Nano-selenium alleviates cadmium-induced cerebellar injury by activating metal regulatory transcription factor 1 mediated metal response

Shao-Shuai Bi a, b, 1, Milton Talukder a, c, 1, Hai-Tao Jin d, Mei-Wei Lv a, Jing Ge a, Cong Zhang a, e, Jin-Long Li a, f, g, *  

a College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, China  
b College of Biotechnology and Pharmaceutical Engineering of West Anhui University, Lu’an 237012, China  
c Department of Physiology and Pharmacology, Faculty of Animal Science and Veterinary Medicine, Patuakhali Science and Technology University, Barishal, 8210, Bangladesh  
d Quality and Safety Institute of Agricultural Products, Heilongjiang Academy of Agricultural Sciences, Harbin 150010, China  
e College of Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, China  
g Quality and Safety Institute of Agricultural Products, Heilongjiang Academy of Agricultural Sciences, Harbin 150010, China  

* Corresponding author. College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, China.  
E-mail address: jinlongli@neau.edu.cn (J.-L. Li).  
1 These authors contributed equally to this study.  

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ABSTRACT  
This study aims to investigate the role of metal regulatory transcription factor 1 (MTF1)-mediated metal response in cadmium (Cd)-induced cerebellar injury, and to evaluate the antagonistic effects of nano-selenium (Nano-Se) against Cd toxicity. A total of 80 chicks (1 d old, male, Hy-Line Variety White) were randomly allocated to 4 treatment groups for 3 months: the control group (fed with a basic diet, n = 20), the Nano-Se group (basic diet with 1 mg/kg nano-Se and 140 mg/kg CdCl2, n = 20), the Nano-Se + Cd group (basic diet with 1 mg/kg Nano-Se and 140 mg/kg CdCl2, n = 20) and the Cd group (basic diet with 140 mg/kg CdCl2, n = 20). The results of the experiment showed that the Purkinje cells were significantly decreased with their degradation and indistinct nucleoli after Cd exposure. Moreover, exposure to Cd caused a significant accumulation of Cd and copper. However, the contents of Se, iron, and zinc were decreased, thereby disturbing the metal homeostasis in the cerebellum. The Cd exposure also resulted in high levels of malondialdehyde (MDA) and down regulation of selenoprotein transcriptome. Furthermore, the expressions of MTF1, metallothionein 1 (MT1), MT2, zinc transporter 3 (ZNT3), ZNT5, ZNT10, zrt, irt-like protein 8 (ZIP8), ZIP10, transferrin (TF), ferroportin 1 (FPN1), ATPase copper transporting beta (ATP7B), and copper uptake protein 1 (CTR1) were inhibited by Cd exposure. However, all these changes were significantly alleviated by the supplementation of Nano-Se. This study proved that Cd could disorder metal homeostasis and induce oxidative stress, whereas Nano-Se could relieve all these negative effects caused by Cd via activating the MTF1-mediated metal response in the cerebellum of chicken.  
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1. Introduction

Cadmium (Cd) is a contaminant to the environment where it ubiquitously exists. In recent decades, pollution caused by Cd has become an acute problem in the agricultural environment. In human bodies, Cd has a half-life of 10 to 30 years and may lead to serious health complications (Maddela et al., 2020). Moreover, the anthropogenic release of Cd into the environment estimated to nearly 30,000 tonnes every year (Forcella et al., 2020; Guo et al., 2020). The agency for toxic substances and disease registry (ATSDR) ranked Cd in the seventh position for most hazardous
materials (Andrade et al., 2017; Ge et al., 2022a, 2022b). Mining activities release Cd into the environment and hence contaminate drinking water and soil. It has been reported that people living near the Panaasqueira mine in Portugal are found to have higher levels of Cd in their bodies when compared to those living in the regulated regions, which suggests that those with higher environmental and occupational exposures to Cd, for example, their engagement in mining activities, face a higher potential risk of absorbing Cd than others (Coelho et al., 2012). Moreover, people who engage in non-occupational activities, such as drinking water, eating food, using cosmetics, breathing air particles, and smoking cigarettes, are also reported to have been exposed to Cd (Dai et al., 2021a, 2021b). It is worth noting that Cd could easily bioaccumulate in the brain of a pilot whale through its blood—brain barrier (BBB) (Gajdosechová et al., 2016). As for the brain of zebrafish, its exposure to Cd would induce cerebral hemorrhage, increase BBB permeability, and promote abnormal vascular formation by promoting production of vascular endothelial growth factor (VEGF) (Zhao et al., 2022c). It has been demonstrated that Cd-dependent alteration of BBB contributed to the pathogenesis of neurodegeneration (Branca et al., 2020). Altogether, these evidences suggest that the central nervous system (CNS) is a critical position at which Cd-toxicity targets. However, the mechanisms underlying Cd-induced neurotoxicity in the cerebellum are yet to be further investigated.

