Biol Trace Elem Res (2018) 184:463–473
https://doi.org/10.1007/s12011-017-1193-x

Ameliorative Effects of Selenium on Cadmium-Induced Injury in the Chicken Ovary: Mechanisms of Oxidative Stress and Endoplasmic Reticulum Stress in Cadmium-Induced Apoptosis

Na Wan[1] · Zhe Xu[1] · Tianqi Liu[1] · Yahong Min[1] · Shu Li[1]

Received: 9 September 2017 / Accepted: 24 October 2017 / Published online: 31 October 2017

Abstract Despite the well-established toxicity of cadmium (Cd) to animals and the ameliorative effects of selenium (Se), some specific mechanisms in the chicken ovary are not yet clarified. To explore the mechanism by which the toxicity effect of Cd is induced and explore the effect of supranutritional Se on Cd toxicity in female bird reproduction, forty-eight 50-day-old Isa Brown female chickens were divided randomly into four groups. Group I (control group) was fed the basic diet containing 0.2 mg/kg Se. Group II (Se-treated group) was fed the basic diet supplemented with sodium selenite (Na_2SeO_3), and the total Se content was 2 mg/kg. Group III (Se + Cd-treated group) was fed the basic diet supplemented with Na_2SeO_3; the total Se content was 2 mg/kg, and it was supplemented with 150 mg/kg cadmium chloride ($CdCl_2$). Group IV (Cd-treated group) was with the basic diet supplemented with 150 mg/kg $CdCl_2$. The Cd, estradiol (E2), and progestogen (P4) contents changed after subchronic Cd exposure in chicken ovarian tissue; subsequently, oxidative stress occurred and activated the endoplasmic reticulum (ER) pathway to induce apoptosis. Further, Se decreased the accumulation of Cd in ovarian tissue, increased the E2 and P4 contents, alleviated oxidative stress, and reduced apoptosis via the ER stress pathway. The present results demonstrated that Cd could induce apoptosis via the ER stress pathway in chicken ovarian tissue and that Se had a significant antagonistic effect. These results are potentially valuable for finding a strategy to prevent Cd poisoning.

Keywords Cadmium · Selenium · Oxidative stress · Endoplasmic reticulum stress · Apoptosis · Chicken ovary

Introduction

Cd is toxic heavy metal that is widely distributed in the environment [1]. It comes mainly from environmental pollution caused by industrial and agricultural production. Exposure of humans and animals to Cd arises from air, food, and water. After Cd enters the body, there is little excretion due to the lack of an effective Cd elimination pathway in the organism, and the remaining Cd is accumulated in target organs. Therefore, Cd can cause long-term toxic effects.

Many researchers have shown that Cd causes severe morphological and biochemical lesions to organs in mammals, including liver, brain, heart, and lung [2–4]. Other studies have shown that dietary exposure to Cd increases toxicity to immune organs, liver, and kidney in poultry [5–7]. Important here, the reproduction organs are one of the most important target organs for Cd accumulation and intoxication. Previous research results indicated that Cd exposure causes a variety of physiological changes in cock testes and hen ovary [8–10].

A previous study showed that Cd might have an obvious adverse effect on the secretion or/and release of ovary endocrine hormones [11]. The regular cyclic changes of the ovary are mainly stimulated through the hypothalamic-pituitary-ovarian axis. The luteinizing hormone of pituitary stimulates the secretion of E2 and P4 and from the corpus luteum. Conversely, E2 and P4 occupy key roles in the control of ovarian function through positive and negative feedback and the indirect action by the hypothalamic-pituitary-ovarian axis.

All of the authors have read the manuscript and agreed to submit it in its current form for consideration for publication in the *Biological Trace Element Research*.

✉ Shu Li
lishu@neau.edu.cn

[1] College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, People's Republic of China

Cd exposure suppressed E2 and P4 synthesis in rat granulosa and luteal cells [12]. Cd also inhibited E2 and P4 release in ovaries of rats, which might be an important mechanism of endocrine disruption [11].

Increasing evidence has suggested that oxidative stress participates in the pathological processes induced by Cd in various tissues [3, 13, 14]. Many studies have found that the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) decreased after Cd exposure, whereas the methane dicarboxylic aldehyde (MDA) content, nitric oxide (NO) levels, and inducible nitric oxide synthase (iNOS) activity increased in chicken kidney, liver, ovaries and serum [5, 6, 10]. Oxidative stress triggers apoptosis through a variety of signaling pathways, including the ER stress response [15]. Yokouchi M indicated that ER stress locates downstream of oxidative stress in apoptotic processes triggered by Cd and that Cd causes ER stress via an increase in reactive oxygen species (ROS), leading to apoptosis [16, 17].

Se is an essential micronutrient and is considered one of the most efficient metals that mediate Cd toxicity [18]. Se could protect the renal and hepatic tissues against the toxicity of Cd via an antioxidative effect because it reduces lipid peroxidation (LPO) and increases the activities of antioxidant enzymes in these tissues [19]. A study by Li demonstrated that Se attenuates the Cd-induced hepatotoxicity by decreasing oxidative stress, NO overproduction, and iNOS activity [6]. In addition, a previous study showed that Cd-induced ER stress was inhibited by antioxidants [16]. Several studies reported ameliorative effects of Se on Cd-induced oxidative stress, ER stress, and apoptosis in the chicken splenic lymphocytes, kidney, and testis [5, 8, 20]. This finding suggests that Se can be used to alleviate the ER stress and apoptosis caused by Cd through its antioxidant effect.

There are many studies on the toxic effects of Cd on many organs in mammals and on the antagonistic effect of Se. An epidemiological investigation found that a large amount of Cd accumulates in poultry in the polluted area [21, 22]. Although the toxic effects of Cd on birds and Se role in fertility are well known for the male reproductive system and spermatogenesis have been studied, whether supranutritional Se can alleviate the toxic effects of Cd on chicken ovarian tissue has not been reported. To explore a possible mechanism of Cd-induced apoptosis in chicken ovary, in the present study, we replicated a model that supplements the basic diet with Cd, which causes ovarian tissue injury and pathological changes. We also explored the ameliorative effects of supranutritional Se on Cd-induced apoptosis via the oxidative stress—ER stress—apoptosis pathway. In general, our experiments contributed to the study of Cd on reproductive toxicology and further elucidated the mechanism by which supranutritional Se decreases the activity of iNOS and the harmful effects of ROS, reduces the accumulation of Cd, and ameliorates Cd-induced oxidative stress, ER stress, and apoptosis.

Materials and Methods

Chemicals

Na_2SeO_3 and $CdCl_2$, which were analytical grade (≥ 99%), were obtained from the Sigma Aldrich Chemical Co. (St. Louis, Missouri, USA). Trizol reagent was purchased from Invitrogen, America. mRNA reverse transcription kit was provided by Thermo Fisher Scientific Inc., and other chemicals were provided by Technology Co., Ltd.

