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Selenium nanoparticles reduce oxidative stress-induced cardiomyocyte apoptosis in ascites syndrome in broiler chickens via the ATF6-DR5 signaling pathway

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Abstract

Broiler ascites syndrome (AS) is one of the main diseases threatening the health of broilers. It is well documented that myocardial hypertrophy and failure is one of the key mechanisms of broiler ascites syndrome. Therefore, prevention of cardiac hypertrophy and failure would be one goal to reduce broiler ascites syndrome incidence. Myocardial hypertrophy and failure are closely related to endoplasmic reticulum stress (ERS) in cardiac myocytes, and the endoplasmic reticulum stress signaling system (ATF6-DR5) is one of the important pathways of myocardial apoptosis. Excessive hypertrophy will affect the heart muscle's normal contraction and diastole function, and the heart will turn from compensated to decompensate thus causing myocardial injury. Myocardial apoptosis is a core component of the pathological changes of this myocardial injury. Nano-selenium is a kind of red elemental selenium nanoparticle. Due to its excellent physical, chemical and biological properties, it has attracted extensive academic attention in recent years. It has been proven to have excellent antioxidant, antibacterial, antitumor, antihypertrophic, and antiapoptotic abilities. Herein, nano-selenium (1 μmol/L) can inhibit hydrogen peroxide (H_2O_2)-induced oxidative stress in broiler primary cardiomyocytes, and at the same time reduce cardiomyocyte apoptosis. *In vivo*, nano-selenium can reduce broiler myocardial injury-related enzyme indicators (AST, CK and LDH), and alleviate myocardial injury. It can also activate the antioxidant enzyme system (SOD, GSH-Px and CAT) and reduce MDA, and make the recovery of T-AOC ability in the organization. Meanwhile, nano-selenium can down-regulate the genes and proteins expression of ATF-6, GRP-78, CHOP and caspase 12 in the ERS-related signaling pathway, and inhibit that of downstream-related caspase 3, Bax and caspase 9, and increase that of the downstream anti-apoptotic Bcl-2, thereby maintaining the homeostasis of the endoplasmic reticulum and alleviating cardiomyocyte apoptosis. It can be seen that nano-selenium can protect the damaged myocardium in the broiler ascites caused by high-salt drinking by regulating the ATF6-DR5 signaling pathway. This study was performed in chickens and cardiomyocyte cells and attempted to demonstrate that selenium nanoparticles can protect the damaged myocardium in broiler ascites. This paper provides a new idea for preventing and treating broiler ascites syndrome.

Keywords Broiler ascites syndrome, Cardiomyocyte apoptosis, Nano-selenium, ATF6-DR5 signaling pathway

Introduction

Broiler ascites syndrome (AS) is a metabolic disease characterized by the accumulation of large amounts of yellow fluid in the abdominal cavity with varying degrees of damage to the heart, lungs, kidneys, and other organs (Wideman et al. 2013). It is also known as pulmonary

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hypertension syndrome (PHS) in broilers (Shi et al. 2017). The etiology of AS is complex and usually results from multiple causative factors (Hassanzadeh et al. 2014). Broiler breeders primarily reduce the incidence through integrated control, the primary means of which are optimal feeding management and pharmacological control (Qiao et al. 2019; Yang et al. 2007). There is no specific drug for the treatment of broiler ascites. Instead, it is treated based on the disease's causative mechanism, with the appropriate drugs used to prevent and control it.

Myocardial hypertrophy and failure are one of the core pathological processes in the development of broiler ascites, and excessive apoptosis of cardiomyocytes can cause irreversible damage to the myocardium (Zeng et al. 2022). Pulmonary hypertension causes oxidative stress in the cardiovascular system, which will produce excessive oxygen free radicals, leading to damage to the heart, lungs, and other vital organs, which in turn leads to broiler ascites. And myocardial apoptosis is an important component of the pathological changes of this cardiac injury. Studies have shown that endoplasmic reticulum stress (ERS) is involved in the development of various cardiac diseases, such as myocardial hypertrophy and heart failure, and ERS is a key process that regulates the death/survival mechanism of cardiomyocytes in response to stress stimuli, thereby triggering heart failure (Wang et al. 2018). ERS is one of the apoptotic pathways in cardiac myocytes, and glucose-regulated protein78 (GRP-78) is a signature protein of endoplasmic reticulum stress (Hu et al. 2022). Transcriptional activation factor 6 (ATF-6) is a transmembrane sensor of the endoplasmic reticulum that is activated upon dissociation from GRP-78 (Hotamisligil and Davis 2016). Activated ATF-6 can enter the nucleus and induce the expression of CCAAT enhancer binding proteins (CHOP) (Elimam et al. 2015; Huang et al. 2020). Overexpression of CHOP can activate the DR5 receptor, which can only trimerize the receptor by adhering to tumor necrosis factor apoptosis-related ligands to form the death-inducing signaling complex (DISC) (Zhang et al. 2022). Then the caspase cascade reaction is activated and caspase 3 was directly activated by caspase 8 and caspase 9 (Lu et al. 2014; Wada et al. 2018). Endoplasmic reticulum stress was shown to be a key process regulating the death/survival mechanism of cardiomyocytes under stress (Wang et al. 2018). Therefore, inhibiting ERS development and myocardial apoptosis is a critical strategy for preventing myocardial injury which could lead to the development of AS.

Because of its size, shape, and unique physicochemical properties, nano-selenium has become a trending research topic in recent years, and its preparation methods, properties, and potential applications in life sciences have piqued the interest of many researchers (Khurana et al. 2019; Patra and Lalhriatpuii 2020). Studies have shown that nano-selenium not only has strong antioxidant properties but also can exert its antioxidant function by regulating multiple signaling pathways such as p38 MAPK and ASK1/JNK, removing excess reactive oxygen species (ROS) and inhibiting lipid peroxidation, thereby inhibiting oxidative stress-induced apoptosis (Sadek et al. 2017). However, it remains to be seen whether nano-selenium supplementation protects against myocardial injury caused by ascites syndrome in broiler chickens, as well as the underlying molecular mechanisms.

Given above, we want to investigate that whether selenium nanoparticles could inhibit apoptosis of cardiomyocytes caused by broiler ascites syndrome by modulating the endoplasmic reticulum-related signaling pathway (ATF6-DR5). This study can provide new ideas for the clinical prevention and treatment of broiler ascites syndrome, and broaden the application range of nano-selenium, a new anti-oxidation and anti-apoptosis nanomaterial.

Results

Preparation and characterization of nano-selenium

The selenium nanoparticles were red in color which prepared by reducing sodium selenite with ascorbic acid and using chitosan as a stabilizer (Fig. 1a). The selenium nanoparticles were dispersed spherical particles with an average particle size of 117.3 nm and a zeta potential of +60.5 mV (Fig. 1b-e). The selenium content in chitosan nano-selenium (CS-SeNP) was determined to be 1,726,535 μg/L using inductively coupled plasma mass spectrometry (ICP-MS) (Table 1).

Results of CCK-8 assay of cardiomyocyte activity

The CCK-8 method was used to screen the optimal peroxide modeling concentration, and the IC_{50} calculated by GraphPad Prism 7.0 software was 169.4 μmol/L, so 200 μmol/L was selected as the modeling concentration (Fig. 2a). Low concentrations of CS-SeNPs (0.001, 0.01, 0.1 and 1 μmol/L) had neither toxic nor pro-proliferative effects on primary broiler cardiomyocytes. When the concentration of CS-SeNPs was increased to 5 μmol/L, it had a highly significant cellular proliferative effect on cardiomyocytes ($p < 0.01$) (Fig. 2b). For the follow-up experiments, a nano-selenium concentration of 1 μmol/L was chosen to investigate nano-selenium's anti-apoptotic effect on chicken cardiomyocytes.

