

Selenium regulates Nrf2 signaling to prevent hepatotoxicity induced by hexavalent chromium in broilers

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ABSTRACT Hexavalent chromium (Cr(VI)) is considered to be a common environmental pollutant, which widely exists in industrial effluents and wastes and then potentially noxious effects to the health of the poultry. Studies have reported that selenium (Se), which is one of the essential trace elements of the poultry and participates in the oxidative metabolism, can alleviate Cr(VI)-induced organ damage by inhibiting oxidative stress, but its specific molecular mechanism remains unclear. Herein, animal models of Cr(VI)- and Se-exposure were constructed using broilers to investigate the antagonistic mechanism of Se to Cr(VI)-induced hepatotoxicity. In this experiment, the four groups of broiler models were used as the research objects: control, Se, Se plus Cr, and Cr groups. Histopathology and ultrastructure liver changes were observed. Liver-somatic index, serum biochemistry,

oxidative stress, Nrf2 pathway related factors, and autophagy-related genes were also determined. Overall, Se was found to ameliorate the disorganized structure, hepatic insufficiency, and oxidative damage caused by Cr(VI) exposure. Electron microscopy analysis further showed that the number of autophagosomes was obviously decreased after Se treatment compared to Cr group. Furthermore, gene and protein expression analyses illustrated that the levels of Nrf2, glutathione peroxidase 1 (GPx-1), NAD(P)H: quinone oxidoreductase 1 (NQO1), and mechanistic target of rapamycin (mTOR) in the Se&Cr group was upregulated, along with decreased expression of Beclin 1, ATG5 and LC3 compared to the Cr group. These suggest that Se can repair the oxidative lesion and autophagy induced by Cr(VI) exposure in broiler livers by upregulating the Nrf2 signaling pathway.

Key words: autophagy, hepatotoxicity, hexavalent chromium, Nrf2 signaling pathway, selenium

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INTRODUCTION

Chromium (Cr) compounds have been widely used in the industry since Cr was first discovered by the French chemist Vauquelin in 1797. However, Cr-rich industrial wastes represent potentially harmful pollutants that can promote serious environmental and health problems (Alvarez et al., 2021; Pushkar et al., 2021). Being mainly present in the form of Cr lead (Pb) ore, Cr has multiple valence states, with only Cr(III) and Cr(VI) existing stably in nature. Cr(VI) shows good water solubility, significant oxidizing capacity, and is easily accumulated, which makes it is more toxic than Cr(III) (Paithankar et al., 2021). Therefore, Cr(VI) toxicological profile has attracted significant attention as the main toxic form of Cr. Cr(VI) exists as CrO_4^{2-} , which is

structurally similar to SO_4^{2-} ; thus, it can enter cells through nonspecific SO_4^{2-} carriers. Once inside the cell, Cr(IV) is converted into Cr(III) forms by reducing some cellular substances such as ascorbic acid, cytochrome c, and glutathione (GSH) (DesMarais and Costa, 2019). Moreover, an increasing number of ROS are generated during Cr(VI) reduction, thereby contributing for cellular oxidative stress (Wu et al., 2016).

Many studies demonstrated that oxidative stress caused by heavy metals could lead to autophagy. For example, the experimental results of Zeng et al. illustrated that excessive copper would lead to oxidative stress, increase ROS and MDA content, and activate mitochondrial autophagy (Zeng et al., 2020). Li et al. also showed that arsenic (As) and copper exposure induced cardiac trauma and autophagy in chickens, which was mediated by oxidative stress (Li et al., 2018). In addition, Zhang et al. believed that cadmium (Cd) exposure destroyed ion homeostasis as well as caused endoplasmic reticulum stress and autophagy (Zhang et al., 2020a). Recent studies have also shown that Cr(VI) (Zheng et al., 2020), Cd (Chen et al., 2018; Shi et al., 2019) as well as manganese (Jiang et al., 2020)

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exposure could lead to oxidative stress in chicken kidney and spleen, and then induce autophagy. Hence, autophagy induced by oxidative stress also plays an important role in heavy metal-mediated toxic injury of tissues and organs.

Selenium (Se) is one of the essential trace elements that participates in important physiological and biochemical activities. Many researches have proved that Se could antagonize the effects of Cr(VI) (Chen et al., 2017), Pb (Xu et al., 2016), and Cd (Zhang et al., 2018) in chicken liver by regulating homeostasis of the different elements. Se is also an important component of GPx, which can scavenge H₂O₂ and organic peroxides by improving the activity of antioxidant enzymes, thereby ameliorating the damage induced by oxidative stress (Gopalakrishna et al., 2016). What's more, researches have shown that the antioxidant system regulated by Se is closely related to the Nrf2 pathway. Nrf2 is an important regulator of redox homeostasis and the key transcription factor regulating antioxidant and detoxification enzyme genes, thereby protecting organs from oxidative damage (Basak et al., 2017). Indeed, Se can effectively alleviate chicken oxidative damage and autophagy caused by ochratoxin A (Li et al., 2020), high fluorine (Ju et al., 2021), and Cd (Zhang et al., 2017) by upregulating Nrf2 pathway and its downstream antioxidant enzymes.

However, to date, it remains unclear whether Se can prevent Cr(VI)-induced autophagy in liver tissues. Moreover, Se impact on Nrf2-mediated signals in Cr(VI)-induced chicken liver tissue damage remain to be explored. In this study, an in vivo model of Cr(VI)-poisoning and Se-treatment was established in chickens to illustrate whether the Nrf2 pathway is participated in the beneficial effects of Se to alleviate Cr(VI)-induced autophagy injury.

MATERIALS AND METHODS

All experiments were complied with the Institutional Animal Care and Use Committee of the Shanxi Agricultural University (SXAU-EAW-2019C012004).