Preparation of Animals and Treatment

All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University. Previous studies reported that the LD50 of Cd for chicken was 218.44 mg/kg [6, 20, 23]. To exacerbate the ovarian toxicity of Cd and reveal the detoxification effects of Se, the dose levels of Cd and Se were 150 mg $CdCl_2$/kg dry weight of feed and 2 mg Na_2SeO_3/kg dry weight of feed (supernutritional Se but not Se toxicity), respectively. The dose and duration of this study have been described previously [23]. In brief, forty-eight 50-day-old Isa Brown female chickens were divided randomly into four groups (n = 12 per group). Birds were housed in the Laboratory Animal Center, College of Veterinary Medicine, Northeast Agricultural University, China. The animal room was maintained at 18–26 °C, and the birds were kept on 16 h light/8 h dark cycle and given free access to standard food and water about 12 weeks. Group I (control group) was fed with the basic diet containing 0.2 mg/kg Se [24]. Group II (Se-treated group) was fed with the basic diet supplemented with Na_2SeO_3, and the total Se content was 2 mg/kg. Group III (Se + Cd-treated group) was fed with the basic diet supplemented with Na_2SeO_3; the total Se content was 2 mg/kg and supplemented with 150 mg/kg $CdCl_2$. Group IV (Cd-treated group) was fed with the basic diet supplemented with 150 mg/kg $CdCl_2$. Following euthanasia, the tissue samples were immediately excised, blotted, and then rinsed with ice-cold 0.9% NaCl solution. They were dried with filter paper and weighed. A slice of tissue fixed in 2.5% glutaraldehyde was used for transmission electron microscope observation. The blood collected via cardiac puncture was allowed to clot and the serum was obtained by centrifugation at 1000 g for 10 min. 1.0 g tissue and serum via digestion were used for determination of Se and Cd content. Fractions of tissue were homogenized using glass Teflon homogenizer in cold 0.9% NaCl solution. The homogenates were centrifuged at 2000 g for 10 min at 4 °C. The supernatant was collected and used for the various reagent kit. The remaining tissue was frozen in liquid nitrogen and stored at − 80 °C before used to RT-PCR and protein assay.

Estimation of the Ratio of Ovary Weight to Body Weight and the Content of Cd and Se Assay in Serum and Ovary

After euthanasia, the ovary from experimental chickens were collected and weighed. The ratio of ovary weight to body weight was calculated for each chicken. The concentration of Cd and Se in the ovarian tissue and serum were determined using inductively coupled plasma mass spectrometry ICP-MS (Thermo iCAPQ, USA). The instrumental parameters were as follows: frequency 27.12 MHz, reflect power 1.55 kW, carrier gas flow 1.05 L/min, sampling depth 5.0 mm, torch-H 0.01 mm, torch-V-0.39 mm, nebuliser pump 40 rpm, S/C temperature 2.7 °C (spray chamber temperature), oxide ions (156/140) < 2.0%, doubly charged (70/140) < 3.0%, and nebuliser type is concentric. The mineral element concentrations were determined in the acid digest of the samples according to the method of Uluozlu et al. [25]. One gram of each sample was digested with 5 mL of HNO_3 (65%) and 2 mL of H_2O_2 (30%) in a microwave digestion system and diluted to 10 mL with deionized water. A blank digest was carried out in the same way. All of the sample solutions were clear. The digestion conditions for the microwave system were applied at 3 min for 1800 W at 100 °C, 10 min for 1800 W at 150 °C, and 45 min for 1800 W at 180 °C. The digested samples were adjusted with ultrapure water to the final volume before analysis by ICP-MS [26].

Determination of Estradiol and Progestogen Level in Serum

E2 and P4 were assayed by using Chicken E2 ELISA Kit and Chicken P4esterone (P4) ELISA Kit (R&D Systems, USA). E2 and P4 were determined based on according to the manufacturer's protocol, use purified chicken P4/E2 antibody to coat microtiter plate wells, make solid-phase antibody, then add P4/E2 to wells, combined P4/E2 antibody which with HRP labeled, become antibody-antigen-enzyme-antibody complex, after washing completely, add TMB substrate solution, TMB substrate becomes blue color at HRP enzyme-catalyzed; reaction is terminated by the addition of a sulphuric acid solution, and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of P4/E2 in the samples is then determined by comparing the O. D. of the samples to the standard curve.

Measurement of Oxidative Stress

The MDA and NO contents (Nanjing Jiancheng Bioengineering Institute, PR, China) and the SOD, GSH-Px and iNOS activities were assayed using kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer's protocol, and with a spectrophotometer (7230G, Shanghai Jinghua, Shanghai, China). The SOD activity was measured by the xanthinoxidase method, GSH-Px activity by DTNB color rendering, iNOS activity by colorimetry, NO content by the nitric reductase method, and MDA content by the thiobarbituric acid method.

Quantitative Real-Time PCR Analysis

Total RNA was isolated from ovarian tissue using Trizol according to the manufacturer's instructions (Invitrogen, USA). RNA concentrations were determined using a GeneQuant 1300. Primers for iNOS, glucose-regulated protein 78 (GRP78), cysteine-aspartic protease 3 (caspase-3), activating transcription factor 4 (ATF4), activating transcription factor 6 (ATF6), and inositol requiring enzyme (IRE) (Table 1) were designed using the Prime 5 Software (PREMIER Biosoft International, USA) based on known chicken sequences. Chicken β-actin (GenBank accession number L08165.1), a housekeeping gene, was used as an internal reference. The primers were synthesized by Invitrogen Biotechnology Co. Ltd. Reverse transcription reactions were performed as previously described [20]. RT-PCR was used to detect the expression of genes in tissue using Fast Universal SYBR Green Master mix (Roche, Basel, Switzerland), and RT-PCR was done using the Light Cycler® 480 RT-PCR system (Roche, Basel, Switzerland). The PCR procedure was performed as previously described [27]. Relative messenger RNA abundance was calculated using the ΔΔCt method, which accounts for gene-specific efficiencies, and was normalized to the mean expression of the abovementioned index.

Table 1 Gene-special primers for iNOS, GRP78, caspase-3, ATF4, ATF6, IRE, and β-actin used in the real-time quantitative reverse transcription PCR

Gene	Primer sequence
iNOS	Forward 5′-CTGAAGGCTCCTGGTTTA-3′
	Reverse 5′-TGCCACTCTGCGATTTAC-3′
GRP78	Forward 5′-GAATCGGCTAACACCAGAGGA-3′
	Reverse 5′-CGCATAGCTCTCCAGCTCATT-3′
Caspase-3	Forward 5′-CTGAAGGCTCCTGGTTTA-3′
	Reverse 5′-TGCCACTCTGCGATTTAC-3′
ATF4	Forward 5′-GAATCGGCTAACACCAGAGGA-3′
	Reverse 5′-CGCATAGCTCTCCAGCTCATT-3′
ATF6	Forward 5′-CGTCGTCTGAACCACTTACTGA-3′
	Reverse 5′-CCTTCTTTCCTAACAGCCACAC-3′
IRE	Forward 5′-CTACAGGTCGCTCCTCACATC-3′
	Reverse 5′-ATCAGTCCTTCTGCTCCCATCT-3′
β-actin	Forward 5′-ACCGCAAATGCTTCTAAACC -3′
	Reverse 5′- CCAATCTCGTCTTGTTTTATGC-3′

Protein Analysis of iNOS, GRP78, Caspase-3, ATF4, ATF6, and IRE

The protein concentrations were measured according to Bradford and using bovine serum albumin (BSA) as standard. Equal amounts of protein (80 mg) were resolved in 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes [28]. The membranes were blocked with 5% nonfat dry milk in PBST for 2 h at 37 °C. The membranes were incubated overnight at 4 °C with primary antibodies specific for iNOS, GRP78, caspase-3, ATF4, ATF6, and IRE that were diluted to their optimum concentrations in PBST + 5% nonfat dry milk. After three 5-min washes in PBST, the membranes were incubated for 1 h at 37 °C with peroxidase-conjugated secondary antibodies against rabbit IgG (1:1000, Santa Cruz, USA). After three 5-min washes, bound antibodies were visualized by chemiluminescence using ECL-plus reagent (GE Healthcare, Buckinghamshire, UK). The GAPDH content was analyzed as a control using a rabbit anti-chicken polyclonal antibody (Sigma).

Ultrastructural Observations

The ovarian tissue samples were fixed with 2.5% glutaraldehyde and were rinsed twice for 15 min in 0.2 M phosphate buffer (pH = 7.2). Samples were post-fixed in 1% buffered osmium tetroxide for 1 h, dehydrated through graded alcohols, and embedded in epoxy resin. According to the method of Xu et al., ultrathin sections were stained with uranyl acetate before examination under a transmission electron microscope [29].