Content of H_2O_2-induced ROS reduced by nano-selenium

Myocardial hypertrophy and failure are accompanied by a sustained increased oxygen radical response, such as the excessive ROS, which is also linked to myocardial apoptosis. Figure 3 showed the detection results of ROS content in each group of primary broiler cardiomyocytes. Compared with the control group, ROS content

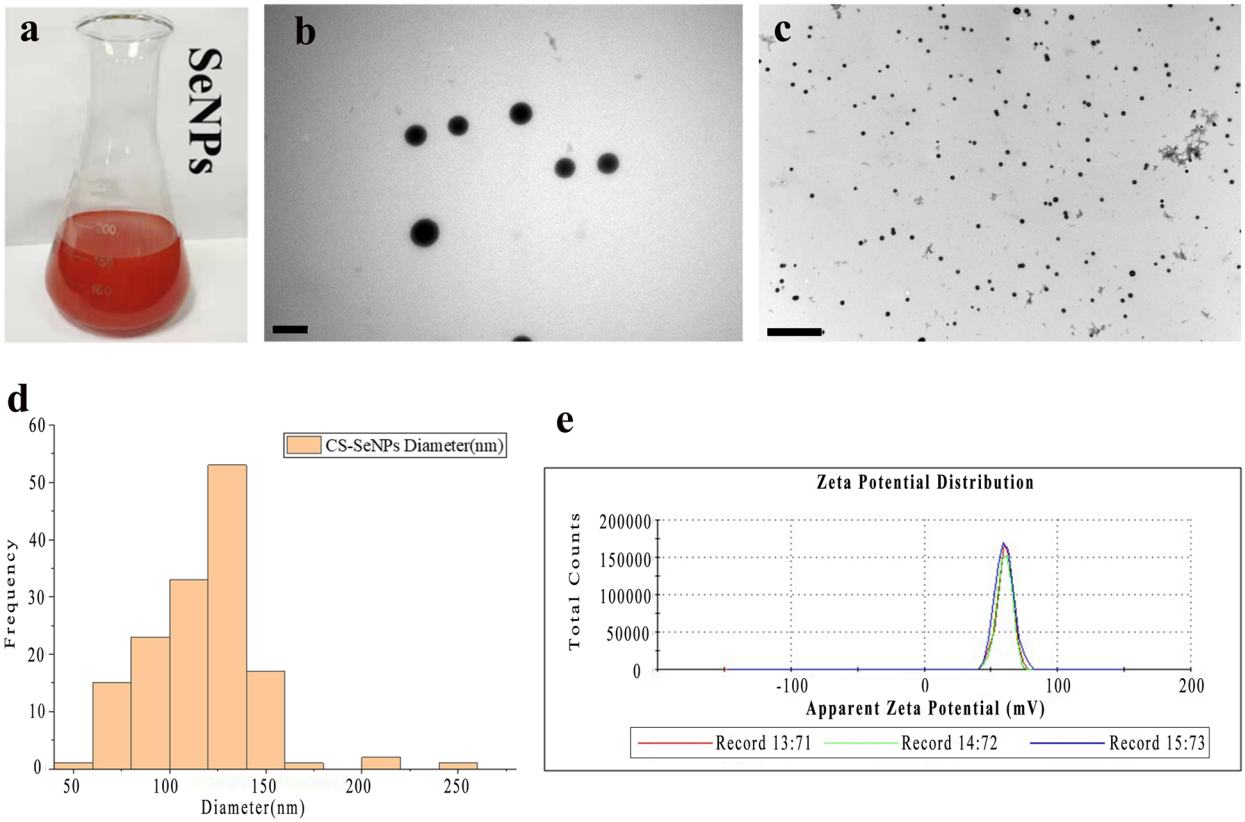

Fig. 1 Results of chitosan nano-selenium preparation. **a** The original selenium nanoparticles. **b**, **c** The transmission electron microscope image. **d** The particle size distribution of nano-selenium. **e** The zeta potential results of nano-selenium. Bar of figure b means 200 nm, μbar of figure c means 2 μm

Table 1 Analysis of the results of selenium content determination by ICP-MS

Name of sample	Selenium concentration (μg/L)	RSD/%
CS-SeNP-1	17,052.96	0.78
CS-SeNP-2	17,456.22	0.81
CS-SeNP-3	17,286.88	0.57
Average	**17,265.35**	

The sample is diluted 100 times and measured 3 times

in the H_2O_2 groups were highly significant increase ($p < 0.01$), while the ROS content of the Se + H_2O_2 groups was significantly reduced ($p < 0.01$). This indicated that the early uptake of CS-SeNPs by cardiomyocytes prevented H_2O_2-induced oxidative stress in cardiomyocytes.

H_2O_2-induced apoptosis of broiler primary cardiomyocytes attenuated by nano-selenium

As shown in Fig. 4, the technique of double-staining Annexin v-fitc/PI in primary broiler cardiomyocytes detected by flow cytometry was used to detect the inhibitory effect of CS-SeNPs on H_2O_2-induced apoptosis of broiler cardiomyocytes. compared with the control group, the apoptosis rate was highly significant higher in the H_2O_2 group ($p < 0.01$), and the apoptosis rate in the Se + H_2O_2 group was significantly declined compared with the H_2O_2 group ($p < 0.01$). The results indicated that CS-SeNPs could inhibited the peroxide-induced cardiomyocyte apoptosis, although it cannot recover the same as the control group.

High salt-induced broiler ascites cardiac index reduced by nano-selenium

Subsequently, nano-selenium was also used to evaluate its inhibitory effect on myocardial apoptosis in broiler ascites syndrome in vivo. At the same time, nano-selenium was also used to evaluate its inhibitory effect on myocardial apoptosis in broiler ascites syndrome in vivo. Broiler ascites heart index (AHI) is the core index to judge whether broiler ascites syndrome occurs or not and reflects the degree of broiler right heart hypertrophy. When the AHI value is higher than 0.28 and there is a large amount of yellow jelly-like liquid in the abdominal cavity, it can be judged that the broiler tax syndrome has occurred (Cheng et al.

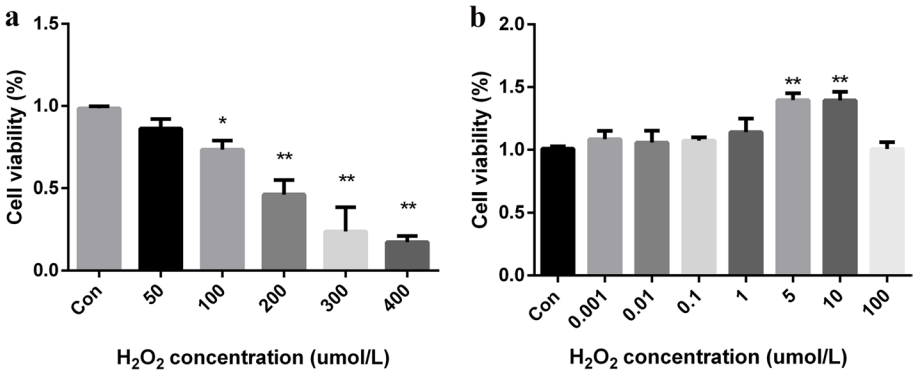

Fig. 2 Effects of hydrogen peroxide and nano-selenium on the activity of chicken cardiomyocytes. Select 200 μmol/L hydrogen peroxide and 1 μmol/L selenium nanoparticles were applied to chicken cardiomyocytes. **a** The cell viability under different H_2O_2 concentrations. **b** The cell viability under different nano-selenium concentrations. Data were presented as the mean ± SD. * denotes a significant difference ($p < 0.05$) vs. the control group. ** denotes a highly significant difference ($p < 0.01$) *vs*. the control group