Animal Experiment

Hundred one-day-old broilers were divided into 4 groups regardless of sex (25 broilers/group, 5 replicates/group, and 5 broilers/replicate): control (C), Se-exposure (Se), detoxification (Se&Cr), and poisoning (Cr) groups after 7 d. The average weight \pm standard deviation of broilers was 0.19 ± 0.01 kg before adding Se or Cr. As described previously, the concentration of 0.3 mg/kg \cdot diet Se and 37 mg/kg \cdot body weight K₂Cr₂O₇ were used (Liu et al., 2019; Zhao et al., 2022; Zhang et al., 2020b). C group was fed basal diet which provided by Shanxi Daxiang Agriculture and Animal Husbandry Group Co. (Lvliang, China), and drank sterilized distilled water. Se group was fed basal diet supplemented with Se-enriched yeast (SeY; Angel, Hubei,

China). Se&Cr chickens were given SeY-supplemented basal diet and K₂Cr₂O₇ (Xiya Reagent, Shandong, China) added to the drinking water. Broilers in the Cr group received K₂Cr₂O₇ via the drinking water.

All broilers were fed routinely and drank freely. In order to maintain the normal metabolic activity and physiological function of broilers, the compound feed of corresponding ages was provided at different growth stages. The ambient temperature was adjusted with the age of the chicken. After the experimental period (42 d), the chickens in each group were fasted overnight, euthanized by injecting sodium pentobarbital, and weighted. The livers were collected and weighted, then washed with normal saline solution. Small pieces of liver tissues were respectively fixed in 4% paraformaldehyde solution (Biosharp, Shanghai, China) and 2.5% glutaraldehyde solution (Biotopped, Beijing, China) for microstructure and ultrastructure observation. Other liver samples were immediately frozen, and then stored at -80°C for subsequent experiments. Meanwhile, the serum was also obtained by the low-speed centrifuge for biochemical analysis.

Body Weight and Liver-Somatic Index Assessment

The weight of broilers was recorded, and the liver-somatic index was analyzed according to the organ weight (g)/body weight (g) ratio.

Histopathological Analysis

After dehydration with different concentrations of ethanol and transparent xylene, the liver tissues were embedded in paraffin. Cut the liver paraffin into 3- μm thin slices, and then assessed by hematoxylin and eosin staining. All liver slices were observed and pictured using a light microscope (Nikon, Tokyo, Japan) (Zhao et al., 2019).

Ultrastructure Analysis

Fixed liver tissue samples, with a volume of 1 mm³, continued to be dehydrated with different concentrations of alcohol, infiltrated, embedded, polymerized, and sectioned. Finally, the ultrathin sections were stained, and fixed on cuprum grids. Under accelerating voltage, the ultrastructure of hepatocytes was observed with transmission electron microscope (Wang et al., 2020).

Detection of Biochemical Indexes

Biochemical serum indexes, such as alnine amiotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), total protein (TP), and total bilirubin (TBIL), were measured using an automatic biochemical analyzer (VetScan VS2; Zoetis, Parsippany, NJ) (Andleeb et al., 2020; Tao et al., 2020).

