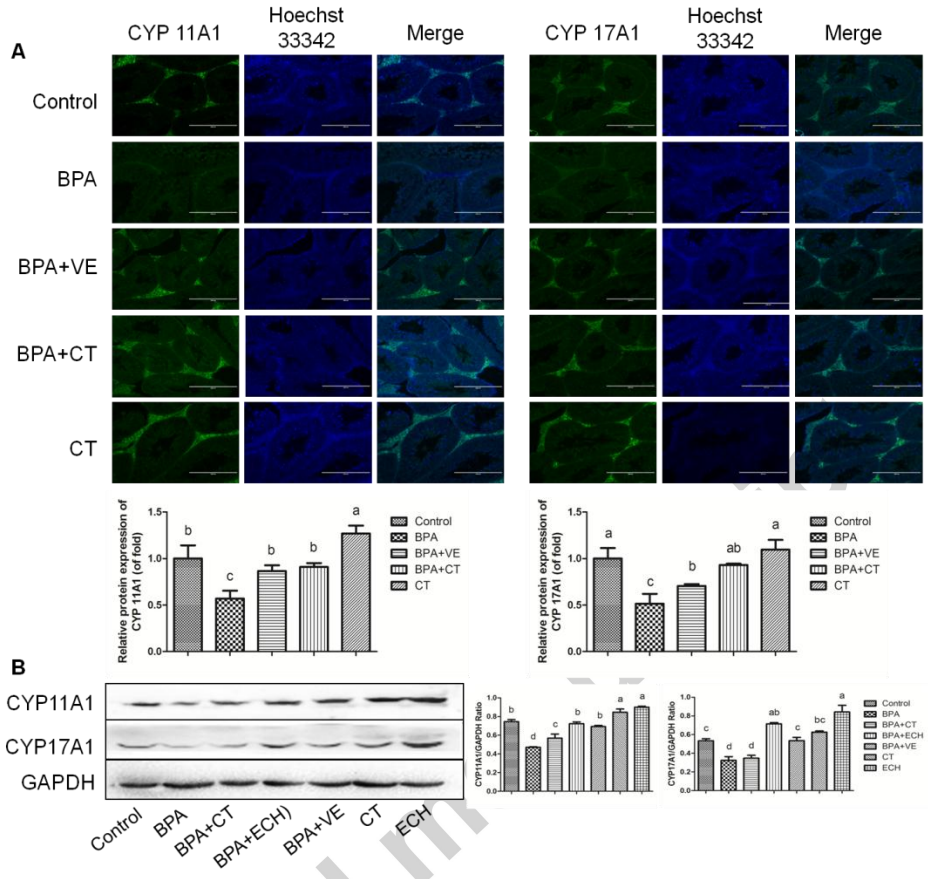


Fig. 6 Investigation on the expression of testosterone-associated proteins

Notes: (A) immunofluorescence analysis of testosterone-associated proteins. Green fluorescence represents CYP11A1 and CYP17A1, and Hoechst 33342 stained for nuclei. (B) Western blot analysis of CYP11A1 and CYP17A1 in testis. GAPDH was set as loading control. The down panel is a density metrically scanned histogram of protein bands, n=6.

4. Discussion

Exogenous endocrine disruptors possess growing impact on human health due to their adverse effects on development and/or function of reproductive organs (Liu et al.,

2015). In this study, BPA (as a typical exogenous endocrine disruptor) was used for developing male reproductive damage model. CT and ECH treatment could effectively increase the sperm motility (Fig. 2), sperm number and DSP (Table 1). The changes of sperm parameters were associated with sperm energy, steroid hormone synthesis and oxidative stress (Verma et al., 1993) and (Sikka et al., 1996). As compared to VE, ECH showed stronger steroidogenesis, suggesting that they may have different pathways in reducing testicular and sperm toxicity by considering that sex hormone-related levels were significantly increased compared to VE.

LDH-*x* is a major energy source required for sperm motility (Gupta et al., 1999). Therefore, reduced LDH-*x* activity is positively correlated with reduced sperm motility and count (Verma et al., 1993). Administration of ECH enhanced LDH-*x* activity, and normalized the BPA induced sperm abnormal motility. These results indicated that ECH was a sperm motility accelerant.

Steroid hormonal (such as testosterone) levels are also responsible for sperm quality. Testosterone selectively binds and activates androgenic receptors in Sertoli cells, resulting in initiation of the spermatogenic process and hindrance of germ cell apoptosis (Sikka et al., 1996). In line with previous reports, our study evidenced that BPA decreases the levels of some sex-related hormones (Peretz et al., 2013) and (Nakamura et al., 2010). The changes in sex hormone levels could cause subsequent reproductive dysfunction by interfering with the feedback regulatory mechanisms of the HPG-axis (Xi et al., 2011). Interestingly, the co-administration of BPA and ECH significantly improved BPA-induced testosterone and LH increase (Fig. 4). In studies

of gonadotrophin production, the production of testosterone by testicular leydig cells is strongly regulated by the HPG-axis, forming a homeostatic feedback loop. Increased testosterone levels have been shown to suppressing the frequency and magnitude of LH secretion whereas decreased testosterone levels enhancing release of LH from the pituitary (O'Hara et al., 2015). The results suggest that, ECH can elevate hormone insufficient symptom and effect on the feedback loop. It has reported that androgen receptor (AR) participate the regulation of negative feed loop (Holdcraft et al., 2004) and (Walters et al., 2010), and ECH may competitively bind to AR and less testosterone bind to AR, which decreased the stimulation of HPG-axis, the level of LH therefore were increased. However, further studies are needed to demonstrate this hypothesis.

Steroidogenesis is a tightly controlled, essential process for the development of spermatogenesis and male reproduction. Metabolism of steroidogenesis is dependent on steroidogenic enzymes such as CYP11A1, 3β-hydroxysteroid dehydrogenase (3β-HSD), aromatase, and the steroidogenic protein StAR (Luu-The, 2013), which converts cholesterol into testosterone (Ye et al., 2011).The mRNA levels of the genes encoding these enzymes were tested using qRT-PCR, and this data shown that BPA decreases the expression of CYP11A1, 3β-HSD, CYP17A1 and 17β-HSD and StAR. Administration of ECH increased the levels of CYP11A1, 3β-HSD, CYP17A1 and17β-HSD, and the expression of StAR mRNA did not change compared with control group; suggesting ECH had no effect on the cholesterol transfer to the inner membrane of mitochondria, while promoted the cholesterol converted into

testosterone. It has been demonstrated that a change in the expression of CYP11A1, 3β-HSD, CYP17A1 and 17β-HSD may directly affect the levels of testosterone (Barone et al., 2013; Gregoraszczuk et al., 2013 and Robic et al., 2014). Our results show that ECH affects testosterone biosynthesis. However, the CT significantly increased the levels of StAR, suggesting some other active ingredients of CT improve the cholesterol transfer to the inner membrane of mitochondria.

Conclusively, the CT and ECH have inhibited the BPA-induced deleterious effects and protected rat sperm and testis from injury through regulation of steroid hormone synthesis effect. ECH is the major active ingredient of CT to effect on sperm and testis injury and can be considered as a potential natural resource to develop reproductive agent.

Conflict of interest statement

The authors declare no conflict of interest.

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Author contribution

Zhi-hui JIANG designed experiments, performed experiments and wrote the paper; Jian WANG analyzed data; Xin-ping LI designed experiments and analyzed data; Xiao-ying ZHANG designed experiments, analyzed data and constructive discussions.

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Graphical abstract