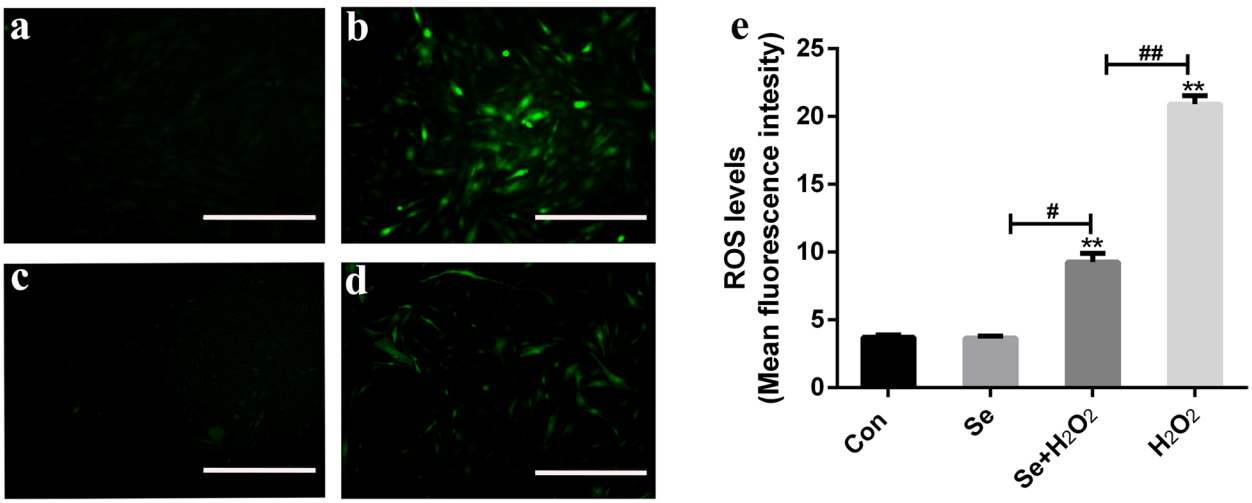

Fig. 3 The ROS levels in each group. **a**-**e** The ROS levels of the con group, the H_2O_2 group, the Se group and the Se + H_2O_2 group. ** denotes a highly significant difference ($p < 0.01$) *vs*. the Control group. # denotes a significant difference between the two groups ($p < 0.05$), and ## denotes a highly significant difference between the two groups ($p < 0.01$). Bar means 400 μm

2021a). Figure 5 showed that the AHI of the Y group was extremely significantly higher than that of the control group ($p < 0.01$), and the AHI values of all broilers in group Y were higher than 0.28, indicating that the model of broiler ascites syndrome induced by high salt was successful. Although the AHI of most broilers in the Se + Y group was still higher than 0.28, the AHI of the Se + Y group was significantly lower than that of the Y group ($p < 0.05$).

Broiler myocardial injury-related enzyme indexes reduced by nano-selenium

The results of serum detection of broiler heart function-related enzymes were shown in Fig. 6. The serum CK, AST and LDH values of group Y were significantly higher than those of control group ($p < 0.05$). The serum CK, AST and LDH values of the Se + Y group were significantly lower than those of the Y group ($p < 0.05$), and there was no significant difference compared with the control group. The results showed that nano-selenium could inhibit the increase of enzyme indexes related to myocardial injury and, to some extent, reduce the degree of myocardial injury.

Broiler antioxidant enzyme system activated by nano-selenium

Figure 7 depicted the results of antioxidant function indexes in serum. In the Y group, CAT, GSH-Px, SOD and T-AOC activities were significantly lower than in the control group, while MDA levels were significantly

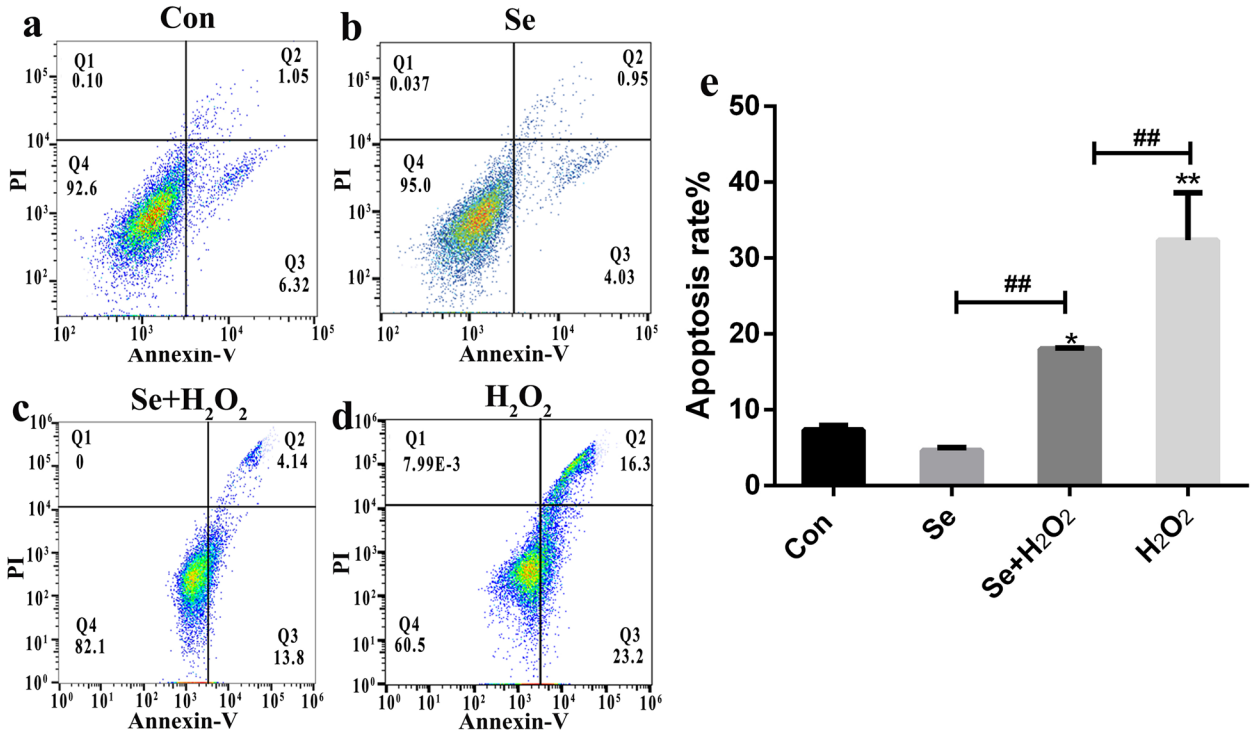

Fig. 4 Flow cytometry was used to examine apoptosis. **a**-**e** The apoptosis rate of the con group, the H_2O_2 group, the Se group and the Se + H_2O_2 group. * denotes a significant difference ($p < 0.05$), ** denotes a highly significant difference ($p < 0.01$) *vs*. the control group. # denotes a significant difference between the two groups ($p < 0.05$), and ## denotes a highly significant difference between the two groups ($p < 0.01$)