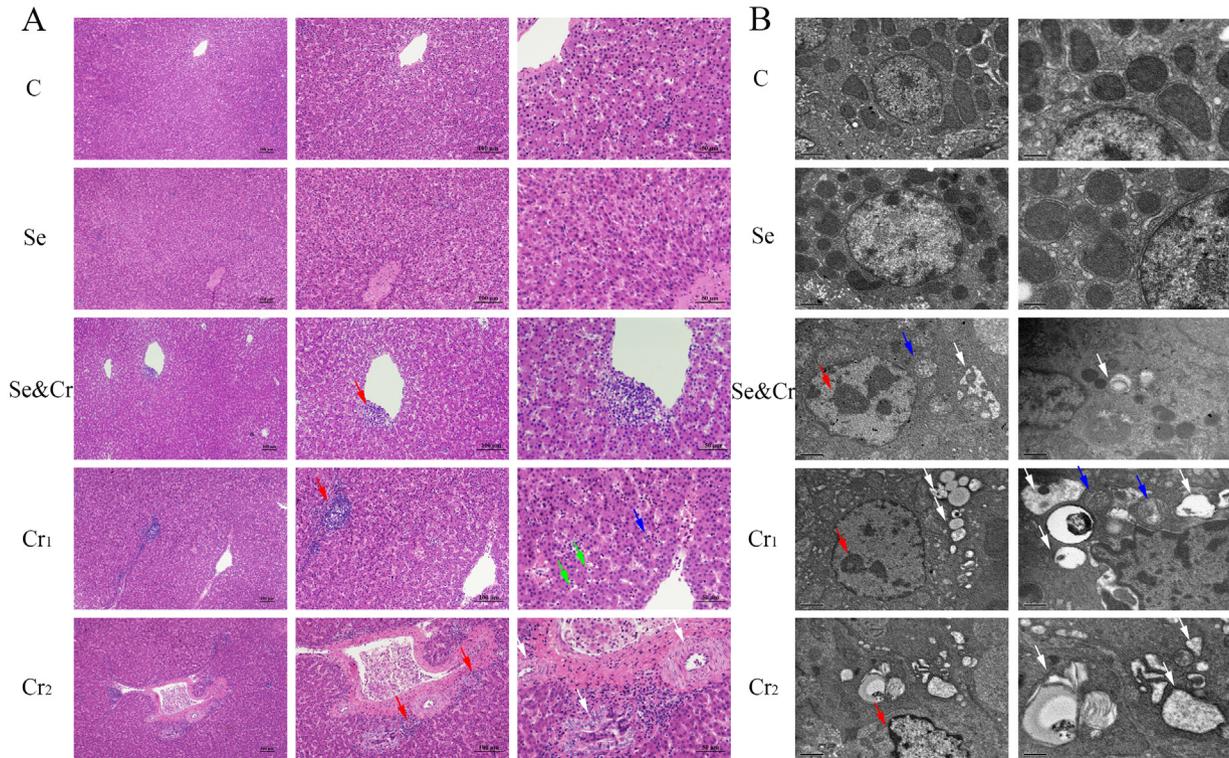


Figure 1. Influences of Cr(VI) and Se on the histopathology and ultrastructure of broiler liver tissues. (A) Representative images showing the histopathological findings assessed by hematoxylin and eosin staining (magnifications from left to right: 100 \times , 200 \times , and 400 \times). Red, blue, green, and white arrows represent infiltration of inflammatory cells, congested sinusoids, vacuolar degeneration, and muscular hypertrophy in blood vessels and bile duct hyperplasia, respectively. (B) Representative images of the ultrastructural tissue changes (magnification from left to right: 15,000 \times and 30,000 \times). Red, blue, and white arrows represent chromatin aggregation, mitochondrial cristae dissolution, and autophagosome, respectively.

Determination of Oxidative Stress Parameters

Firstly, 10% liver tissue homogenate in normal saline solution was prepared. Next, absorbances of TP, total superoxide dismutase (**T-SOD**), GSH, and malondialdehyde (**MDA**) were measured at specific wavelengths using a microplate reader or spectrophotometer (Unico, Shanghai, China), as described in the kit instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Finally, the levels of the oxidative stress parameters in liver tissues were separately calculated with its corresponding formula (Fan et al., 2020).

qPCR Analysis

Total RNA was obtained from 0.1 g broiler liver tissue samples using 1 mL of Trizol reagent. Next, RNA templates were reverse transcribed into cDNA. All the specific primers used are shown in [Supplementary Table 1](#). Finally, the SYBR Green I chimeric fluorescence method (Vazyme, Nanjing, China) was used to perform the qPCR reaction. The levels of the target genes were analyzed with $2^{-\Delta\Delta CT}$ method evaluation.

Western Blot Analysis

The protein sample was obtained from 0.1 g tissue samples with 1,000 μ L RIPA lysis buffer containing 10 μ L

phenylmethanesulfonyl fluoride. The target proteins were isolated with SDS-PAGE and transferred onto PVDF membranes. Next, the membranes were sealed with 5% skim milk, and following primary antibodies were incubated overnight in a refrigerator (4 $^{\circ}$ C): β -tubulin (1:1,800), β -actin (1:3,000), mTOR (1:600), ATG5 (1:1,000), and LC3B (1:1,000) from Abmart (Shanghai, China); Nrf2 (1:1,000) from Bioss (Beijing, China); Keap1 (1:1,800), GPx-1 (1:1,000), NQO1 (1:1,000), HO-1 (1:1,000), and p62 (1:800) from Wanleibio (Shenyang, China); and Beclin 1 (1:1,200) from ABclonal (Wuhan, China), all diluted in primary antibody dilution buffer (Beyotime, Shanghai, China). The membrane was incubated with the secondary goat anti-mouse antibody (1:5,000) and anti-rabbit IgG antibody (1:8,000), both from Bioss, for 1 h at 28 $^{\circ}$ C. Lastly, the protein was identified using a hypersensitive chemiluminescence kit (Beyotime), and scanning and imaging was performed using a gel imaging system. Densitometric analysis of the protein bands was analyzed with ImageJ (Version 1.38) (Ali Shah et al., 2021).

Immunohistochemical Analysis

Firstly, paraffin sections of broiler livers were dewaxed using xylene and ethanol gradient solutions, washed with distilled water, and then treated with ethylenediaminetetraacetic acid (pH 9.0) antigen retrieval buffers (Servicebio, Hubei, China) and repaired in a microwave oven. Next, the primary antibodies LC3B (Abmart) and p62