Selenium (Se) is an element with strong antioxidant properties which makes it essential to animals (Zhang et al., 2020a, 2022). It has been noted that low doses of Se possess antioxidant properties which could protect animals from contracting multiform diseases (Zwolak, 2020). It can alleviate injuries to cells via the reactive oxygen species (ROS) scavenging system (Ge et al., 2022a, 2022b). Furthermore, Se is a component of co-enzyme, which is an antioxidant (Jiang et al., 2021; Li et al., 2021). Notably, deficiency of Se is associated with diseases and necroptosis in the nervous system (Cui et al., 2022; Li et al., 2021). A previous study demonstrated the fact that Se could play a protective role against cypermethrin-induced neurotoxicity in rats’ brains (Ali et al., 2020). However, traditional Se supplements (mainly provided through sodium selenite) remain a concern to the supplement takers due to their high toxicity and low bioavailability (Zhang et al., 2020d). For this reason, nano-selenium (Nano-Se) has emerged as a promising agent which is therapeutic and can treat a number of diseases. Nonetheless, the protective effect that Nano-Se has against Cd-induced neurotoxicity in the cerebellum is subjected to further study.

In recent years, nanoparticles have become a popular subject of research due to their puny sizes, fascinating shapes, and unique and intrinsic physicochemical properties. The biosynthesis method has become a new direction and hotspot for synthesizing Nano-Se. Specifically, the biosynthesis method refers to the use of redox systems of bacteria to reduce high-valent Se to low-valent Se, hence forming stable and uniformly arranged Nano-Se particles under actions of intracellular or extracellular proteins. Indeed, Nano-Se is more soluble and bioavailable than its organic or non-organic forms (Ge et al., 2022a, 2022b). Nano-Se is a zero-valent Se, yet it differs from zero-valent elemental Se in the traditional sense. The traditional element of Se is a gray or black solid with metallic luster, which engages in few biological activities (Jia et al., 2005). Nano-Se, however, is an elemental Se with red color and zero valence which reaches the Nano-level with excellent nano-related characteristics. Compared with other forms of Se, Nano-Se displays the biological characteristics of being mildly toxic and highly efficient (Kumar and Prasad, 2021). The Nano-Se particles uniformly distributed and small in volume, whereas their surface area is large, the characteristics of which not only improve the adhesion of the gastrointestinal mucosa, but also make themselves easily absorbable by an animal’s intestine. Enzymes and other enzymatic-active centers enable enzymes to conduct more of activities, which is the main reason why their biological characteristics are so apparent. In a nutshell, Nano-Se is an important particle due to its abundant bioavailability and high digestive efficiency (Rana, 2021). Nonetheless, the protective effect that Nano-Se has towards Cd-induced neurotoxicity in the cerebellum is subject to further examination.

Metal regulatory transcription factor 1 (MTRF1) participates in redox homeostasis and heavy metal detoxification via regulating the expression of metal-responsive genes (Talukder et al., 2021). It is not only modulated metallothionein (MT) but also regulates other metal transporters involved in iron (Fe), copper (Cu), and zinc (Zn) homeostasis (Hardyman et al., 2016; Zhao et al., 2021c; Zhang et al., 2020c). It is worth noting that MTs have neuroprotective effects (Forcella et al., 2020; Guo et al., 2020). Moreover, Zn, Fe, and Cu are elements that are essential to some enzymes in the development of central nervous system (Qian et al., 2012; Zhao et al., 2021c; Stalke et al., 2020). Notably, MTRF1-mediated metal response plays an important role in relieving oxidative stresses and making cellular responses to homeostasis in metals (Guntert et al., 2012; Park and Jeong, 2018). However, the role of MTRF1-mediated metal response to Cd-induced cerebellar lesions and the effect that Nano-Se has to words it are subject to further examination.