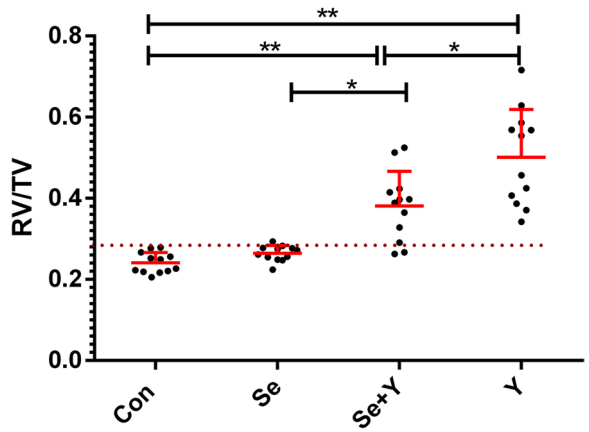

Fig. 5 RV/TV value measurement ($n = 12$). * denotes a significant difference between the two groups ($p < 0.05$), and ** denotes a highly significant difference between the two groups ($p < 0.01$)

higher. However, after nano-selenium treatment (the Se + Y group), these changes were significantly reversed ($p < 0.05$), and serum total antioxidant capacity was significantly increased ($p < 0.05$), but GSH-Px and SOD activities were not significantly reversed. To summarize, nano-selenium may reduce the oxidative stress in the body caused by high salt-induced ascites syndrome in broilers.

Expression of apoptosis-related genes in the ATF-6-DR5 signaling pathway antagonized by nano-selenium

The mRNA expression of related genes in ATF-6-DR5 signaling pathway in heart tissues was detected by real-time fluorescence quantitative PCR, and the relative quantification was taken to process the results. The results showed (Fig. 8) that the mRNA expressions levels of caspase3, caspase9, Bax, ATF-6, GRP-78 and CHOP in the Y group were extremely significant increase than those in the control group ($p < 0.01$). The mRNA expression levels of caspase3, caspase9, ATF-6, GRP-78 and CHOP were significantly lower in the Se + Y group compared with the Y group ($p < 0.05$).

Expression of apoptosis-related proteins in the ATF-6-DR5 signaling pathway antagonized by nano-selenium

Figure 9 showed that, compared with the control group, BAX, ATF-6, GRP-78, CHOP, caspase 3, caspase 9 and caspase 12 protein expression were significant increase in the Y group ($p < 0.05$), while Bcl-2 protein expression was significant reduction in the Y group ($p < 0.05$). Compared with the Y group, BAX, ATF-6, GRP-78, CHOP, caspase 9 and caspase 12 protein expression were significantly lower in the Se + Y group ($p < 0.05$), and Bcl-2 protein expression was significantly higher in the Se + Y group ($p < 0.01$).

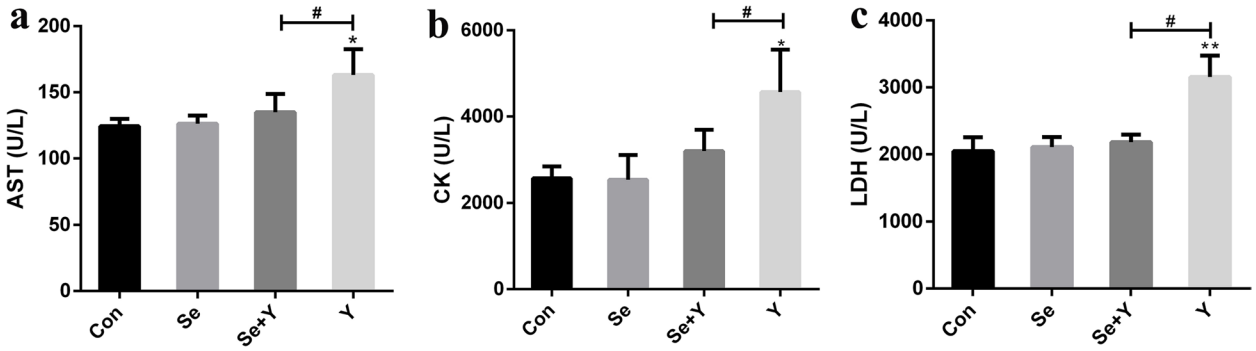

Fig. 6 The serum cardiac function test results. **a**-**c** AST, CK and LDH activities. * denotes a significant difference ($p < 0.05$), ** denotes a highly significant difference ($p < 0.01$) *vs*. the control group. # denotes a significant difference between the two groups ($p < 0.05$)

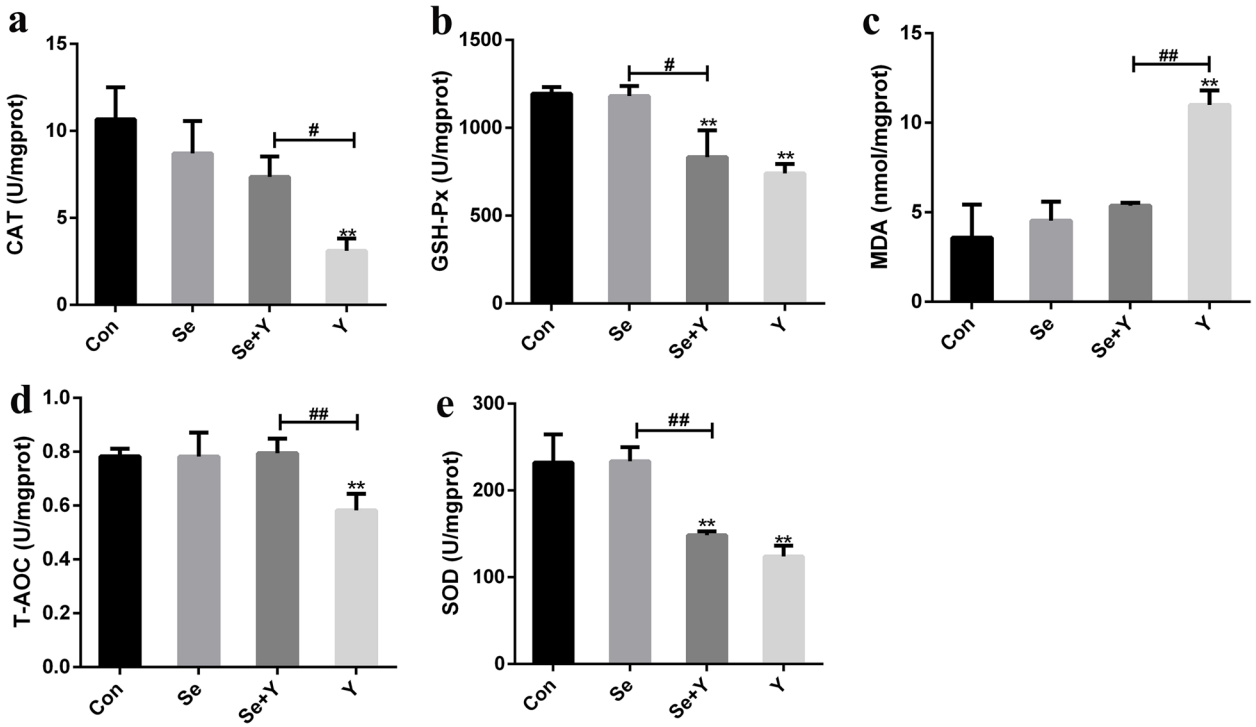

Fig. 7 Effect of nano-selenium on antioxidant function in broiler serum. **a**-**e** CAT, GSH-Px, MDA, T-AOC and SOD activities. ** denotes a highly significant difference ($p < 0.01$) *vs*. the control group. # denotes a significant difference between the two groups ($p < 0.05$), and ## denotes a highly significant difference between the two groups ($p < 0.01$)

These findings suggested that nano-selenium inhibited apoptosis in broiler cardiomyocytes via the endoplasmic reticulum stress ATF-6-DR5 signaling pathway.