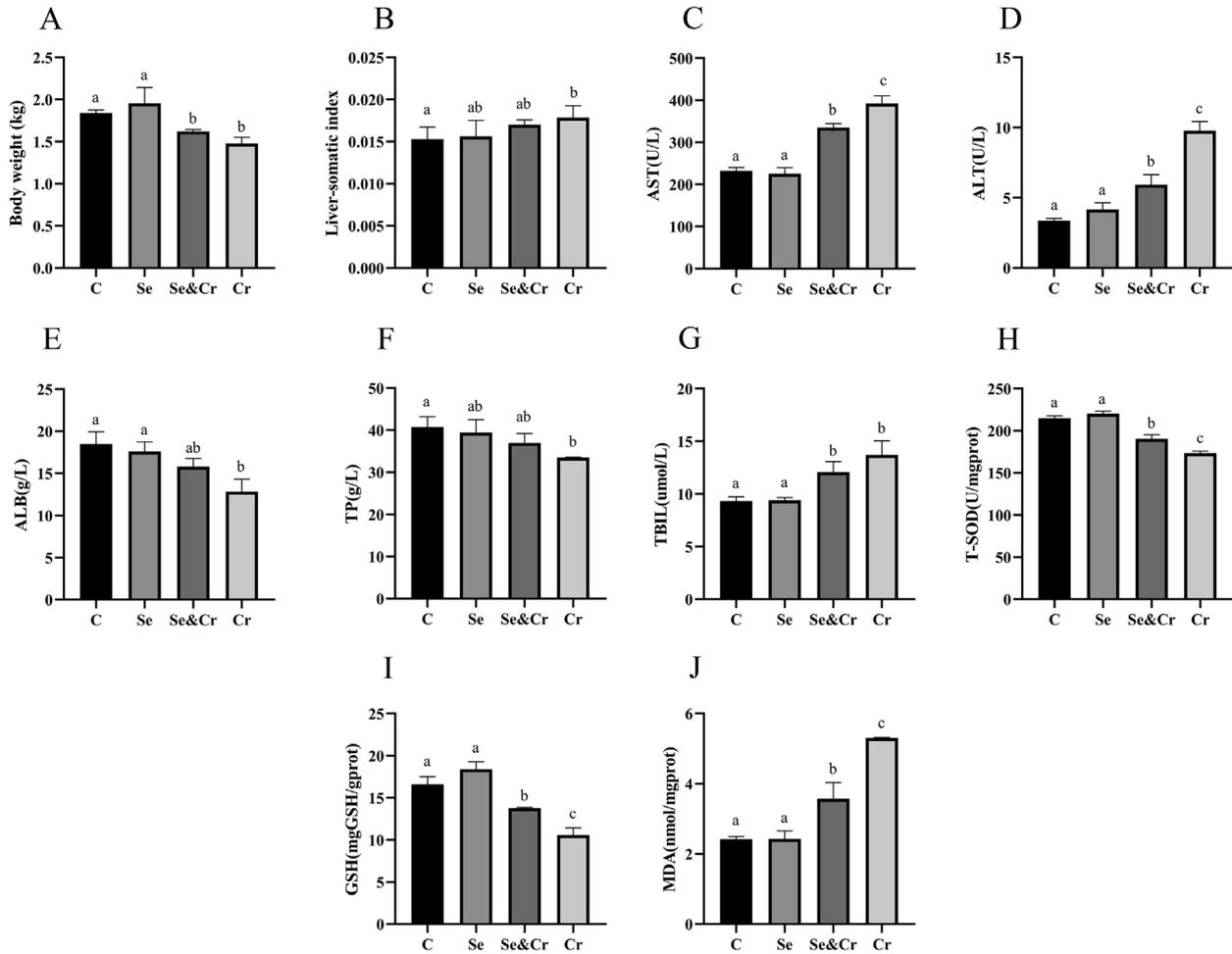


Figure 2. Influences of Cr(VI) and Se on liver injury indicators in broilers. (A) Body weight index, (B) liver-somatic index, (C–G) biochemical indicators of liver function, and (H–J) oxidative stress parameters in broiler tissues. Different lowercase letters in the column diagrams indicate statistical difference ($P < 0.05$).

(Wanleibio) were incubated at 4°C, and the secondary antibodies were incubated the next day. Finally, 3,3'-diaminobenzidine color rendering and neutral resin sealing were performed. All liver immunohistochemical sections were observed and photographed using the Nikon eclipse99 E100 light microscope (Nikon, Tokyo, Japan) (Yin et al., 2019).

Statistical Analysis

GraphPad Prism 8.0.2 and Origin 2018 were applied for analyzing the data in this study. The differences comparison among groups was calculated by one-way analysis of variance. The mean \pm standard deviation was used for all experimental results.

RESULTS

Influences of Cr(VI) and Se on Liver Histopathology

As shown in Figure 1A, no marked histopathological finding was observed from the groups C and Se, and the typical architecture of hepatic cords and their radiation from the central vein were observed in the broiler liver

tissues. However, the derangement of the hepatic cords, congestive dilatation of sinusoids, pyknosis or dissolution of nucleus, cytoplasmic vacuolation, infiltrations of inflammatory cells, and the endothelial hyperplasia of bile duct with newly formed bile duct were observed in liver tissues of animals exposed to Cr. Overall, the characteristic changes of portal triad were vascular wall thickening and fibrinoid degeneration, with inflammatory cells infiltrations around the vessels. Noteworthy, the histopathological injury of liver induced by Cr was less serious after Se treatment, thus indicating that Se may repair the histopathological lesion induced by Cr(VI) exposure.

Influences of Cr(VI) and Se on Liver Ultrastructure

The ultrastructure of liver tissues from 4 groups is shown in Figure 1B. In C and Se groups, the hepatocytes had normal mitochondria with a clear mitochondrial crista, and the nuclear double membranes and evenly distributed chromatin were clearly visible. In contrast, severe destruction of liver cell morphology was observed in the Cr group, with irregular chromatin condensation, nuclear membrane shrinkage, mitochondrial cristae dissolution, vacuolar degeneration, and accumulation of autophagosome.

However, compared with the Cr group, the aforementioned cell structure damage induced by Cr(VI) was attenuated by Se administration, with the number of autophagosomes being markedly reduced in the Se&Cr group.

Influences of Cr(VI) and Se on Body Weight and Liver-Somatic Index in Broilers

The body and liver developments are summarized in Figures 2A and 2B. The body weight of broilers in the Se group was elevated to some extent but not significantly compared to that of the C group, whereas it has an appreciable decline in the Se&Cr and Cr groups. In addition, the liver-somatic index in the Se and Se&Cr groups was similar to that of the C group, but that of the Cr group was increased ($P < 0.05$). After supplementary Se, the rising trend of liver-somatic index induced by Cr(VI) was reduced. The results suggested that Se can enhance the stunted growth of broiler induced by Cr(VI).

Influences of Cr(VI) and Se on Serum Liver Function Indicators

AST and ALT are sensitive indexes reflecting hepatocyte damage, reflecting increased enzymatic activity,

and release of the enzymes to circulation after hepatocyte damage. Furthermore, the synthesis and metabolic ability of liver can be partly reflected by ALB, TP, and TBIL serum levels. Data of the serum indicators of liver function are shown in Figures 2C–2G.