As stated above, while several studies have already been done to investigate the role that Se plays in alleviating Cd-elicited damage to the neural system, the role that MTRF1 plays with respect to the mechanism that underlies Nano-Se’s mitigation of Cd-induced injuries to the cerebellum is not yet clear. To prove that this hypothesis is valid, this study has been designed to examine the role that Nano-Se could play in combating Cd-induced neurotoxicity. Specifically, histopathological examinations and the detection of the contents of metals, oxidative stress indexes, and the changes over MTRF1-mediated metal responses were performed in this study.

2. Materials and methods

2.1. Animal treatment and ethics

In the study, Nano-Se (purity ≥99%, size averages 100 nm) was obtained from Omega Biotech Co., Ltd, Shanghai, China. CdCl2 (purity ≥98%, an analytical reagent) was obtained from Tianjin Zhiyuan Chemical Reagent Co., Ltd. Chicks that were male, of Hy-Line Variety, and White (1 d old, n = 80) were separately grouped into four: the control group (fed with a basic diet, n = 20), the Nano-Se group (basic diet with 1 mg/kg Nano-Se, n = 20), the Nano-Se + Cd group (basic diet with 1 mg/kg Nano-Se and 140 mg/kg CdCl2, n = 20) and Cd group (basic diet with 140 mg/kg CdCl2, n = 20). The median acute lethal dose of Cd to chickens through oral feeding is 218.44 mg/kg. Thus, this study chose the dose to be 140 mg/kg (3/5 LD50). The choice of the doses for Nano-Se and Cd followed Ge and Bi et al. studies (Bi et al., 2021; Ge et al., 2021a). In this study, we set up a subchronic Cd-exposure model, and chose 3 months as the time period for Cd-exposure. After 3 months, chicks were sacrificed. Then, cerebellum from each chicken was taken out, collected, and stored at −80 °C for study during the subsequent experiments. All procedures relating to the treatment of animals were performed in strict accordance with the Guidelines for Care and Use of Laboratory Animals of Northeast Agricultural University, and all experiments had been approved of by the Animal Ethics Committee of Northeast Agricultural University (NEAUEC20200346).

2.2. Histopathology assessment

The process of this assessment can be stated briefly as follows: firstly, the cerebellar tissue was immersed in a 4% fixative paraformaldehyde solution to generate paraffin sections; second, the
nuclei were stained with hematoxylin, the cytoplasm was stained with eosin, and the sections were stained in an eosin-staining solution for one to 3 min, and thereafter dehydrated and mounted; finally, the specimens of the cerebellum were visualized through the use of Aperio Image Scope v12.0.1 (Zhao et al., 2020b, 2021a).

2.3. Detection of trace elements by inductively coupled plasma-mass spectroscopy (ICP-MS)

The contents of Cd, Cu, Zn, Fe, and Se in the cerebellum were determined in accordance with Jin et al. (2018), and the method of inductively coupling with plasma spectroscopy (ICP-MS) was used for this process of determination. Weigh accurately 1 g of cerebellar tissue, add 5 mL of nitric acid (with 65% concentration) and then 2 mL of hydrogen peroxide (with 30% concentration), and place it into a microwave-digestion apparatus for digestion, which was programmed as follows: 120 °C, 3 min; 190 °C, 40 min. After the temperature dropped to below 60 °C, drive off the acid, adjust the volume to 50 mL using deionized water, and mix the solution to be measured later. The elements detected by the indicators were Cd, Cu, Zn, Fe, and Se.

2.4. Detection of oxidative stress

The malondialdehyde (MDA), total antioxidant capacity (T-AOC), catalase (CAT), and glutathione peroxidase (GPx) assays were carried out through use of assay kits (Nanjing Jiancheng Biotechnology Institute, P.R. China) in accordance with the specifications set out by the manufacturer (Zhao et al., 2020a, 2021b).