Discussion

An effective inhibitory effect of nano-selenium on H_2O_2-induced oxidative stress and apoptosis in broiler primary cardiomyocytes

Broiler ascites syndrome is a disease caused by metabolic disorders that is common and poses a serious threat to broiler health while also limiting the economic benefits of broiler farming (Zhang et al. 2018). Myocardial hypertrophy and failure are critical links in the development of broiler ascites syndrome (Zeng et al. 2022). The onset of cardiac hypertrophy and failure is accompanied by a sustained increased oxygen radical response, such as the production of large amounts of ROS and lipid hydroperoxide, which can cause cell membrane damage and in turn cause DNA damage, thereby eventually inducing cardiomyocyte death via apoptosis (Del Re et al. 2019).

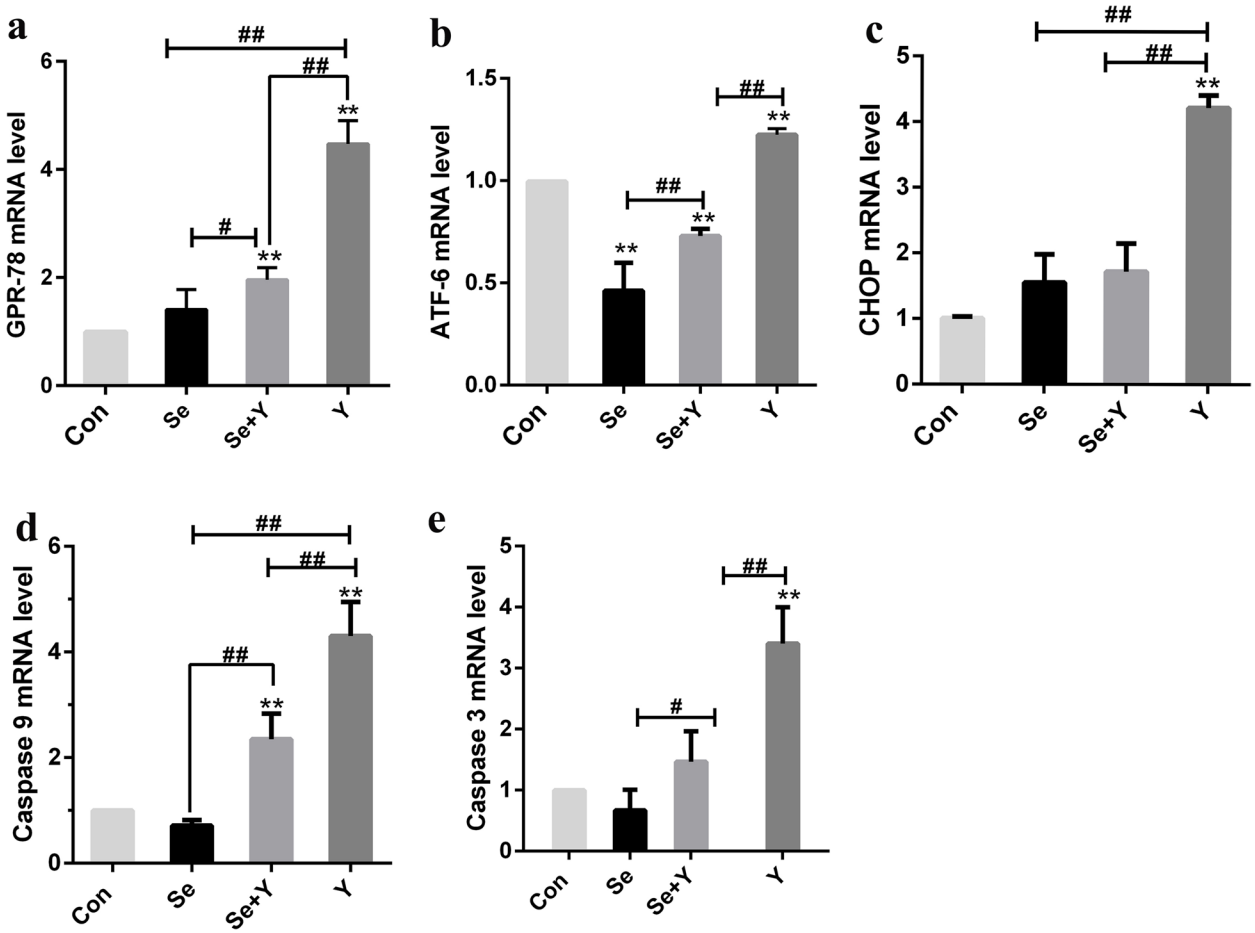

Fig. 8 Effect of nano-selenium on apoptosis-related genes in broiler myocardium. **a**-**e** the mRNA expression level of GPR-78, ATF-6, CHOP, caspase 3 and caspase 9. * denotes a significant difference ($p < 0.05$), ** denotes a highly significant difference ($p < 0.01$) *vs*. the control group. # denotes a significant difference between the two groups ($p < 0.05$), and ## denotes a highly significant difference between the two groups ($p < 0.01$)

Hence, tons of researchers identify with that apoptosis of cardiomyocytes is a key component of this pathogenesis (Cheng et al. 2021b; Fan et al. 2021; Wan et al. 2022). Excessive apoptosis of cardiomyocytes causes irreversible myocardial damage to the heart, resulting in cardiac hypertrophy and failure, ascites, and even death (Wang et al. 2018).

Herein, Cardiomyocyte apoptosis model was established by hydrogen peroxide (Chu et al. 2020; Wang et al. 2015). The H_2O_2 concentration close to the IC_{50} (200 μmol/L) was discovered to be the optimal concentration for modeling by measuring CCK-8. In this study, 1 μmol/L CS-SeNPs were used to verify the effect of nano-selenium on H_2O_2-induced cardiomyocyte apoptosis. The findings showed that 1 μmol/L nano-selenium effectively reduced cardiomyocyte apoptosis caused by H_2O_2 simulation. In addition, the ROS assay was used to assess the effect of nano-selenium on H_2O_2-induced oxidative stress in chicken cardiomyocytes. The fluorescence intensity of the Se + H_2O_2 group was significantly lower than that of the H_2O_2 group, implying that nano-selenium inhibited hydrogen peroxide-induced oxidative stress in cells. Selenium (Se), an essential trace element in humans and animals, is involved in numerous important physiological processes and exerts biological effects in the form of selenoprotein (Estevez et al. 2021). SeNPs have become a new selenium source that has attracted extensive attention in recent years because of their size, shape, and unique physical and biological properties. To maintain the body's normal functioning, selenium nanoparticles scavenge ROS by converting into selenoproteins with antioxidant functions and promoting the synthesis of antioxidant enzymes (Rocca et al. 2019).