Statistical Analysis

Statistical analysis of all data was performed using graph pad prism (version 5.0, GraphPad Software Inc., San Diego, CA, USA). All data showed a normal distribution and passed equal variance testing. Differences between means were assessed using Tukey's honestly significant difference test for post hoc multiple comparisons. Data are presented as the means ± S.D. and values were considered statistically significant if $P < 0.05$. In addition, we use a, b, c... to indicated significant differences between groups.

Results

Ovary Weight/Body Weight Ratios and the Content of Se and Cd in Chicken Ovarian Tissue and Serum

After 12 weeks of exposure to Cd in feed, there were no significant differences in growth rate between groups. However, as expected, the final body weight was less in the Cd group than in the control, Se-treated and Se + Cd-treated group. Relative ovary weight was reduced by subchronic Cd administration and increased when supplement with Se (Table 2). The contents of Se and Cd in ovarian tissue and serum for diets with different Se and Cd concentrations are shown in Table 2. The Se content was highest in the chicken ovarian tissue and serum for the Se-treated groups and was the lowest in the Cd-treated groups; the content in the Se + Cd-treated group was between those of Se- and Cd-treated groups. In contrast, in the chicken ovarian tissue, the Cd content in the Cd-treated group was nearly 50 times higher than that of control group. Similarly, in the serum, the Cd content in the Cd-treated group was almost 500 higher than that

Table 2 Ovary weight/body weight ratios and the content of Se, Cd, estradiol, and progestogen in chicken ovarian tissue and serum

Sample	Parameters	Control	Se	Se + Cd	Cd
Ovary (μg/g)	Se	0.231 ± 0.014	0.274 ± 0.018	0.201 ± 0.023	0.176 ± 0.011
	Cd	0.287 ± 0.006a	0.243 ± 0.015a	8.764 ± 0.034 b	14.607 ± 0.067 c
Serum (ng/ml)	Se	0.256 ± 0.014	0.274 ± 0.009	0.234 ± 0.012	0.207 ± 0.008
	Cd	0.007 ± 0.0001a	0.004 ± 0.0001a	2.476 ± 0.011c	3.698 ± 0.021d
Serum	E2(pM)	87.946 ± 4.081a	94. 713 ± 4.661a	64.376 ± 3.147b	49.847 ± 5.615c
	P4(nM)	214.63 ± 9.443a	237.0 ± 10.014a	185.16 ± 8.675b	139.407 ± 7.082c
Ovary	SOD (nu/mg.pr)	32.408 ± 2.014a	31.15 ± 2.438a	24.61 ± 1.483b	19.046 ± 3.247c
	GSH-Px (u/mg.pr)	11.907 ± 0.643a	13.04 ± 0.714a	7.083 ± 0.145b	4.672 ± 0.096c
	MDA (nmol/mg.pr)	2.207 ± 0.016a	2.038 ± 0.024a	3.496 ± 0.027b	4.675 ± 0.032c
Ratio of ovary/body weight (%)		8.688 ± 0.211a	8.730 ± 0.154a	3.048 ± 0.128b	0.576 ± 0.034b

Se and Cd contents in serum and ovary and the ratios of ovary/body weight of the normal and experimental chicken. The content of estradiol and progestogen in serum. Effects of oxidation indexes in ovary. Values are expressed as mean ± SD, $n = 12$. Statistically significant differences: means with different lowercase letters within columns are significantly different ($P < 0.05$)

in the control group. However, the Cd content in the ovarian tissue and serum of the Se + Cd-treated group was 40 and 33% lower, respectively, than that of the Cd-treated groups.

E2 and P4 Levels in Chicken Serum

The E2 and P4 levels were measured in chicken serum by ELISA Kit to determine the potential effects of Cd and Se. As shown in Table 2, supplementation with 2 mg/kg of Na_2SeO_3 alone had no significant impact on the E2 and P4 contents in serum. Exposure to Cd led to the E2 and P4 contents decreased by 55 and 24%, respectively, compared to the control group. However, treatment with both Se and Cd resulted in a decrease in E2 and P4 contents of 29 and 33% compared to the Cd treatment, respectively.

Variation in Antioxidant Activity

To examine whether Cd exposure could cause oxidative stress in ovarian tissue, we examined the activity of SOD, GSH-Px, and MDA in ovarian tissue (Table 2). Compared with control group, SOD activity had a decreasing trend and GSH-Px activity had an increasing trend in Se-treated groups, but there were no significant differences ($P > 0.05$). However, the SOD and GSH-Px activity in the Se + Cd- and Cd-treated groups decreased significantly ($P < 0.05$). After treatment with Cd alone, the activity of SOD and GSH-Px decreased by 41 and 61% compared to the control group, respectively. However, after Se and Cd co-administration, the SOD and GSH-Px activities increased by 29.2 and 51.6%, respectively, compared to the Cd-treated group. Additionally, treatment with both Se and Cd resulted in a decrease in MDA content of 25.2% compared to the Cd treatment.

NO Content and iNOS Expression

The NO content in the Cd-treated group was significantly higher than that in the control group by nearly 2.7-fold ($P < 0.05$) (Fig. 1a). Se supplementation resulted in a decrease in NO content of 42.8% compared to the Cd-treated group.

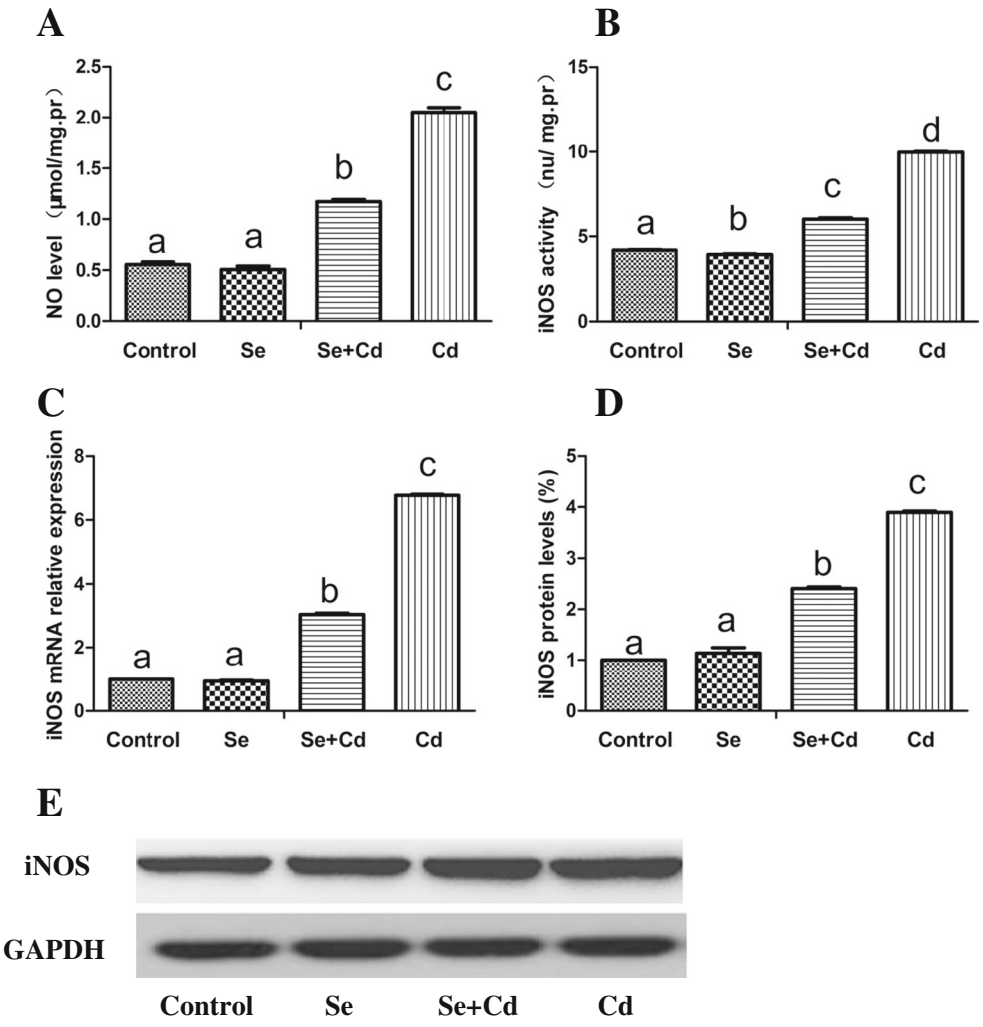

Fig. 1 NO level and iNOS activity. **a** Effects of Se administration on the content of NO in ovary tissues of Cd-induced chickens. **b** Effects of Se administration on the activity of iNOS in ovary tissues of Cd-induced chickens. **c** Effects of Se administration on the mRNA levels of iNOS gene in ovary tissues of Cd-induced chickens. Each value is the mean ± SD of 12 individuals. Statistically significant differences: means with different lowercase in different groups at the same time point are significantly different ($P < 0.05$). **d**, **e** Effects of Se administration on iNOS protein expression in the ovary of Cd-induced chickens