2.5. RNA extraction and qRT-PCR analysis

The method of extracting total RNA from each group was administered in accordance with the manufacturer’s specifications (RNA-out reagent: Beijing TransGen Biotech, China). The specific oligonucleotide primers are shown in Table S1. The qRT-PCR was performed through use of the LineGene 9600 qRT-PCR detection system (Bioper Technology Co., Ltd). The transcript level was analyzed through use of the 2^(-ΔΔCt) method (Zhang et al., 2019; Zhao et al., 2021a, Zhao et al., 2022a).

2.6. Protein extraction and Western blot analysis

Proteins were extracted from tissues of cerebellum and then quantified through use of commercially available kits (Beyotime Institute of Biotechnology, P.R. China). Detailed methods of treatment were adopted as specified in Xue et al. (Zhao et al., 2020c; Talukder et al., 2021). The MTFL (1:700) antibody was purchased from Nan-Jing AnYan Biotechnology Co., Ltd; the Sep (O-phosphoseryl-tRNA (Sec) kinase (PSTK), and selenoprotein including SECIS-binding protein 2 (SBP2), seryl-tRNA synthetase (SARS), SepSecS, selenophosphate synthetase (SPS), ophosphoseryl-tRNA (Sec) kinase (PSTK), and selenocysteine tRNA 1 associated protein (SepC3-1) were significantly enhanced through treatment of Nano-Se (Fig. 3A–F) (P < 0.01 or P < 0.001). Moreover, Cd significantly inhibited the mRNA levels of SBP2, SARS, SepSecS, PSTK, and SepC3-1 when compared to the control group (Fig. 3A–C, E, and F) (P < 0.01 or P < 0.001). Moreover, the mRNA levels of SPS were inhibited by Cd, but not significantly. However, in the Cd + Nano-Se group, these

3. Results

3.1. Effect of Cd and Nano-Se on histopathological alterations

As shown in Fig. 1, the histopathological results showed that nerve cells had normal structures in the control and Nano-Se group (Fig. 1A). However, Cd-exposure caused Purkinje’s cells to degrade with karyopyknosis and indistinct nuclei. In addition, the number of Purkinje cells decreased significantly (Fig. 1B) (P < 0.001). However, in the Cd + Nano-Se groups, Purkinje cells increased dramatically, and neuronal injuries partially recovered in the Cd + Nano-Se groups when compared with the Cd groups in Fig. 1A and B (P < 0.05). Moreover, the PCA showed that Nano-Se had an antagonistic effect towards protecting the Cd-induced neuronal injuries in the cerebellum (Fig. 1C). These results show that Nano-Se can alleviate Cd-induced cerebellar injury in chickens.

3.2. Effect of Cd and Nano-Se on concentrations of trace elements

To determine whether Cd would disturb metal homeostasis, concentrations of 5 trace elements were detected by ICP-MS. The result showed that Cdexposure caused Cdaccumulation in the cerebellum. Moreover, Cd indeed disturbed metal homeostasis in the cerebellum, in which contents of Se, Zn, and Fe decreased significantly in Fig. 2B, C, and E (P < 0.05, P < 0.01), whereas the contents of Cd and Cu increased significantly in Fig. 2A and F (P < 0.001). In addition, in Cd + Nano-Se group, contents of Se, Zn, and Fe increased significantly in Fig. 2B, C, and E (P < 0.01, P < 0.001) when compared to the Cd group. These data suggested that Nano-Se had an antagonistic effect towards Cd-accumulation and inhibited Cd-induced metal dyshomeostasis. In addition, the correlation analysis showed that there were 5 trace elements in the cerebellum, which suggested that Cd had a negative correlation with Fe, Zn, and Se, but a positive correlation with Cu (Fig. 2D). The heat map showed the 5 trace elements in the cerebellum (Fig. 2K).

Furthermore, to investigate Cd-induced oxidative stress in the cerebellum, we detected oxidative-stress-related indexes. As shown in Fig. 2, the T-AOC level and the activities of anti-oxidative enzymes (CAT and GSH-Px) decreased significantly in the Cd-group in Fig. 2H–J (P < 0.001) returned nearly to normal levels in the Cd + Nano-Se group. The content of MDA was higher in the Cd group than in the control and Nano-Se groups (Fig. 2G) (P < 0.01), which returned nearly to normal levels in the Cd + Nano-Se group. These data showed that Nano-Se had a strong, powerful, antioxidant, and anti-stress ability.