Compared with the C group, the levels of AST, ALT, and TBIL in serum were upregulated, whereas those of ALB and TP were reduced ($P < 0.05$) caused by Cr(VI) exposure. After Se treatment in broilers exposed to Cr(VI), the activities of AST and ALT were significantly decreased. No significant differences were noted between ALB and TP serum levels in the Cr and Se&Cr groups; however, their contents in the Se&Cr group were upregulated to a certain extent as compared with the Cr group. These revealed that the hepatic insufficiency caused by Cr(VI) exposure can be ameliorated by Se administration.

Influences of Cr(VI) and Se on Oxidative Stress Indicators in Broiler Liver

The indicators of oxidative stress in broiler liver tissues are shown in Figures 2H–2J. No significant difference in the activity of T-SOD, as well as GSH and MDA contents existed between C and Se groups.

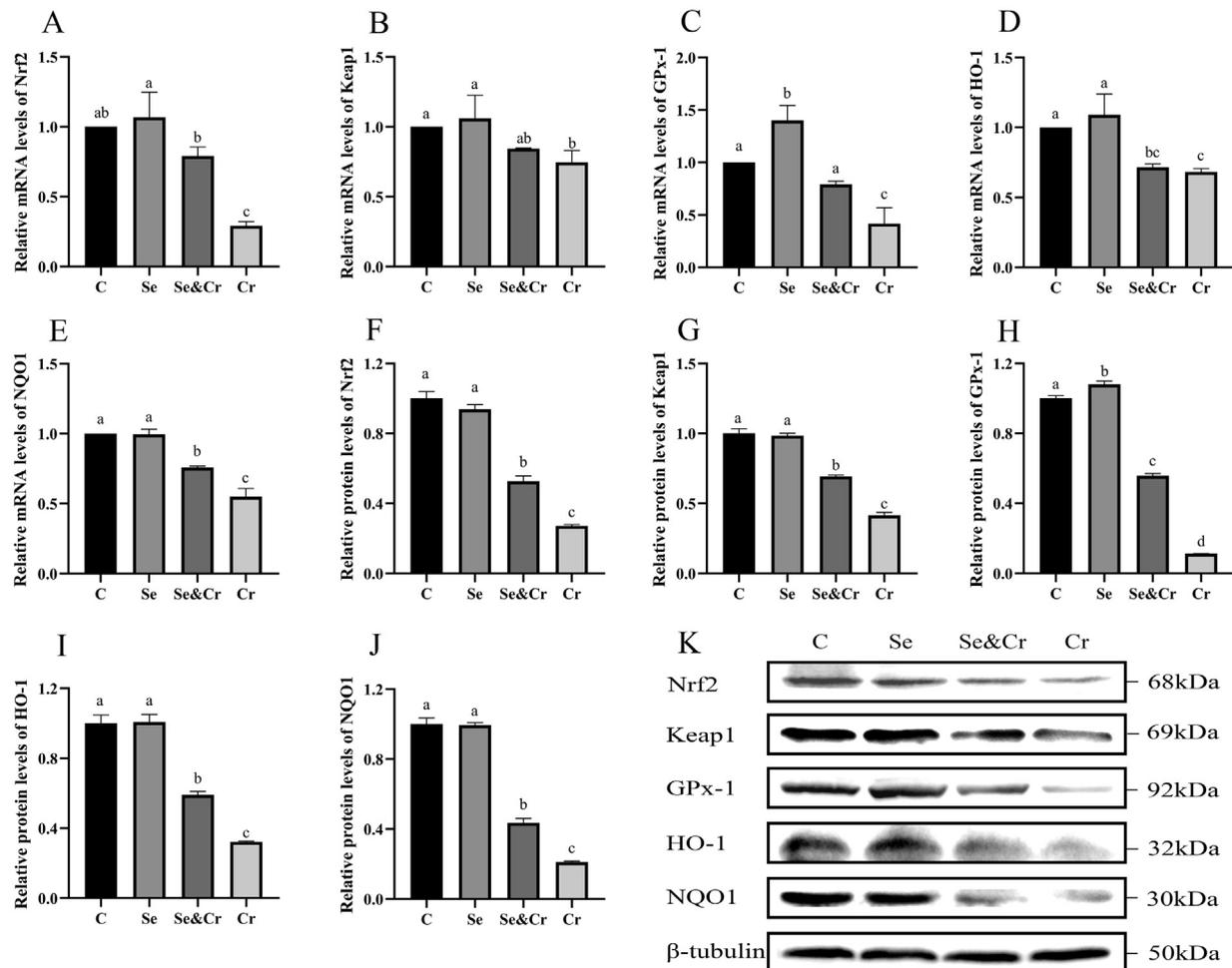


Figure 3. Influences of Cr(VI) and Se on the Nrf2 pathway in broiler liver. (A) Relative mRNA and (B) protein levels of Nrf2 signaling pathway elements. Different lowercase letters in the column diagrams indicate statistical difference ($P < 0.05$).