iNOS activity in the Cd-treated group was nearly 1.4-fold higher than that in the control group (Fig. 1b). However, iNOS activity in the Se + Cd-treated group was lower than that in the Cd-treated group by 39.6%. The mRNA levels of iNOS in the ovary are shown in Fig. 1c; the mRNA levels of iNOS in the Se + Cd-treated group was significantly lower than those in the Cd-treated group ($P < 0.05$). The iNOS protein levels in the ovary are shown in Fig. 1d, e; the expression of iNOS protein in the Cd-treated group was significantly higher than that in the control group in the chicken ovarian tissue. And the protein expression levels of iNOS in the Se + Cd-treated group were nearly 38.19% lower than those in ovarian tissue from the Cd-treated group. In addition, the protein expression levels of iNOS were not significantly different between the control and Se-treated groups ($P > 0.05$).

Expression of the Endoplasmic Reticulum Related Genes (GRP78, ATF4, ATF6, and IRE)

The mRNA levels of ER stress-related factors were measured in chicken ovarian tissue by RT-PCR to determine the potential effects of Cd and Se on ER stress. Our results showed that the mRNA levels of GRP78, ATF4, ATF6, and IRE in the Cd-treated group were significant higher than those of the control group ($P < 0.05$) (Fig. 2a). However, in the Se + Cd-treated group, the mRNA levels of GRP78, ATF4, ATF6, and IRE were significantly lower than in the Cd-treated group. In addition, the mRNA levels of these genes were not significantly different between the control and Se-treated groups ($P > 0.05$). The expression of GRP78, ATF4, ATF6, and IRE proteins in Cd-treated groups was significantly higher than in the control groups in ovarian tissue (4.16-, 5.05-, 3.02- and 2.02-fold, respectively) (Fig. 2b, c). Exposure to Cd led to a marked increase for ER stress-related genes ($P < 0.05$) compared to the control, Se-treated, and Se + Cd-treated group, respectively. However, after Se and Cd co-administration, the protein expression levels of GRP78, ATF4, ATF6, and IRE were 16.36, 31.85, 54.38, and 34.13% lower than in the Cd-treated group, respectively ($P < 0.05$).

The Results of Ultrastructural Observations in Ovary

The ovary cells in the control group and Se-treated group were normal and had smooth rounded nuclei and normally distributed chromatin; further, the inner and outer nuclear membranes were complete, and pathological structures were rarely observed (Fig. 3a, b). Ovarian cells from the Cd-treated group had morphological characteristics of apoptosis, including nuclear chromatin condensation that appeared as crescent-shaped bodies with a clear boundary under the nuclear envelope and chromatin margination (red arrows) and nuclear membrane fusion (green arrows) (Fig. 3d). Se administration during Cd exposure significantly reduced the ultrastructural changes in the ovary induced by Cd (Fig. 3c). Taken together, these ultrastructural analyses indicate that Cd exposure induces variations in cell structure that were alleviated by Se supplementation.

The mRNA and Protein Levels of Caspase-3

As shown in Fig. 4a, the mRNA levels of caspase-3 in the Cd-treated were 3.24-fold higher than control group. Exposure to Cd led to a marked increase for the caspase-3 mRNA level ($P < 0.05$) compared to the control. However, after Se and Cd co-administration, the caspase-3 mRNA level was lower compared to the control and Se treatment groups ($P < 0.05$). In addition, the protein level of caspase-3 in Cd-treated group was significant increase; however, after Se and Cd co-administration, the protein level of caspase-3 in ovarian tissue was 32.59% lower than that of the Cd-treated group. And the protein expression levels of caspase-3 were not significantly different between the control and Se-treated groups ($P > 0.05$).

Discussion

Cd is considered a typical environmental estrogen [30]. It possesses distinct female reproductive toxicity. Vrsanská et al. found that cigarette Cd markedly inhibited the follicle-stimulating hormone induction of cumulus cell progesterone production in porcine follicles [31]. In vivo, the administration of 10–15 mg/kg of Cd to estrus rats decreased the pituitary gonadotropin, ovarian progesterone secretion, and ovulation rates [32]. Zhang found that Cd can inhibit E2 and P4 release in ovaries and has a direct adverse effect, which might be an important mechanism of endocrine disruption [11]. In this study, the chicken showed weight gain is slow, mental depression, reduced ingestion and drinking water, lethargy, claws appear pale, and anemia symptoms, feather fluffy and irregular after Cd exposure. And the results showed that the ratio of ovary to body weight changed after Cd exposure. Moreover, we found that the Cd content in the ovary and serum increased after treatment with Cd. These results indicated that Cd is absorbed by the blood and is deposited in ovarian tissue, where it damaged the ovary, and further confirmed that the ovary is one of the target organs of Cd accumulation. Moreover, the results of subchronic Cd exposure significantly decreased the serum E2 and P4 levels in chickens suggested that Cd induces reproductive endocrine disorder in female chickens.

The antioxidant enzymes in the body include SOD and GSH-Px. These enzymes are generally considered sensitive biomarkers of an organism's antioxidant response and they protect cells against ROS toxicity and LPO [33, 34]. NO is a free radical and can be generated by iNOS; the overproduction of NO leads to LPO [35]. MDA formation is considered a