3.3. Effect of Cd and Nano-Se on factors of biosynthesis of selenoprotein

As shown in Fig. 3, the results showed that the 6 factors of biosynthesis of selenoprotein including SECIS-binding protein 2 (SBP2), seryl-tRNA synthetase (SARS), SepSecS, selenophosphate synthetase (SPS), ophosphoseryl-tRNA (Sec) kinase (PSTK), and selenocysteine tRNA 1 associated protein (SepC3-1) were significantly enhanced through treatment of Nano-Se (Fig. 3A–F) (P < 0.01 or P < 0.001). Moreover, Cd significantly inhibited the mRNA levels of SBP2, SARS, SepSecS, PSTK, and SepC3-1 when compared to the control group (Fig. 3A–C, E, and F) (P < 0.01 or P < 0.001). Moreover, the mRNA levels of SPS were inhibited by Cd, but not significantly. However, in the Cd + Nano-Se group, these
Fig. 1. Effect of cadmium (Cd) and nano-selenium (Nano-Se on the cerebellum in terms of histopathological changes undergone. (A) The histopathological morphology in Con, Nano-Se, Nano-Se + Cd, and Cd groups (H&E, 200×, 400×). (B) The number of Purkinje cells in Con, Nano-Se, Nano-Se + Cd, and Cd groups. (C) The principal component analysis (PCA) of Purkinje cells in Con, Nano-Se, Nano-Se + Cd, and Cd groups. */# indicates statistically significant differences when compared to the Con/Cd group for */# P < 0.05, **/## P < 0.01 and ###/### P < 0.001.

Fig. 2. The analysis of 5 trace elements in chicken’s cerebellum under exposure to cadmium (Cd). (A) The content of cadmium in chicken’s cerebellum. (B) The content of selenium in chicken’s cerebellum. (C) The content of zinc in chicken’s cerebellum. (D) Correlation analysis among Cd, copper, iron, zinc, and selenium. (E) The content of iron in chicken’s cerebellum. (F) The content of copper in chicken’s cerebellum. (G) The content of malondialdehyde (MDA) in chicken’s cerebellum. (H) The level of total antioxidant capacity (T-AOC) in chicken’s cerebellum. (I) The activities of catalase (CAT) in chicken’s cerebellum. (J) The activities of glutathione peroxidase (GSH-Px) in chicken’s cerebellum. (K) Heat map of cadmium, copper, zinc, selenium, and iron in chicken’s cerebellum. From –1 (red) to +1 (green) relative to the values for chicken’s cerebellum in the Con group. The color scale represents the relative mRNA levels, with green indicating up-regulated genes, red indicating down-regulated genes, and black indicating unchanged genes. From –1 (brown) to +1 (blue) relative to correlation analysis for 5 trace elements in chicken’s cerebellum, with brown indicating negative correlations, blue indicating positive correlations, and white indicating no correlations. Values are expressed as mean ± standard deviation (SD). */# indicates statistically significant differences when compared to the Con/Cd group for */# P < 0.05, **/## P < 0.01 and ###/### P < 0.001.
changes became more apparent when compared to the Cd group (Fig. 3A–F) (P < 0.001). These results showed that Nano-Se could alleviate the inhibition of biosynthesis of selenoproteins which was caused by Cd.

3.4. Effect of Cd and Nano-Se on selenoproteins transcriptome

As shown in Fig. 4, the results showed that Cd significantly inhibited selenoprotein transcriptome, including thioredoxin reductase 1 (Txnrd1), Txnrd2, Txnrd3, GPx1, GPx2, GPx3, GPx4, iodothyronine deiodinase 1 (Dio1), Dio2, Dio3, selenoprotein S (SelS), Sel15, SelI, SelD, SelH, SelK, SelT, Sepp1, Sepp2, Sehw, Selm, Seln, Selr, Selt (Fig. 4A) (P < 0.05, P < 0.01 or P < 0.001). However, the supplement of Nano-Se significantly activated the selenoprotein transcriptome (especially for Dio1, Dio3, GPx4, Seh, Sepp1, Selw) when compared with the Cd group (Fig. 4A) (P < 0.05, P < 0.01, P < 0.001). The heat map showed 24 selenoprotein transcriptomes in the cerebellum (Fig. 4B). Moreover, it was also shown that the protein levels of SepSecS and Gpx4 were consistent with those recorded on the transcript (Fig. 4C and D). These results suggested that Nano-Se could alleviate the inhibition of transcriptome of selenoproteins which was caused by Cd.