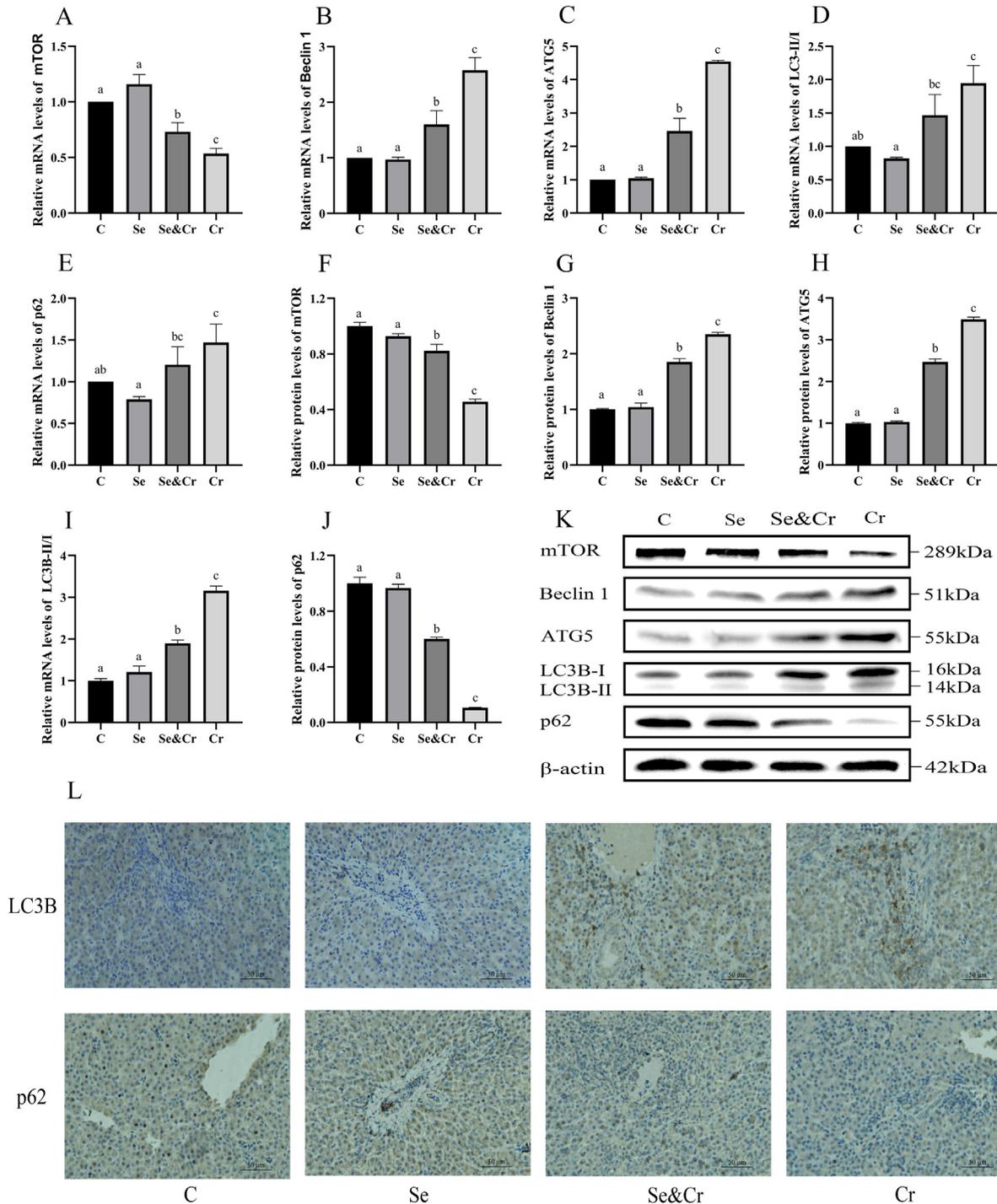


Figure 4. Influences of Cr(VI) and Se on autophagy in broiler liver. (A-E) Relative mRNA and (F-K) protein expression of autophagy-related genes. (L) Immunohistochemical analysis of LC3B and p62 (magnification: 400 \times). Different lowercase letters in the column diagrams indicate statistical difference ($P < 0.05$).

But in comparison with C group, T-SOD activity and GSH content were conspicuously decreased in Se&Cr and Cr groups, and their levels in the Se&Cr group were conspicuously elevated by comparison with Cr group. MDA contents in Se&Cr and Cr groups were conspicuously upregulated by comparison with C group, but its content in Se&Cr group were lower ($P < 0.05$) than that in Cr group. These further indicate that Cr(VI) exposure-induced oxidative liver damage can be alleviated by Se.

Influences of Cr(VI) and Se on the Nrf2 Pathway in Broiler Liver

The results of Nrf2 signaling pathway are shown in Figure 3. Except for GPx-1, no significant differences were detected in the expression of other genes between C and Se groups. Importantly, the mRNA expression of Nrf2, Keap1, GPx-1, HO-1, and NQO1 was conspicuously reduced in Cr-exposed liver tissues compared with control samples. No notable differences in mRNA

expression of Nrf2, Keap1 and GPx-1 were found between C and Se&Cr groups, but HO-1 and NQO1 showed lower expression patterns in Se&Cr group than that control animals. In addition to Keap1 and HO-1, the transcriptional levels of Nrf2, GPx-1 and NQO1 in Se&Cr group were significantly elevated by comparison with Cr group.

Further analysis of the protein levels of Nrf2, Keap1, GPx-1, HO-1, and NQO1 by Western blotting confirmed that, except for GPx-1, no significant difference on Nrf2 signals were found between C and Se groups. Noteworthy, the levels of Nrf2, Keap1, GPx-1, HO-1, and NQO1 in the Se&Cr and Cr groups were conspicuously decreased by comparison with the C group, but all proteins were elevated in the Se&Cr group by comparison with that in Cr-exposed animals. Taken together, these results suggest that Se treatment upregulates the Nrf2 signaling pathway to tackle Cr(VI)-induced oxidative stress.

Influences of Cr(VI) and Se on Autophagy in Broiler Liver

The results of autophagy pathway are exhibited in Figure 4. No notable changes in mRNA expression of mTOR, Beclin1, ATG5, LC3-II/I, and p62 were found between C and Se groups. Importantly, the mRNA expressions of mTOR in Se&Cr and Cr groups were decreased

comparison with that in control samples. Moreover, Beclin1, ATG5, LC3-II/I, and p62 transcription levels were conspicuously upregulated in Cr group by comparison with the control samples. The Beclin1 and ATG5 transcription levels were conspicuously decreased in Se&Cr group compared with Cr-exposed broilers, and the LC3-II/I and p62 transcription levels decreased but have no significant differences in the corresponding groups.