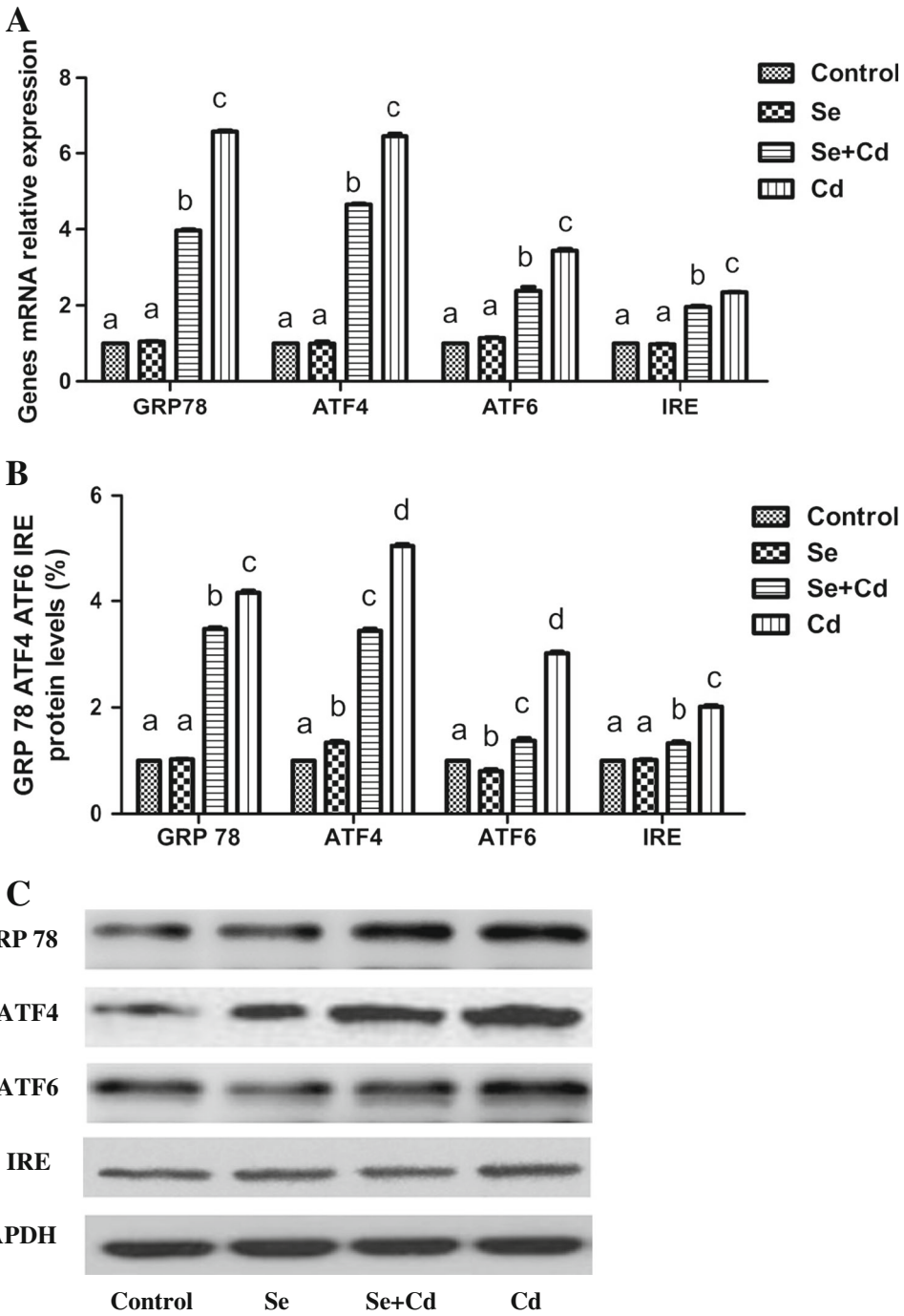

Fig. 2 The levels of ER stress-related genes GRP78, ATF4, ATF6, and IRE. **a** Effects of Se administration on the mRNA levels of GRP78, ATF4, ATF6, and IRE genes in ovary tissues of Cd-induced chickens. Each value is the mean ± SD of 12 individuals. Statistically significant differences: means with different lowercase in different groups at the same time point are significantly different ($P < 0.05$). **b**, **c** Effects of Se administration on GRP78, ATF4, ATF6, and IRE protein expression in the ovary of Cd-induced chickens

general indicator of LPO [36]. Previous studies found that oxidative stress participates in the pathological processes of various tissues induced by Cd [3, 13, 14]. Oxidative stress occurs when ROS increases, which causes an imbalance between antioxidative defense measures and ROS [37–39]. Cd causes oxidative damage in two ways: LPO and changes in intracellular antioxidant function [40]. Cd increases ROS in numerous cell types and can alter the antioxidant defense system both in vivo and in vitro [3, 41]. Cd toxicity modifies the antioxidant/prooxidant ratio in the serum and heart in a time-of-exposure-dependent way [42]. Cd treatment reduces antioxidants in the renal tissues of rats, increases LPO, and elevates the activity of the pro-apoptotic factor caspase-3 [43]. Poultry studies found that oxidative stress-related factors (SOD, GPx, MDA, NO, and iNOS) change in the chicken kidney after Cd exposure [5]. Significant reductions in antioxidant enzyme capacity and increases in both LPO and NO production were found in chicken livers [6]. In addition, Yang found that in the ovary and serum, the activity of SOD

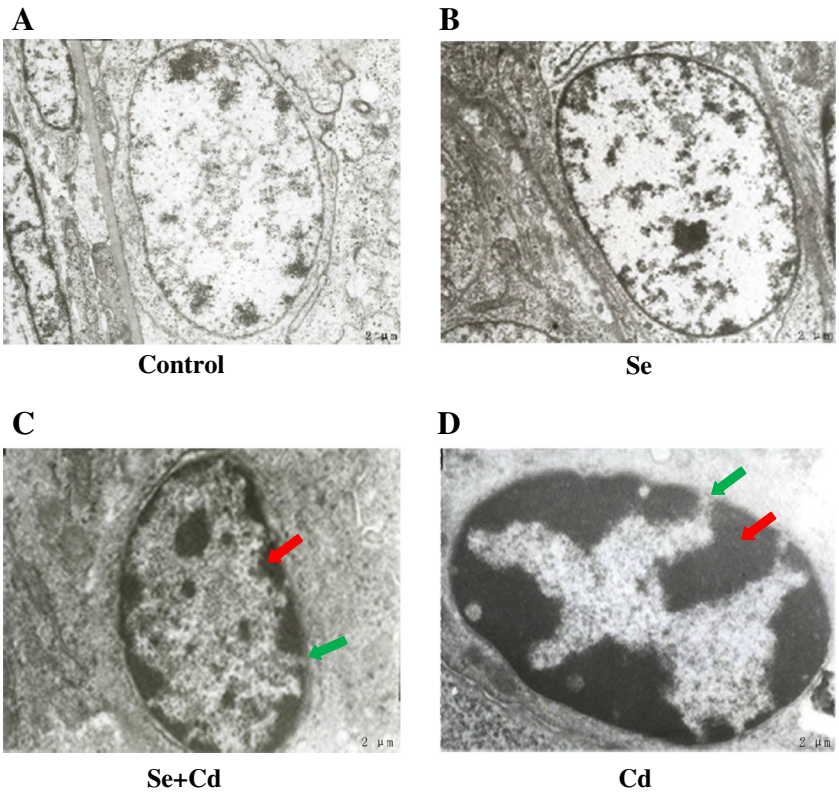

Fig. 3 Ultrastructural changes in ovary. Effects of Se administration on the ultrastructural changes in cortex of ovarian outer of Cd-induced chickens. **a** Ovary of the control group. **b** Ovary of the Se-treated group. **c** Ovary of the Se + Cd-treated group. **d** Ovary of the Cd-treated group. a, b, c, d × 15,000

and GPx decreased after Cd exposure, while the MDA content, NO levels, and iNOS activity increased [10]. In the current study, our results confirm those of previous studies, which clearly suggest that Cd exposure leads to high levels of MDA and NO production and high iNOS activity in chicken ovarian tissue and that the activity of antioxidant enzymes (SOD and GSH-Px) are significantly lower in the Cd-treated group than in controls.