3.5. Effect of Cd and Nano-Se on expressions of MTF1, MT1, MT2, and DMT1

As shown in Fig. 5, the results showed that MTF1, MT1, MT2, and divalent metal ion transporter 1 (DMT1) were inhibited dramatically under their exposure to Cd in Fig. 5A–C, and E (P < 0.01, P < 0.001). By contrast, simultaneous treatment with Nano-Se significantly activated expressions of MTF1, MT1, MT2, and DMT1 when compared to the Cd-group in Fig. 5A–C, and E (P < 0.001). Similarly, the protein level of MTF1 was detected also, which was consistent with the level of MTF1 (Fig. 5D) as recorded on the transcript. These results suggested that Nano-Se could alleviate the inhibition of expressions of MTF1, MT1, MT2, and DMT1 which was caused by Cd.

3.6. Effect of Cd and Nano-Se on Zn, Fe, and Cu-related transporter expressions

As shown in Fig. 6A, the transcripts level of zinc transporter 3 (ZNT3), ZNT5, ZNT10, zrt, irt-like protein 8 (ZIP8), ZIP10, transferrin (TF), ferroportin 1 (FPN1), ATPase copper transporting beta (ATP7B), and copper uptake protein 1 (CTR1) were inhibited significantly in the Cd group (Fig. 6A) (P < 0.05, P < 0.01, P < 0.001), whereas the simultaneous treatment with Nano-Se activated the Zn, Fe, and Cu-related transporters noticeably when compared with the Cd group (Fig. 6A) (P < 0.01, P < 0.001).

Furthermore, the correlation analysis showed that there were certain correlations among MTF1, Zn, Fe, and Cu-related transporters in the cerebellum. Specifically, Cd had a negative correlation with ZNT3 and ZNT5, and a positive correlation with MT1, MT1, DMT1, ZNT10, ZIP8, ZIP10, TF, FPN1, and ATP7B (Fig. 6B). In addition, the heat map showed that MTF1-mediated genes responded to related genes in the cerebellum (Fig. 6C). These results suggested that Nano-Se could alleviate the inhibition of Zn, Fe, and Cu-related transporters caused by Cd in the cerebellum.

4. Discussion

Cd is a hazardous element, which is toxic to humans and animals (Zhang et al., 2020a, 2020c), both environmental and occupational exposures to Cd are related to neurotoxicity. Exposure of CNS to Cd could induce the destruction of neurons synaptic branches (Branca et al., 2020). Se is an element that is essential to animals; it is well known for its antagonistic interaction against toxicity which is caused by Cd. It can protect animals from undergoing oxidative stress (Rodriguez-Moro et al., 2020). It is worth noting that excessive and chronic exposure to Cd could cause neurotoxicity and depletion of Se. Se-deficiency in poultry is characterized by the poultry’s weight loss and infection with diseases. However, chelating agents consisting of heavy metals such as Se and vitamin E could protect the brain and nervous system of humans and animals (Talukder et al., 2021). However, traditional Se
exists in the compound form of sodium selenite, which is poorly absorbed by humans or animals. It has a low bioavailability and a high excretion rate. Nano-Se is prepared since its active components have stability, liberation profile, sieving method, and emulsion-droplet coalescence. It has been reported that the size of Nano-Se ≤ 100 nm can penetrate the endothelium of various tissues and organs (Rana, 2021). Nano-Se has been regarded as a potential supplement for poultry due to its high catalytic efficiency and antioxidative capacity (Bi et al., 2021). It is highly absorbable by animals, Nano-Se is more permeable through capillary walls and less toxic than Sodium Selenite (Ge et al., 2019). It has primarily been used to effectively improve the feed-conversion ratio, promote chicken growth, combat Cd toxicity, and reduce mortality of poultry (Zhang et al., 2020b; Ge et al., 2021a). The range between nutritional and toxic doses of Nano-Se is significantly greater than that of sodium selenite (El-Deep et al., 2020; Ge et al., 2022a, 2022b), making it safer to take by poultry. Nano-Se has a higher bioavailability and a lower toxicity when compared to other forms of Se (Gulyas et al., 2017). In this study, the content of Cd in the basic diets within the specified range that is no more than 0.1 mg/kg, and the content of Se in the basic diets within rational range. We used Nano-Se ≤ 100 nm which has a higher bioavailability indeed. Nano-Se alleviates the clinical symptoms of Cd-induced chickens, such as rhythmic tremors of the head and tail, turning in circles, turning the head to one side, etc.