The biological effects of nano-selenium are dependent on the dose and duration of action (Lesnichaya et al. 2021). Chicken cardiomyocytes were treated with different concentrations of selenium nanoparticles for 24 h. The CCK-8 results showed that there was no significant

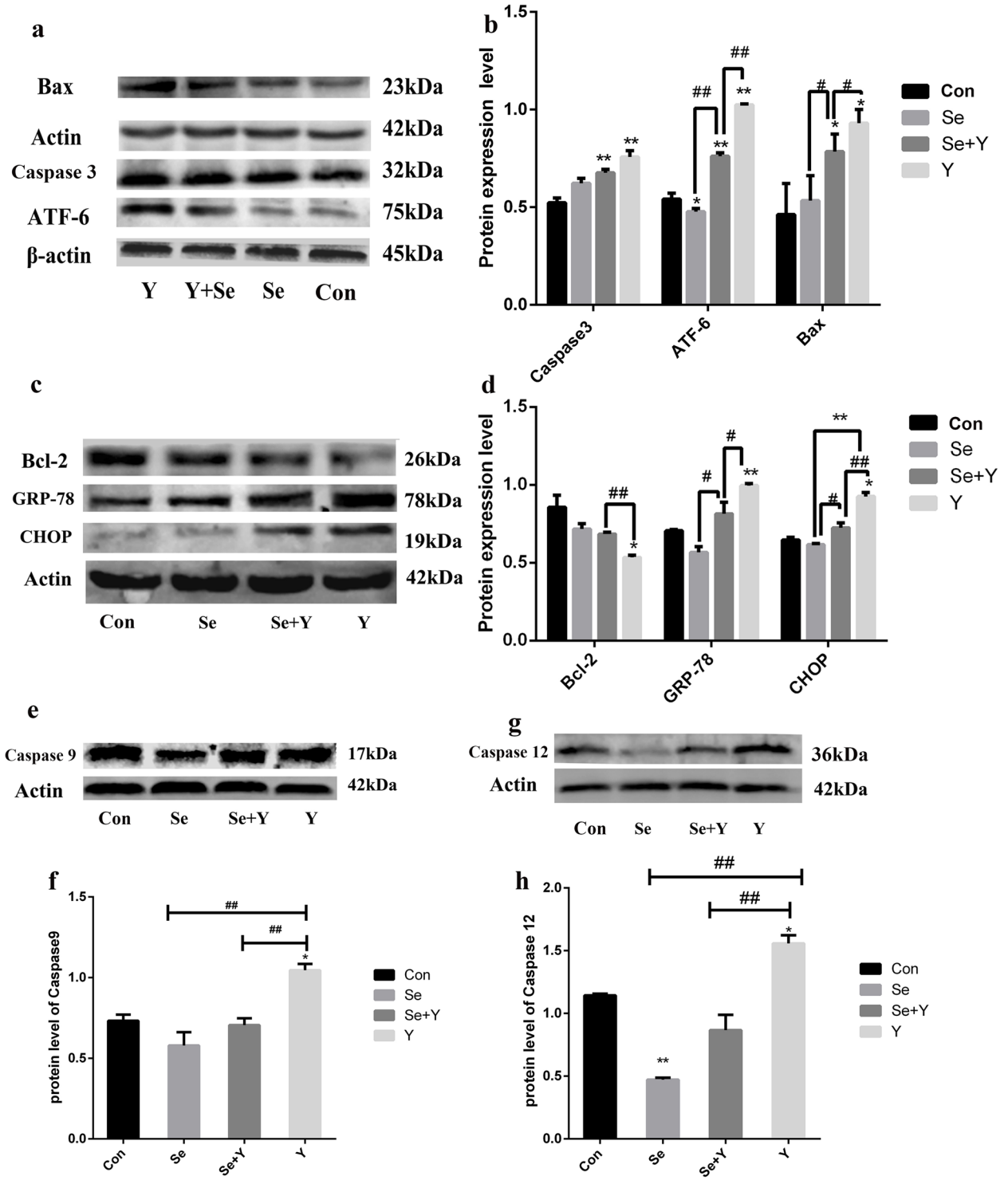

Fig. 9 Effect of nano-selenium on apoptosis-associated proteins in broiler myocardium. **a**, **b** The protein expression level of Bax, caspase 3 and ATF-6. **c**, **d** The protein expression level of Bcl-2, GRP-78 and CHPO. **e**, **f** The protein expression level of caspase 9. **g**, **h** The protein expression level of caspase 12. * denotes a significant difference ($p < 0.05$), ** denotes a highly significant difference ($p < 0.01$) *vs*. the control group. # denotes a significant difference between the two groups ($p < 0.05$), and ## denotes a highly significant difference between the two groups ($p < 0.01$)

effect on the cellular activity of chicken cardiomyocytes when they were treated with lower concentrations of selenium nanoparticles. When the concentration of nano-selenium gradually increased to 5 μmol/L. Nano-selenium had a significant proliferative effect on chicken cardiomyocytes. When the concentration of nano-selenium reached 10 μmol/L, the effect on the survival rate of chicken cardiomyocytes tended to the blank control group. When the selenium concentration reached 100 μmol/L, there was a tendency to inhibit cell activity. Although nano-selenium is biocompatible and has a low toxicity, a high concentration of nano-selenium acting on cells may cause a Trojan horse effect, and the small selenium nanoparticle size allows for uncontrolled passage across the cell barrier, which may have negative effects on cells (Surai and Kochish 2020).

An effective inhibitory effect of nano-selenium on oxidative stress caused by high-salt drinking water-induced broiler ascites syndrome

It has been demonstrated that antioxidant levels of broiler body are proportional to selenium levels within a certain range, and GSH-Px activity in broiler serum and tissues increases with increasing selenium levels in the diet (Boostani et al. 2015; Chen et al. 2014). When GSH-Px activity in serum reaches a plateau, GSH-Px activity does not increase further with increasing selenium concentration in the diet (Hu et al. 2012). When 0.15 mg/kg −1 mg/kg selenium was added to the diet, the activation response of broiler GSH-Px reached its peak (Hu et al. 2012). In this study, the activities of SOD, GSH-Px and CAT in broiler serum were not significantly different between the Se and control groups because the feed fed to the control group also had the basic selenium content of broilers. Herein, high-salt drinking water reduced the antioxidant capacity of broiler chickens, while the antioxidant capacity of broiler chickens in the Se + Y group was significantly restored. This showed that oral administration of nano-selenium could effectively resist the oxidative damage of the body caused by high-salt drinking water-induced broiler ascites syndrome by restoring or improving the body's antioxidant capacity.

Furthermore, the occurrence of oxidative stress is associated with numerous selenoproteins. For example, GPx1, GPx4 and TRx1 expression levels have been shown to be upregulated during oxidative stress (Touat-Hamici et al. 2014). The expression of their associated cardioprotective selenoproteins and selenases has been shown to increase during cardiovascular oxidative stress (Hoffmann et al. 2011), and mRNA and protein levels of MSRB1, TRx1, GPx3 and GPx4 were significantly increased in T3 or isoproterenol-induced myocardial hypertrophy (Kim 2013). Although there was no statistical difference in the serum SOD and GSH-Px activities between the Se + Y group and the Y group, it can be seen that the Se + Y group had an obvious upward trend compared with the Y group. Additionally, the results of MDA, T-AOC and CAT of the Se + Y group and the Y group were significantly different. T-AOC reflects the total antioxidant level composed of various antioxidant substances and antioxidant enzymes in the body, while SOD, CAT and GSH-Px are important antioxidant enzymes in the body, and they participate in a variety of oxygen free radical scavenging processes. The content of MDA is an important parameter reflecting the body's antioxidant capacity, which can reflect the degree and rate of lipid peroxidation in the body. In summary, nano-selenium can reduce oxidative stress in broiler chickens caused by high-salt drinking water by increasing the activity of antioxidant enzymes.