Western blot analysis further confirmed that no notable differences between C and Se groups was noted concerning mTOR, Beclin 1, ATG5, LC3B-II/I, and p62 levels. Nonetheless, mTOR and p62 levels were conspicuously decreased in Se&Cr and Cr groups comparison with control animals, whereas the other autophagy-related proteins showed the opposite trend. In particular, mTOR and p62 levels were elevated in Se&Cr group by comparison with those of the animals exposed to Cr(VI) ($P < 0.05$); however, Beclin 1, ATG5, and LC3B-II/I were downregulated ($P < 0.05$). Immunohistochemical analysis of LC3B and p62 showed consistent results with those of Western blotting. These findings demonstrate that the excessive autophagy induced by Cr(VI) exposure can be reduced by Se administration.

Analysis of Differential Expression

The clustered heatmap illustrates differential gene and protein expression patterns in liver tissues (Figure 5).

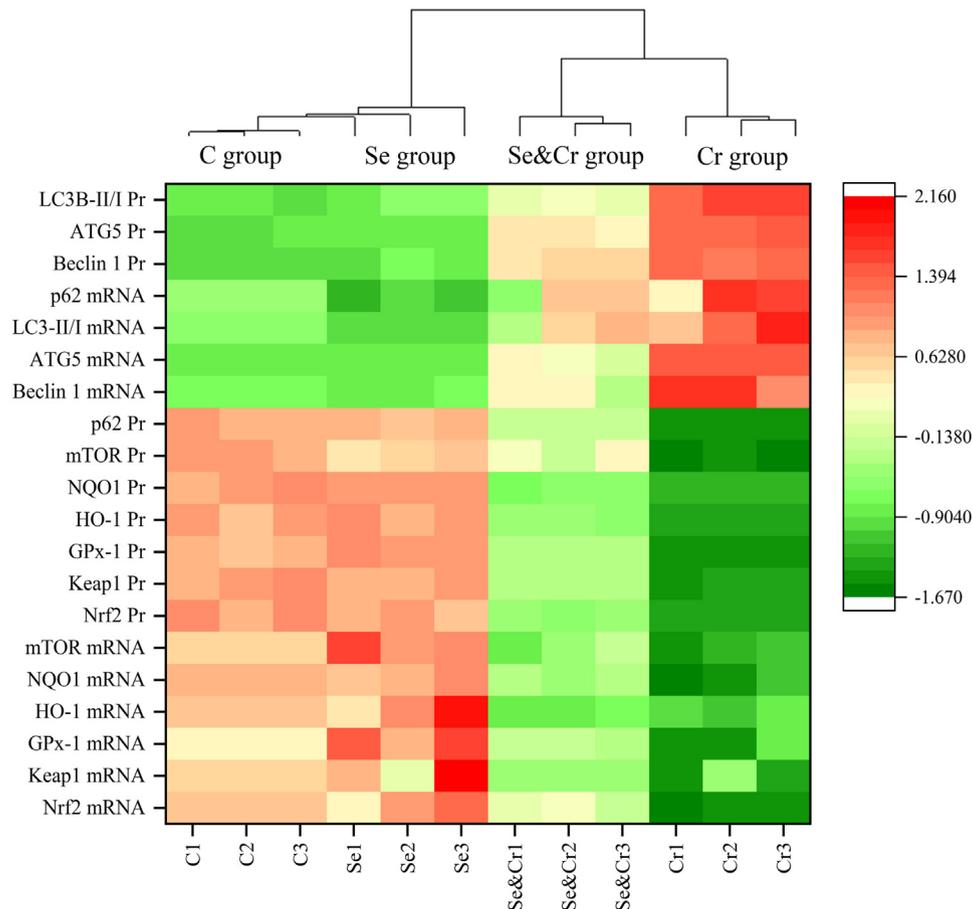


Figure 5. The clustered heatmap of Nrf2 pathway and the autophagy in the broiler livers by Cr(VI) and Se. Red represents high expression, and green represents low expression.

There was little difference in the expression of Nrf2 signaling pathway and autophagy related genes between C and Se groups. The Se&Cr and Cr groups were obviously different from the control and Se treated broilers according to that displayed results of cluster. The transcription and translation levels of Nrf2 related factors and mTOR in liver tissues of broilers in Se&Cr and Cr groups were lower than those in the C group. In comparison with Cr group, the expressions of Nrf2 related factors and mTOR in Se&Cr group were relatively increased. In addition, the expressions of Beclin 1, ATG5 and LC3-II/I were contrary to the above results. It was noteworthy that the expression of p62 was distinct between mRNA and protein expression from the other genes. In comparison with C group, the mRNA expression of p62 in Se&Cr group and Cr group was relatively increased, while the protein expression was relatively decreased. Between the Se&Cr and Cr group, the mRNA expression pattern of p62 was different from protein expression, which might be due to the degradation of p62 by excessive autophagy. The results show that Se mediates Nrf2 signaling pathway to alleviate autophagy induced by Cr(VI) in broiler livers.