Recent studies show that Nano-Se is an effective antioxidant with strong bioavailability properties in place to prevent oxidative injuries (Han et al., 2021). Notably, it has been confirmed that Nano-Se has protective effects on Cd-poisoning in chickens (Zhang et al., 2020c). Dietary supplement with Nano-Se alleviates Cd-toxicity and minimizes Cd-induced health risk in chickens (Ge et al., 2021b). However, there have been few studies thus far on Cd-toxicity in the cerebellum of chicken, and its molecular mechanism is not yet clear. This study investigated into the mechanism of Cd-induced injury to cerebellum and possible protective measures amid such injuries. The results of this study showed that Nano-Se alleviated Cd-caused clinical manifestations, including staggering gait, swinging left and right, and more. Moreover, Cd could cause Purkinje cells to degrade and decrease in number, inducing cerebellar injury. This study examined the role that MTF1-mediated metal response played through comparing the expressions of related proteins and genes in the cerebellum of chicken. From this examination, we explored the protective effects that Nano-Se had towards the Cd-induced injuries to cerebellum. The result of this examination also demonstrated the fact that Nano-Se could against Cd-caused metal dyshomeostasis by regulating MTF1-mediated metal responses.

The cerebellum plays an essential role in executing behavioral functions (Sereno et al., 2020). One of the reports observed that after 28 d of exposure to Cd, motor function and structural integrity were lost, and oxidative balance was disrupted in the cerebellum of rats (P et al., 2019). What is noteworthy in that report is that Cd exerted a detrimental effect onto the CNS, the process of which was related to the production of ROS (Branca et al., 2020). Additionally, the number of endothelial barrier antigens decreased under their acute exposure to Cd in the cerebellum of rats, which proved that Cd carried neurotoxicity (Ibiwoye et al., 2019). It is interesting to note that Nano-Se had many benefits such as improved...
absorbability, higher bioavailability, and engagement into antimicrobial activities (Surai et al., 2017; Surai and Kochish, 2020). Meanwhile, it had powerful functions of promoting poultry growth, improving their feed-conversion ratio, and reducing their mortality rate (Patra and Lalhriatpuii, 2020). Notably, Se exerts antioxidative effects primarily through the incorporation with selenoproteins (Zhang et al., 2020a), and reduces ROS generation and lipid peroxidation, thus maintaining a structural integrity of the cerebellum in rats (Talukder et al., 2021; Bi et al., 2021). Furthermore, whereas Se can alleviate these symptoms and reduce the accumulation of Cd and Cu and depleted the contents of Se, Fe, and Zn (Park and Chung, 2009). Moreover, it’s worth noting that Cd intoxication is associated with the dysregulation of Fe homeostasis (Tsuii, 2020). Moreover, the cellular-iron uptake is regulated by the DMT1 (Zhao et al., 2022b; Zhu et al., 2022). And Cd intoxication is associated with the dysregulation of Fe homeostasis (Park and Chung, 2009). Moreover, it’s worth noting that Cd