Nano-selenium antagonizes cardiomyocyte apoptosis in broiler chickens with ascites syndrome through ERS-related signaling pathway

The protein expression of ERS-related signaling pathways in broiler heart tissues was detected using Western Blot in this in vitro. The results revealed that the expression levels of GRP-78, ATF-6, CHOP, Bax, Caspase 3, Caspase 9 and Caspase 12 were significant increase in the Y group compared to the control group, indicating that broilers with ascites syndrome induced by high-salt drinking water experienced myocardial apoptosis, and at the same time, ERS-related signaling pathways were activated. When ERS occurs, high levels of CHOP induce the expression of apoptotic proteins such as GADD34 and DR5 where the DR5 receptor forms the death-inducing signaling complex (DISC), which activates the caspase cascade reaction (Cao et al. 2021). However, the results revealed that oral nano-selenium significantly inhibited the activation of ERS-related signaling pathways and inhibited myocardial apoptosis, indicating that nano-selenium could inhibit the activation of apoptotic proteins associated with the downstream of the ATF6-DR5 signaling pathway, thereby alleviating the occurrence of apoptosis in vivo. Overexpression of CHOP can also promote the production of ROS, which causes long-term endoplasmic reticulum stress and mediates apoptosis (So 2018). High levels of CHOP protein can cause a decrease in Bcl-2 expression, which can also result in translocation of Bax from the cytoplasm to the mitochondria and the initiation of the mitochondrial apoptotic pathway (Guo et al. 2021).

Conclusion

In summary, nano-selenium can inhibit cardiomyocyte apoptosis induced by oxidative stress in vitro. More importantly, nano-selenium can maintain endoplasmic

reticulum homeostasis by inhibiting the expression of GRP-78, ATF-6, CHOP and caspase 12 and its downstream apoptosis-related proteins caspase 9, caspase 3, Bax and increasing the expression level of Bcl-2, thereby alleviating cardiomyocyte apoptosis and reducing myocardial injury in broiler chickens. The above suggests that nano-selenium may maintain endoplasmic reticulum homeostasis by inhibiting ATF6-DR5 apoptosis signaling pathways, thereby antagonizing broiler cardiomyocyte apoptosis.

Materials and methods

Synthesis of selenium nanoparticles

Nano-selenium was synthesized and surface-modified using chemical methods described previously (Yang et al. 2023). Briefly, 5 mL of 20 mmol/L sodium selenite (Na_2SeO_3) solution (China, Sinopharm Group Chemical Reagent Co., Ltd.) was added in 5 mL of 30 mmol/L potassium iodide solution (China, Sinopharm Group Chemical Reagent Co., Ltd.) and mix well, then put it on a magnetic stirrer. 5 mL of 60 mmol/L ascorbic acid solution (China, Sinopharm Group Chemical Reagent Co., Ltd) was added in the solution for reaction, and the color of the solution gradually changed from colorless to orange-red. After the reaction, the above colloidal solution was continued to be aged for 24 h at 4℃. The synthesized nano-selenium was dialyzed for 2–3 d to remove the excess ascorbic acid, sodium selenite. To modify the selenium nanosurface,0.64 mg/mL chitosan (Feiyang Bio, China) was used.

Characterization of selenium nanoparticles

The selenium content was measured using an inductively coupled plasma emission spectrometer (Agilent, USA) in high-energy helium mode (HE He). The surface morphology and particle size of selenium nanoparticles were observed and photographed using a transmission electron microscope (HITACHI, Japan) at an accelerating voltage of 80 kV. Electrophoretic light scattering with a zeta potential analyzer (Malvern, UK) was used to determine the zeta potential of selenium nanoparticles at a measurement temperature of 25 °C, an incidence angle of 90°, and a solvent refractive index of 1.33. Each measurement had a total of 100 measurements, and each sample was repeated three times.

Primary culture of broiler cardiomyocytes

The12-day-old embryonic chicken eggs (Shandong Texas 817 crossbred white broiler breeder eggs) were used to isolate cardiomyocyte. Under aseptic conditions, embryonic hearts were removed and 1/2 of hearts were minced, and the tissue was repeatedly rinsed with Hanks' solution. The supernatant was discarded and the precipitate was retained. The tissue blocks were digested in 0.12% collagenase (Biosharp, China) for approximately 10 min (on a magnetic stirrer). The supernatant was transferred into another sterile centrifuge tube after each digestion and sedimentation before the first digestive juice was discarded, and the digestion was terminated by adding cold medium containing 10% fetal bovine serum (China, HYCEZMBIO), and the process was repeated five times. The supernatant was collected in a centrifuge tube, centrifuged at 1200 r/min for 8 min. Then, the precipitate was resuspended by DMEM-F12 medium containing 10% fetal bovine serum and 0.1 mmol/L 5-Brdu (5-Bromoo2'-deoxyuridine, China, Biosharp) and incubated for 90 min in a 5% CO_2 constant temperature (37°C) incubator. After 90 min of incubation, the fibroblasts quickly adhered to the wall and sank to the bottom of the bottle, whereas the cardiomyocytes did not. The cell suspension was aspirated and transferred to another culture bottle, yielding cardiomyocytes with a purity of 95% or higher. When the cells had achieved approximately 80% fusion and pulsating cardiomyocytes, the myocardial cells were digested with 0.25% trypsin and used in subsequent experiments.

Cell experiments grouping and processing

The cardiomyocytes were divided into 4 groups: control (con) group, selenium (Se) group, selenium + hydrogen peroxide ($Se + H_2O_2$) group, and hydrogen peroxide (H_2O_2) group. Cardiomyocytes in the Se group were treated with nano-selenium for 24 h. In the $Se + H_2O_2$ group, cardiomyocytes were treated with nano-selenium for 24 h in advance, then the cardiomyocytes were treated with H_2O_2 for 4 h. The H_2O_2 group applied H_2O_2 directly to cardiomyocytes for 4 h.

CCK-8 assay

The CCK-8 kit (Biosharp, China) was used to detect cells viability to verify the effect of H_2O_2 and nano-selenium on broiler cardiomyocyte. 10 μL of CCK-8 solution was added to each well, then the cardiomyocyte was incubated for 2 h without light. The medium was added to the wells without cells, and same operations as the above, these wells were seen as control. After the incubation, the OD value of each well was measured at 490 nm, referring to the calculation formula in the manual of CCK-8kit.

ROS assay

DCFH-DA was diluted 1:1000 with serum-free medium to a final concentration of 10 μmol/L (ROS assay kit,

Bain Marie, China). A sufficient volume of diluted DCFH-DA was added to adequately cover the cells, and the cells were incubated in the dark at 37 °C for 20 min. Cells were washed three times with serum-free medium before being observed under a fluorescence microscope (Japan Nikon).

Apoptosis rate analysis

According to the Annexin V-FITC/PI Apoptosis Assay Kit's instructions (Biosharp, China), cardiomyocytes were digested with EDTA-free trypsin for 2 min before being centrifuged at 1200 rpm/min for 10 min. The cells were collected and twice washed in PBS solution. After resuspending the cell precipitates in 100 μL Binding Buffer working solution, 5 μL of Annexin V-FITC (C1062L-1, Biyuntian Bio-Tech Co. ShangHai) and 5 μL of propidium iodide (PI C1062L-3, Biyuntian Bio-Tech Co. ShangHai) were added. After 15 min at room temperature without light, cells were analyzed by flow cytometry (Beckman Coulter, USA).

Animals and treatment

Forty-eight white feather broilers were approved for feeding in the animal house of Huazhong Agricultural University. After 1 week of normal feeding, 48 broilers were randomly divided into four groups: the saline + selenium group (Se + Y) and the saline group (Y) received drinking containing 5 mg/L sodium chloride (NaCl), while the control and selenium groups (con and Se) received purified water (0 mg/L NaCl). Broilers in the Se and the Se + Y groups received 1 mg/kg of nano-selenium colloidal solution daily. During the 28-day feeding period, there were no dietary restrictions. After 28 d, anesthesia was performed by intramuscular injection using 846 anesthetic. Samples were collected from chickens with ventricular tissue, blood.