DISCUSSION

The liver plays an indispensable metabolite filtration role, protecting the body from heavy metal toxicity (Reinke and Asher, 2016; Davies et al., 2020). However, a large number of heavy metals including Cd (Cong et al., 2019), As (Ren et al., 2021), copper (Sharaf et al., 2021), Pb (Amin et al., 2021), and Cr(VI) (Wang et al., 2017), can accumulate in the liver and destroy its structure and function in several species. Currently, Cr(VI) dissemination into the environment is largely caused by its wide use in different industrial production and processing applications (Balmer, 2018; Jobby et al., 2018). Once released into the environment, Cr(VI) tends to accumulate in the liver of different animals of the food chain with potentially noxious effects to the health of the animals, consequently causing significant economic losses and representing a serious public health problem. Recent studies have shown that Cr(VI) exposure could induce histopathological and ultrastructural injury, and oxidative damage in the liver tissues (Boşgelmez and Güvendik, 2017; Yang et al., 2020) and cells (Zhong et al., 2017), as well as liver dysfunction (Andleeb et al., 2020; Mohamed et al., 2020). In agreement with these findings, our results showed vacuolation of the cytoplasm and infiltration of inflammatory cells in the liver of broilers orally fed $K_2Cr_2O_7$. In addition, Cr exposure significantly promoted oxidative imbalance in chickens, as shown by decreased activity of T-SOD and contents of GSH, ALB, and TP, as well as significant increased the levels of MDA, AST, ALT and TBIL. Hence, these results confirm the oxidative damage, structural injury and dysfunction induced by Cr(VI) on broiler livers.

Nrf2, is a vital redox transcription factor, can be dissociated from Keap1 by moderate oxidative stress, and

enter into the nucleus, then induce downstream antioxidant enzymes and detoxification enzymes to protect cells from oxidative damage. (Andleeb et al., 2020). However, the continuously intensive oxidative stress can inhibit the activation of Nrf2 pathway (Xue et al., 2021). There were studies have demonstrated that Cr(VI) exposure could lead the downregulation of mRNA and protein expressions of Nrf2, NQO1, and HO-1 in a dose dependent manner (Yang et al., 2021). However, antioxidants enhanced Nrf2 mediated antioxidant defense system to prevent Cr(VI)-induced cardiotoxicity, pulmonary toxicity, and nephrotoxicity in rats (Lv et al., 2020; Awoyomi et al., 2021). Se, as a powerful antioxidant, has been shown to reduce the toxic damage caused by As or Cd by activating the Nrf2 pathway (Zwolak, 2020). The current study demonstrated that Se administration could significantly upregulate Nrf2 levels and of its downstream antioxidant enzymes, counteracting the Cr(VI)-mediated oxidative damage. Further, oxidative damage could destroy the histopathological structure and cell ultrastructure as well as lead to organ dysfunction (Yin et al., 2020; Li et al., 2021). In this experiment, Se supplementation was also found to reduce the histo- and cytopathological structure damages, repaire hepatic insufficiency and improve liver development through improving the antioxidant capacity in broilers exposed to Cr(VI). Overall, Se may decrease the oxidative damage by activating the Nrf2 signaling pathway, to alleviate Cr(VI)-induced hepatotoxic injury in broilers.

Different heavy metals have distinct effects on autophagy of diverse cellular entities. However, it is still unclear whether autophagy is an important factor of cytoprotection or cytotoxicity. A study showed that Cd could damage the structure and function of lysosomes, thereby blocking the autophagic flux to reduce autophagy in mouse neural crest-derived cells (Pi et al., 2017). In turn, Cr(VI) was reported to induce excessive autophagy activation in mitochondria of hepatocytes, impairing their physiological activities (Zhang et al., 2020c). p62, as a selective autophagy substrate protein, is a critical factor regulating the step of substrate clearance, colocalizing with LC3B to enhance the autophagic flux (Lamark et al., 2017), and being ultimately degraded in the middle and late stages of autophagy. LC3B is recognized as an autophagy marker protein, with the LC3B-II/I ratio can estimate the level of autophagy (Zhang et al., 2019). In addition, the recruitment of autophagy-associated proteins and Beclin 1, and the inhibition of mTOR, can induce autophagosome formation (Lu et al., 2018). Herein, Cr(VI) exposure was shown to induce a notable increase in LC3B-II/I, Beclin 1, and ATG5 in broiler livers, and a significant decrease in p62 and mTOR, which suggests that Cr(VI) can activate autophagy through oxidative stress-induced p62 phosphorylation. Therefore, excessive autophagy is a toxic damage response in broiler liver tissues induced by Cr(VI) exposure.

Given its heavy metal antagonist proprieties, whether Se can alleviate the Cr(VI) toxicity by reducing autophagy. In agreement with this hypothesis, Wang et al. showed that Se could exert its cytoprotective effects by

reversing the Cd-promoted impairments on energy metabolism and, thereby, inhibiting Cd-induced autophagy of chicken ovarian cells (Wang et al., 2018). Rahman et al. also suggested that Se could upregulate mTOR levels owing its antioxidant potential and by restraining the accumulation of As in pheochromocytoma PC12 cells, thereby inhibiting autophagy (Rahman et al., 2018). Furthermore, Se was used to reduce inflammation, heat shock reaction and autophagy caused by oxidative stress, to relieved Pb toxicity in cocks testis (Wang et al., 2021). The present findings showed that Se administration significantly increased the translation levels of Beclin1, ATG5, and LC3 in the liver of Cr-exposed broilers, whereas mTOR and p62 levels showed the opposite trend, thereby confirming that Se can alleviate excessive autophagy induced by Cr(VI).

In conclusion, Cr(VI) exposure causes obvious structural damage, developmental hindrance and dysfunction of the liver of chickens, which are (at least in part) induced by oxidative stress-mediated excessive autophagy. This Cr(VI)-induced hepatotoxicity can be counteracted by SeY administration, which enhances Nrf2 signals and its downstream antioxidant effects. Taken together, these finding can pave the way for the use of Se for the prevention and treatment of Cr(VI) poisoning; nonetheless, further cellular and molecular studies are still warranted.

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DISCLOSURES

The authors declared that there is no conflict of interest to this work.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.102335.

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