A number of studies have shown that GRP78 is a marker protein of ER stress and that oxidative stress can induce ER stress, leading to the induction of apoptosis via the ER stress pathway [15, 16]. GRP78 is a central regulator of ER stress due to its role as a major ER chaperone that has anti-apoptotic properties and the ability to control the activation of three major transmembrane transducers that sense ER stress, RNA-dependent protein kinase-like ER kinase (PERK),

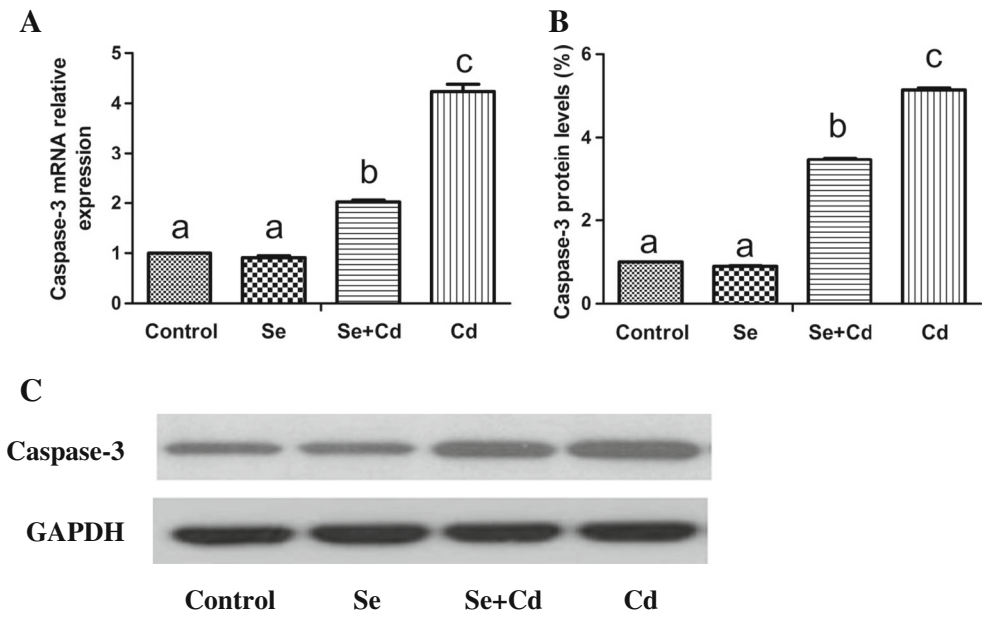

Fig. 4 The mRNA and protein levels of caspase-3. **a** Effects of Se administration on the mRNA levels of caspase-3 gene in ovary tissues of Cd-induced chickens. Each value is the mean ± SD of 12 individuals. Statistically significant differences: means with different lowercase in different groups at the same time point are significantly different ($P < 0.05$). **b**, **c** Effects of Se administration on caspase-3 protein expression in the ovary of Cd-induced chickens

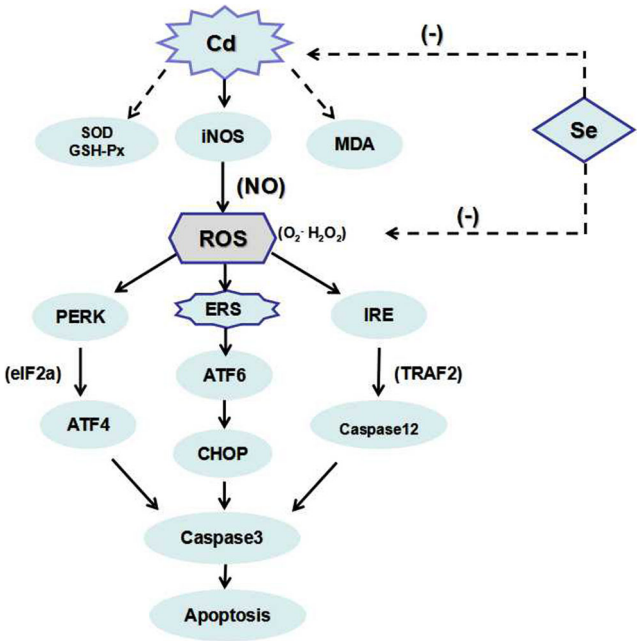

Fig. 5 Cd-induced apoptosis pathway and ameliorative effects of Se. Cd exposure leads to the activities of antioxidant enzymes (SOD and GSH-Px) decrease and MDA productions and the activity of iNOS increase. Cd causes ER stress via generation of ROS. ER stress triggers activation of the PERK-ATF4, ATF6-CHOP, and the IRE1-TRAF2 pro-apoptotic pathways. The activation of caspase-12 through an interaction with IRE1 and TRAF2. ER stress activates caspase-3 through three pathways, leading cells to undergo apoptosis. Se decreases the activity of iNOS and the harmful effects of ROS, and it also reduces the accumulation of Cd, and then ameliorates Cd-induced oxidative stress and ER stress and apoptosis

ATF6, and IRE1, via a binding-release mechanism [44]. In general, Cd induces ER stress and then activates IRE1, PERK, and ATF6 via GRP78 to induce apoptosis [45]. IRE1 pathway-induced apoptosis involves caspase-3 through the intermediate caspase-12 [15]. Interestingly, Cd enhances the production of ROS such as superoxide anion (O^{2-}), which rapidly reacts with NO, yielding the reactive species peroxynitrite ($ONOO^-$) [46, 47]. In addition, NO depletes Ca^{2+}, causes ER stress and leads to apoptosis via the activation of IRE, ATF6, and PERK [48]. A previous study found that the Cd-induced apoptosis of tubular cells occurs via ER stress-dependent and ER stress-independent mechanisms; however, Cd-induced apoptosis was markedly attenuated by antioxidants, which suggests that oxidative stress is a major mechanism in Cd-induced apoptosis. The O^{2-} and $ONOO^-$ produced in Cd-exposed cells can trigger the ATF6-CCAAT/enhancer-binding protein-homologous protein (CHOP) and IRE1-X-box-binding protein 1 (XBP1) pro-apoptotic pathways [16]. Yan-Li Ji reported that a single exposure to Cd induced both ER stress and UPR signaling as well as germ cell apoptosis in the testes of mice and that Cd elevates the level of GRP78 and changes the levels of the downstream targets, phosphorylated eukaryotic initiation factor-2a (eIF2a) and spliced mRNA encoding XBP1, which activate the PERK and IRE1a pathway, respectively [49]. Our results show that subchronic Cd exposure causes an increase in the NO levels, oxidative stress, and GRP78 expression and that it induces ER stress in chicken ovarian tissue, thereby increasing the expression levels of PERK, IRE1, and ATF6. It confirmed that ER stress-apoptosis pathway is activated after Cd exposure. Moreover, the transmission electron microscopy results confirmed that Cd exposure induced apoptosis.

Se, an essential trace element, is an important component of several enzymes and selenoproteins such as GSH-Px, thioredoxin reductases, and selenoprotein P. And previous study indicated that GSH-Px may be a target protein for Se deficiency in chicken [33]. Se can antagonize the toxicity of heavy metals and protect mammals and poultry both in vitro and in vivo against Cd toxicity [50]. In addition, Se displays an antioxidant effect, oxygen free radical scavenging, and transformations involving redox regulation [51–53]. Liu found that Se reduces Cd accumulation in chicken brain and that Se supplementation markedly enhanced the antioxidant defense system, which prevented Cd-caused oxidative damage [7]. Shuang Liu found that Se supplementation enhanced antioxidant systems and inhibited Cd-induced apoptosis in chicken splenic lymphocytes. In addition, Se eliminated the toxic effects of Cd on the activity of antioxidant enzymes (SOD and GSH-Px), prevented or decreased the harmful effects of oxidants and ROS, and significantly decreased the expression of caspase-3 and caspase-9 [54]. Apoptotic characteristics and an increased rate of apoptosis were obtained in the chicken liver of Se-deficient animals, and Se deficiency causes oxidative and ER stress, which can induce oxidative-ER apoptosis pathway [55]. Li et al. demonstrated that dietary exposure to Cd-caused histopathological changes, oxidative stress, endocrine disorders, and apoptosis in cock testes, and that Se ameliorated these effects by enhancing antioxidant systems and decreasing Cd accumulation in the testis [8]. In addition, Se significantly ameliorated oxidative stress and decreased the expression of the ER stress-related genes GRP78, GRP94, ATF4, ATF6, and IRE in the chicken kidney as well as the levels of apoptosis-related genes B cell lymphoma-2 (Bcl-2) and caspase-3 [5]. In this study, the ratio of ovary to body weight changed after exposure to Cd; this change was ameliorated by exposure to selenium. This result suggests that Se reduces Cd-induced ovarian damage. In addition, exposure to Cd led to SOD and GPx activity decreased and the MDA content increased, NO overproduction, iNOS activity increased, and expression of the ER stress-related genes GRP78, ATF4, ATF6, and IRE increased in chicken ovarian tissue; however, after Se and Cd co-administration, these changes were alleviated. These results indicate that Se plays a key role in reducing

Cd-induced oxidative stress and ER stress in the chicken ovaries. Besides, Se also alleviates Cd-induced pathological injuries and the ultrastructural damage caused by apoptosis and modifies the mRNA and protein levels of the apoptosis-related gene caspase-3 caused by after Cd exposure in chicken ovaries.