Calculation of AHI

The ascites heart index (AHI) reflects the degree of right heart hypertrophy which is used to diagnose ascites syndrome in broiler chickens. After dissecting the chicken, the heart was removed and washed with saline to remove the clot. The atrium was incised along the coronary sulcus and the anterior and posterior longitudinal sulci, then the right ventricle and the left ventricle (including the atrial septum) were separated, and the mass ratio of the right ventricle to the entire ventricle (RV/TV) was calculated.

Biochemical assays

Totally 0.5 mL of serum was taken from each sample, and the serum samples were tested using a fully automated biochemical analyzer (Myriad Biomedical Electronics Co., Ltd., China) to determine the content of cardiac function-related indicators: glutamate transaminase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH) (Myriad Biomedical Electronics Co., Ltd., China).

Antioxidant assay

Broiler serum was processed according to the kit instructions to determine the activities of glutathione peroxidase (GSH-PX), malondialdehyde (MDA), total antioxidant capacity (T-AOC), superoxide dismutase (SOD) and catalase (CAT) expression (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, China).

Fluorescent quantitative PCR

Total RNA was extracted and purified from broiler ventricular tissues using Trizol solution (Eric Biotechnology Co., Ltd., China). The total RNA was reversely transcribed into cDNA using TSK302M Goldenstar™ RT6 cDNA Synthesis Kit Ver.2 kit (Beijing DynaScience Biotechnology Co., Ltd., China), and the fluorescent quantitative PCR was performed using TSE202 2×T5 Fast qPCR Mix Kit (Beijing, China). The mRNA expression level analysis was performed using SYBR Premix Ex TaqTM Fluorescence PCR kit and fluorescent fluorescence quantitative PCR instrument. Three replicates of each sample were used as reference genes for comparison correction using β-actin. The relative expression was calculated using the $2^{-\Delta\Delta ct}$ method. The qPCR primers were shown in Table 2.

Western Blot

The protein concentration of broiler ventricular tissues was determined using a BCA kit (Biotian Biotechnology Co., Ltd., China), and the protein samples were denatured by boiling after being added to the loading buffer (Biosharp, China). The protein samples were separated by SDS-PAGE (Wuhan Bachandu Biotechnology Co., Ltd., China), and the proteins on the gel were transferred to PVDF membranes. Then, the membranes were blocked by 5% skimmed milk (Wuhan Bachandu Biotechnology Co., Ltd., China) for 2 h at room temperature, and followed by overnight incubation with primary antibody at 4 °C. After that, the corresponding secondary antibody was added and incubated at room temperature for 1.5 h. the protein expression density was detected with imaging system (Japan, Nikon).

These antibodies are used in this experiment: Caspase3 Rabbit Multi-Antibody (WL02117 Wan Class Biology), Caspase9 Rabbit Multi-Antibody (WL01838Wan Class Biology), Caspase12 Rabbit Multi-Antibody (WL03268 Wan Class Biology)、Bcl-2 Rabbit Multi-Antibody (WL01556 Wan Class Biology), Bax Rabbit Multi-Antibody (WL01637 Wan Class Biology), GRP-78 Rabbit

Table 2 Primer sequences used for the RT-qRCR

Gene	Primer Sequence (5′-3′)
β-actin	Forward 5′- ATGCCTTGCCCCATGCTATT- 3′
	Reverse 5′- AATCTCCCGTTCCGCAGTG- 3′
Caspase3	Forward 5′- CCACCGAGATACCGGACTGT- 3′
	Reverse 5′- ACTGCTTCGCTTGCTGTGAT- 3′
Caspase9	Forward 5′- CTGCCTGCACTGACTTCTGA- 3′
	Reverse 5′- AATCTCCCGTTCCGCAGTG- 3′
Bcl-2	Forward 5′- TCGTCGCCTTCTTCGAGTTC- 3′
	Reverse 5′- CCACAAAGGCATCCCATCCT- 3′
Bax	Forward 5′- ATCGTCGCCTTCTTCGAGTT- 3′
	Reverse 5′- ATCCCATCCTCCGTTGTTCT- 3′
GRP-78	Forward 5′- TCGGAACCACCTACTCTTGC- 3′
	Reverse 5′- ACGGTTTCCTTGGTCGTTGA- 3′
ATF-6	Forward 5′- GACGAGCCCGACTCATTTCA- 3′
	Reverse 5′- GGCTCCGTCTTCACATTTGC- 3′
CHOP	Forward 5′- GGTGCACAGCAGGAAGAAGA- 3′
	Reverse 5′- CTCATCCAGCTCACAGCACA- 3′

Multi-Antibody (WL03157 Wan Class Biology), ATF-6 Rabbit Multi-Antibody (WL02407 Wan Class Biology), CHOP Rabbit Multi-Antibody (WL02407 Wan Class Biology), Actin Mouse Antibody (M20011 Abimat), Actin Rabbit Multi-Antibody (WL0002d Wan Class Biology), HRP-labeled goat anti-rabbit secondary antibody (GB23303 Wuhan Xavier Biotechnology Co., Ltd.).

Data statistics and analysis of the experiment

One-way ANOVA was used to be analyzed by Graph Pad Prism 7.0, and all results were expressed as mean ± standard deviation (Mean + SD). The difference between the experiment groups and control was expressed as * ($p < 0.05$) and ** ($p < 0.01$). The differences between the rest of the groups were indicated by # ($p < 0.05$) and ## ($p < 0.01$).

Abbreviations

AS	Broiler ascites syndrome
ERS	Endoplasmic reticulum stress
H_2O_2	Hydrogen peroxide
PHS	Pulmonary hypertension syndrome
DR5	Death Receptor 5
GRP-78	Lucose-regulated protein78
ATF-6	Transcriptional activation factor 6
DISC	CCAAT enhancer-binding, death-inducing signaling complex
ROS	Reactive oxygen species
MAPK p38	Mitogen-activated protein kinase p38
CS-SeNP	Chitosan nano-selenium
ICP-MS	Inductively coupled plasma mass spectrometry
AHI	Ascites heart index
Se	Selenium
Na_2SeO_3	Sodium selenite
PI	Propidium iodide
AST	Glutamate transaminase
CK	Creatine kinase
LDH	Lactate dehydrogenase
GSH-PX	Glutathione peroxidase
MDA	Malondialdehyde
T-AOC	Total antioxidant capacity
SOD	Superoxide dismutase
CAT	Catalase

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Authors' contributions

Conceptualization, Xiaoqi Yang and Xin Liu; investigation, Xiaoqi Yang and Xin Liu; writing – original draft, Xin Liu; writing – review and editing, Xiaoqi Yang, Jiabin Zhang, Jiaqi Liu, Pei Wu Yang Fu, KYEIN SAN LOON, Mengdi Zhang, Yuxuan Peng; supervision, Donghai Zhou; project administration, Donghai Zhou; funding acquisition, Donghai Zhou. All authors have read and approved the final version of the manuscript.

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Availability of data and materials

The authors confirmed that all data on which the study results are based are available without restriction. The materials and data not presented in this manuscript are available from the corresponding author upon request.

Declarations

Ethics approval

All experimental procedures of animals were approved by the Scientific Ethics Committee of Huazhong Agricultural University.

Consent for publication

All authors approve to publish this study in the Animal Diseases.

Competing interests

The authors state that there are no competing interests.

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