### Fig. 5. Effect of cadmium (Cd) and nano-selenium (Nano-Se) on metal regulatory transcription factor 1 (MTF1), metallothionein 1 (MT1), MT2, and divalent metal ion transporter 1 (DMT1) expressions. (A) The mRNA levels of MTF1. (B) The mRNA levels of MT1. (C) The mRNA levels of MT2. (D) The protein levels of MTF1. (E) The mRNA levels of DMT1. Values are expressed as mean ± standard deviations (SD). */# indicates statistically significant differences when compared to the Con/Cd group for */# < 0.05, */##P < 0.01 and ***#### < 0.001.
reduced the Fe-concentration in serum in pregnant rats (Sowa and Steibert, 1985), and MTF1 is a biomarker of Cu-exposure, which is involved in Cu’s homeostasis (Burke et al., 2008). Cu-transporters (CTR1, ATP7B) are also involved in Cu’s homeostasis, with MTF1 regulating the ATP7B expression through the MRE binding (Stalke et al., 2020). Cu-exposure increased the level of ATP7B in a pig’s liver (Huang et al., 2022). Furthermore, ATP7B responds to copper, and it functions to excrete surplus copper. It is associated with MTF1 (Chen et al., 2018). The function of CTR1 is to transport Cu from an extracellular position to the cytoplasm over the course of Cu-deficiency in the cytoplasm (Kitada et al., 2008). Strikingly, exposure to Cd increased the contents of copper in the brains of mice (Ivanova et al., 2017), and it resulted in the accumulation of Cu in the brains of coxsackievirus-infected mice (Talukder et al., 2021). In agreement with the previous study, this study revealed that exposure to Cd caused Cd and Cu to accumulate, and Zn and Fe to lessen in the cerebellum. However, simultaneous treatment with Nano-Se mitigated these detrimental effects. Nano-Se, through activating MTF1-mediated metal responses, upregulated Cd-suppressed expressions of ZNT3, ZNT5, ZNT10, ZIP8, ZIP10, TF, DMT1, ATP7B and CTR1, and subsequently maintained homeostasis of metals and relieved injuries to cerebellum (Fig. 7).

Fig. 6. Effect of cadmium (Cd) and nano-selenium (Nano-Se) on zinc, iron, and copper related transporter expressions. (A) The mRNA levels of zinc transporter 3 (ZNT3), ZNT5, ZNT10, zrt, irt-like protein 8 (ZIP8), ZIP10, transferrin (TF), ferroportin 1 (FPN1), ATPase copper transporting beta (ATP7B), and copper uptake protein 1 (CTR1). (B) Correlation analysis of metal regulatory transcription factor 1 (MTF1)-mediated and metal-response related genes. (C) Heat map of MTF1-mediated and metal-response related genes. From –1 (red) to +1 (green) relative to the values for chicken’s cerebellum in the Con group. The color scale represents the relative mRNA levels, with green indicating up-regulated genes, red indicating down-regulated genes, and black indicating unchanged genes. From –1 (brown) to +1 (blue) relative to the correlation analysis for MTF1-mediated and metal-response related genes in chicken’s cerebellum, with brown indicating negative correlations, blue indicating positive correlations, and white indicating no correlations. Values are expressed as mean ± standard deviations (SD). */# indicates statistically significant differences when compared to the Con/Cd group for *#P < 0.05, **##P < 0.01 and ***###P < 0.001.
5. Conclusion

In summary, exposure to Cd caused accumulation of Cd, oxidative stress, and dyshomeostasis of Fe, Cu, Zn, and Se in the cerebellum of chicken, and subsequently led to cerebellar injury. Due to its high digestibility as a nutrient and bioavailability, Nano-Se enhanced the anti-oxidative stress capacity and alleviated the Cd-induced metals dyshomeostasis by activating the transcriptome of selenoprotein and MTF1-mediated metal responses. This study shows that supplementation of Nano-Se at 1 mg/kg can effectively improve the Cd-induced damage to cerebellum. This study also reveals the mechanism of Nano-Se antagonized Cd-induced cerebellar toxicity from the perspective of regulation of metals homeostasis.

Author contributions

Shaoshuai Bi and Milton Talukder designed and performed experiments, performed statistical analysis, wrote original draft of the manuscript and review and editing; Haitao Ji analyzed and organized the experimental data; Meimei Lv performed data analysis, review and editing; Jing Ge, Cong Zhang review and editing; Jinlong Li conceptualization, supervision, review and editing.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2022.06.021.

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