In summary (Fig. 5), Cd exposure decreases the activity of antioxidant enzymes (SOD and GSH-Px) and increases MDA production and iNOS activity. Cd causes ER stress via the generation of ROS. ER stress triggers the activation of the PERK-ATF4, ATF6-CHOP, and the IRE1-TNF receptor associated factor 2 (TRAF2) pro-apoptotic pathways. Caspase-12 is activated via interaction with IRE1 and TRAF2. ER stress activates caspase-3 through three pathways, leading cells to undergo apoptosis. Supranutritional Se decreased the activity of iNOS and the harmful effects of ROS, reduced the accumulation of Cd, and relieved Cd-induced oxidative stress and ER stress and apoptosis. Our experimental results show that Cd content in the ovary increases when the diet contains Cd, which results in reduced E2 and P4 as well as oxidative stress, which activates the ER stress pathway, which can induce apoptosis. However, supranutritional Se can decrease the accumulation of Cd in ovarian tissue, increase the content of E2 and P4, alleviate oxidative stress, and reduce the apoptosis induced by the ER stress pathway. The present results show that Cd could induce apoptosis via the ER stress pathway in chicken ovarian tissue and that Se has a significant antagonistic effect.

Acknowledgments The authors thank the Key Laboratory of the Provincial Education Department of Heilongjiang for Common Animal Disease Prevention and Treatment.

Funding This study was supported by the National Natural Science Foundation of China (Grant No.31472161).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Humane Care of Animals The experiments were approved by the Institutional Animal Care and Use Committee of the Northeast Agricultural University under the approved protocol number SRM-06.

Abbreviations *Cd*, cadmium; *Se*, selenium; *E2*, estradiol; *P4*, progestogen; Na_2SeO_3, sodium selenite; $CdCl_2$, cadmium chloride; *ER*, endoplasmic reticulum; *SOD*, superoxide dismutase; *GSH-Px*, glutathione peroxidase; *MDA*, malondialdehyde; *NO*, nitric oxide; *iNOS*, inducible nitric oxide synthase; *LPO*, lipid peroxidation; *ROS*, reactive oxygen species; *GRP78*, glucose-regulated protein 78; *PERK*, PKR-like ER kinase; *ATF6*, activating transcription factor 6; *ATF4*, activating transcription factor 4; *IRE1*, inositol requiring enzyme 1; *Caspase*, cysteine-aspartic protease; *CHOP*, ATF6-CCAAT/enhancer-binding protein-homologous protein; *XBP1*, IRE1-X-box-binding protein 1; *TRAF2* IRE1-TNF receptor associated factor 2

References

1. Kim J, Koo TH (2008) Heavy metal distribution in chicks of two heron species from Korea. Arch Environ Contam Toxicol 54(4): 740–747
2. Bagchi D, Vuchetich PJ, Bagchi M, Hassoun EA, Tran MX, Tang L, Stohs SJ (1997) Induction of oxidative stress by chronic administration of sodium dichromate [chromium VI] and cadmium chloride [cadmium II] to rats. Free Radic Biol Med 22(3):471–478
3. Ferramola ML, Pérez Díaz MF, Honoré SM, Sánchez SS, Antón RI, Anzulovich AC, Giménez MS (2012) Cadmium-induced oxidative stress and histological damage in the myocardium. Effects of a soy-based diet. Toxicol Appl Pharmacol 265(3):380–389
4. Napolitano JR, Liu MJ, Bao S, Crawford M, Nana-Sinkam P, Cormet-Boyaka E, Knoell DL (2012) Cadmium-mediated toxicity of lung epithelia is enhanced through NF-κB-mediated transcriptional activation of the human zinc transporter ZIP8. Am J Physiol Lung Cell Mol Physiol 302(9):909–918
5. Liu L, Yang B, Cheng Y, Lin H (2015) Ameliorative effects of selenium on cadmium-induced oxidative stress and endoplasmic reticulum stress in the chicken kidney. Biol Trace Elem Res 167(2):308–319
6. Li JL, Jiang CY, Li S, Xu SW (2013) Cadmium induced hepatotoxicity in chickens (Gallus domesticus) and ameliorative effect by selenium. Ecotoxicol Environ Saf 96(8):103–109
7. Liu S, Xu FP, Yang ZJ, Li M, Min YH, Li S (2014) Cadmium-induced injury and the ameliorative effects of selenium on chicken splenic lymphocytes: mechanisms of oxidative stress and apoptosis. Biol Trace Elem Res 160(3):340–351
8. Li JL, Gao R, Li S, Wang JT, Tang ZX, Xu SW (2010) Testicular toxicity induced by dietary cadmium in cocks and ameliorative effect by selenium. Biometals 23(4):695–705
9. Wenchang Z, Zhiren W, Huangyuan L, Ezaki T, Koji T (2002) Effects of cadmium as a possible endocrine disruptor upon the serum level of sex steroids and secretion of gonadotropins from pituitary in adult rats. Acta Med Nagasaki 47(1):53–56
10. Yang S, Zhang Z, He J, Li J, Zhang JL, Xing H, Xu S (2012) Ovarian toxicity induced by dietary cadmium in hen. Biol Trace Elem Res 148(1):53–60
11. Zhang W, Pang F, Huang Y, Yan P, Lin W (2008) Cadmium exerts toxic effects on ovarian steroid hormone release in rats. Toxicol Lett 182(1):18–23
12. Paksy K, Varga B, Lázár P (1992) Cadmium interferes with steroid biosynthesis in rat granulosa and luteal cells in vitro. Biometals Int J Role Metal Ions Biol Biochem Med 5(4):245
13. Tribowo JA, Arizal MH, Nashrullah M, Aditama AR, Utama DG (2014) Oxidative stress of cadmium-induced ovarian rat toxicity. Int J Chem Eng Appl 5(3):389–391
14. Wang J, Zhu H, Liu X, Liu Z (2014) Oxidative stress and Ca^{2+} signals involved on cadmium-induced apoptosis in rat hepatocyte. Biol Trace Elem Res 161(2):180–189
15. Kitamura M, Hiramatsu N (2010) The oxidative stress: endoplasmic reticulum stress axis in cadmium toxicity. Biometals Int J Role Metal Ions Biol Biochem Med 23(5):941–950
16. Yokouchi M, Hiramatsu N, Hayakawa K, Okamura M, Du S, Kasai A, Takano Y, Shitamura A, Shimada T, Yao J (2008) Involvement of selective reactive oxygen species upstream of proapoptotic branches of unfolded protein response. J Biol Chem 283(7):42–52
17. Jin Y, Zhang S, Tao R, Huang J, He X, Qu L, Fu Z (2016) Oral exposure of mice to cadmium (II), chromium (VI) and their mixture induce oxidative- and endoplasmic reticulum-stress mediated apoptosis in the livers. Environ Toxicol 31(6):693–705
18. Jihen EH, Imed M, Fatima H, Abdelhamid K (2009) Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity

in the liver of the rat: effects on the oxidative stress. Ecotoxicol Environ Saf 72(5):1559–1564
19. Newairy AA, Elsharaky AS, Badreldeen MM, Eweda SM, Sheweita SA (2007) The hepatoprotective effects of selenium against cadmium toxicity in rats. Toxicology 242(3):23–30
20. Liu LL, Li CM, Zhang ZW, Zhang JL, Yao HD, Xu SW (2014) Protective effects of selenium on cadmium-induced brain damage in chickens. Biol Trace Elem Res 158(2):176–185
21. Han G (1998) Exposure level of cadmium in poultry in cadmium polluted areas. J Environ Health 199(2):111–114
22. Shuvaeva OV, Belchenko LA, Romanova TE (2013) Studies on cadmium accumulation by some selected floating macrophytes. Int J Phytoremediation 15(10):979–990
23. Tan S, Chi Q, Liu T, Sun Z, Min Y, Zhang Z, Li S (2017) Alleviation mechanisms of selenium on cadmium-spiked neutrophil injury to chicken. Biol Trace Elem Res 178(2):301–309
24. Zhang R, Xing L, Bao J, Sun H, Bi Y, Liu H, Li J (2017) Selenium supplementation can protect from enhanced risk of keel bone damage in laying hens exposed to cadmium. RSC Adv 7(12): 7170–7178
25. Uluozlu OD, Tuzen M, Mendil D, Soylak M (2009) Assessment of trace element contents of chicken products from Turkey. J Hazard Mater 163(2):982–987
26. Xu T, Gao X, Liu G (2016) The antagonistic effect of selenium on lead toxicity is related to the ion profile in chicken liver. Biol Trace Elem Res 169(2):365–373
27. Yang Z, Liu C, Liu C, Teng X, Li S (2016) Selenium deficiency mainly influences antioxidant selenoproteins expression in broiler immune organs. Biol Trace Elem Res 172(1):209–221
28. Bradford MM (1976) A rapid and sensitive methods for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72(1–2):248–254
29. Xu J, Lian LJ, Wu C, Wang XF, Fu WY, Xu LH (2008) Lead induces oxidative stress, DNA damage and alteration of p53, Bax and Bcl-2 expressions in mice. Food Chem Toxicol 46(5): 1488–1494
30. Huff MOM, Todd SL, Bleser AS, Riggs KA, Dougherty SM, Klinge CM (2007) Cadmium chloride and sodium arsenate, environmental estrogens in cigarette smoke, activate estrogen signaling pathways to induce proliferation in a human lung adenocarcinoma cell line. FASEB J 21(2):A255–A259
31. Vrsanská S, Nagyová E, Mlynarcíková A, Ficková M, Kolena J (2002) Components of cigarette smoke inhibit expansion of oocyte-cumulus complexes from porcine follicles. Physiol Res 52(3):383–387
32. Paksy K, Varga B, Horváth E, Tátrai E, Ungváry G (1989) Acute effects of cadmium on preovulatory serum FSH, LH, and prolactin levels and on ovulation and ovarian hormone secretion in estrous rats. Reprod Toxicol 3(4):241–247
33. Li JL, Sunde RA (2016) Selenoprotein transcript level and enzyme activity as biomarkers for selenium status and selenium requirements of chickens (Gallus gallus). PLoS One 11(4):e0152392
34. Lei W, Wang L, Liu D, Xu T, Luo J (2011) Histopathological and biochemical alternations of the heart induced by acute cadmium exposure in the freshwater crab Sinopotamon yangtsekiense. Chemosphere 84(5):689–694
35. Pi J, Horiguchi S, Sun Y, Nikaido M, Shimojo N, Hayashi T, Yamauchi H, Itoh K, Yamamoto M, Sun G (2003) A potential mechanism for the impairment of nitric oxide formation caused by prolonged oral exposure to arsenate in rabbits. Free Radic Biol Med 35(1):102–113
36. Wang M, Zhou Q (2006) Effects of herbicide chlorimuron-ethyl on physiological mechanisms in wheat (Triticum aestivum). Ecotoxicol Environ Saf 64(2):190–197
37. Bloom JA, Cox IJ, Miller ML, Min WU, Lin CY, Lui YM (2001) Rotation, scale and translation resilient public watermarking for images. IEEE Trans Image Process 10(5):767–782
38. Wittman R, Hu H (2002) Cadmium exposure and nephropathy in a 28-year-old female metals worker. Environ Health Perspect 110(12):1261–1266
39. Bernard A (2008) Cadmium & its adverse effects on human health. Indian J Med Res 128(4):557–564
40. Fouad AA, Qureshi HA, Al-Sultan AI, Yacoubi MT, Ali AA (2009) Protective effect of hemin against cadmium-induced testicular damage in rats. Toxicology 257(3):153–160
41. Pathak N, Khandelwal S (2006) Oxidative stress and apoptotic changes in murine splenocytes exposed to cadmium. Toxicology 220(1):26–36
42. Ferramola ML, Antón RI, Anzulovich AC, Giménez MS (2011) Myocardial oxidative stress following sub-chronic and chronic oral cadmium exposure in rats. Environ Toxicol Pharmacol 32(1):17–26
43. Hagar H, Malki WA (2014) Betaine supplementation protects against renal injury induced by cadmium intoxication in rats: role of oxidative stress and caspase-3. Environ Toxicol Pharmacol 37(2):803–811
44. Hammadi M, Oulidi A, Gackière F, Katsogiannou M, Slomianny C, Roudbaraki M, Dewailly E, Delcourt P, Lepage G, Lotteau S (2013) Modulation of ER stress and apoptosis by endoplasmic reticulum calcium leak via translocon during unfolded protein response: involvement of GRP78. FASEB J 27(4):1600–1609
45. Yokouchi M, Hiramatsu N, Hayakawa K, Kasai A, Takano Y, Yao J, Kitamura M (2007) Atypical, bidirectional regulation of cadmium-induced apoptosis via distinct signaling of unfolded protein response. Cell Death Differ 14(8):1467–1474
46. Szabó C, Ohshima H (1997) DNA damage induced by peroxynitrite: subsequent biological effects. Nitric Oxide 1(5):373–385
47. Liu J, Qu W, Kadiiska MB (2009) Role of oxidative stress in cadmium toxicity and carcinogenesis. Toxicol Appl Pharmacol 238(3): 209–214
48. Oyadomari S, Takeda K, Takiguchi M, Gotoh T, Matsumoto M, Wada I, Akira S, Araki E, Mori M (2001) Nitric oxide-induced apoptosis in pancreatic cells is mediated by the endoplasmic reticulum stress pathway. Proc Natl Acad Sci U S A 98:10845–10850
49. Jia W, Loria RM, Park MA, Yacoub A, Dent P, Graf MR (2010) The neuro-steroid, 5-androstene 3β,17α diol; induces endoplasmic reticulum stress and autophagy through PERK/eIF2α signaling in malignant glioma cells and transformed fibroblasts. Int J Biochem Cell Biol 42(12):2019
50. Talas ZS, Orun I, Ozdemir I, Erdogan K, Alkan A, Yılmaz I (2008) Antioxidative role of selenium against the toxic effect of heavy metals (Cd +2, Cr +3) on liver of rainbow trout (Oncorhynchus mykiss Walbaum 1792). Fish Physiol Biochem 34(3):217–222
51. Fischer JL, Lancia JK, Mathur A, Smith ML (2006) Selenium protection from DNA damage involves a Ref1/p53/Brca1 protein complex. Anticancer Res 26(2A):899–904
52. Bansal MP, Kaur P (2005) Selenium, a versatile trace element: current research implications. Indian J Exp Biol 43(12):1119–1129
53. Zhang ZW, Wang QH, Zhang JL, Li S, Wang XL, Xu SW (2012) Effects of oxidative stress on immunosuppression induced by selenium deficiency in chickens. Biol Trace Elem Res 149(3):352–361
54. Liu LL, Zhang JL, Zhang ZW, Yao HD, Sun G, Xu SW (2014) Protective roles of selenium on nitric oxide-mediated apoptosis of immune organs induced by cadmium in chickens. Biol Trace Elem Res 159(1–3):199
55. Yao L, Du Q, Yao H, Chen X, Zhang Z, Xu S (2015) Roles of oxidative stress and endoplasmic reticulum stress in selenium deficiency-induced apoptosis in chicken liver. Biometals 28(2): 255–265