



# Zinc Inhibits Lead-Induced Oxidative Stress and Apoptosis of ST Cells Through ROS/PTEN/PI3K/AKT Axis

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## Abstract

Lead (Pb) is a widely distributed toxic heavy metal element known to have strong male reproductive toxicity, which can result in issues such as abnormal count and morphology of sperm. Zinc (Zn) is an essential trace element for the human body that can antagonize the activity of Pb in some physiological environments, and it also possesses antioxidant and anti-inflammatory effects. However, the specific mechanism of Zn's antagonism against Pb remains largely unclear. In our study, we conducted research using swine testis cells (ST cells) and confirmed that the half maximal inhibitory concentration of Pb on ST cells was 994.4  $\mu\text{M}$ , and the optimal antagonistic concentration of Zn was 10  $\mu\text{M}$ . Based on this information, we treated ST cells with Pb and Zn and detected related indices such as apoptosis, oxidative stress, and the PTEN/PI3K/AKT pathway using flow cytometry, DCFH-DA staining, RT-PCR, and Western blot. Our results demonstrated that Pb exposure can generate excessive reactive oxygen species (ROS), disrupt the antioxidant system, upregulate PTEN expression, and inhibit the PI3K/AKT pathway in ST cells. In contrast, Zn significantly inhibited the overproduction of ROS, improved oxidative stress, and decreased PTEN expression, thus protecting the PI3K/AKT pathway compared to Pb-exposed ST cells. Furthermore, we found that Pb exposure exacerbated the expression of genes related to the apoptosis pathway and reduced the expression of anti-apoptotic genes. Furthermore, this situation was significantly improved when co-cultured with Pb and Zn. In summary, our study demonstrated that Zn alleviated Pb-induced oxidative stress and apoptosis through the ROS/PTEN/PI3K/AKT axis in ST cells.

**Keywords** Zn · Pb · Swine testis cells · ROS/PTEN/PI3K/AKT axis · Apoptosis

## Introduction

Lead (Pb) is a common environmental pollutant widely used in the industry. Due to its resistance to degradation and easy accessibility to humans in the environment, Pb can accumulate in the human body over the long term [1, 2]. Pb exposure occurs in daily life when people come into contact with products containing Pb, such as wastewater, tail gas, and puffed food. In China, heavy metal contamination has affected approximately 10.18% of arable land, leading to a series of crop contamination events related to Pb that have jeopardized food security and public health [3, 4]. In the USA, the median Pb content in uncontaminated topsoil is 18 mg, while the Pb content in topsoil in Pennsylvania

has nearly doubled [5]. Mountainous areas around many Pb mines in northern Vietnam are especially contaminated with Pb, with an average lead content in the soil ranging from 689 to 1043 mg, posing serious health risks to the surrounding population [6]. Children and certain occupational groups are at higher risk of Pb exposure due to frequent contact [7].

Pb can cause multi-system and multi-organ damage to the human body. Under high Pb exposure, almost all organs and systems can be damaged to varying degrees, including the nervous system, liver, hematopoietic tissue, immune system, and the reproductive system, which is also a target organ of Pb [1, 8–10]. Studies have shown that exposure to Pb at concentrations above 100 mg for 3 weeks results in significant damage to testicular structures and a significant decrease in sperm viability in adult male mice [11]. Furthermore, Pb can induce oxidative stress and apoptosis in testicular tissue [12–14]. The potent neurotoxicity of Pb can mediate oxidative stress and reduce neuronal density in the motor cortex of mice. Chronic Pb exposure can also lead to excessive

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accumulation of Pb in the kidney and liver of animals, initiating the apoptosis program by generating excess reactive oxygen species [15–17].

Phosphatidylinositol 3-kinases (PI3Ks) are intracellular signaling molecules that play critical roles in regulating cellular functions such as proliferation, differentiation, apoptosis, and protein synthesis. These pathways can induce PI3K to produce phosphatidylinositol 3,4,5-trisphosphate, which provides anchor sites for proteins containing the PH structural domain, including AKT and its upstream activator pyruvate dehydrogenase kinase 1 (PDK1) [18, 19]. Phosphorylated AKT activates or inhibits downstream target proteins such as BCL2-associated agonist of cell death (Bad), cysteinyl aspartate-specific proteinase (Caspase), and nuclear factor kappa-B (NF- $\kappa$ B) through phosphorylation, thereby regulating cell function [20–23]. Among these, phosphorylated AKT can mediate mitochondrial apoptosis by regulating downstream genes Bcl-2/BAX and Caspase [24–26]. Phosphatase and tensin homolog (PTEN), a tumor suppressor gene, is widely believed to greatly inhibit the expression of PI3K as well as AKT [27]. The PTEN/PI3K/AKT pathway is important for cells, contributing to cell growth, survival, and metabolism, and enabling genomic integrity [28, 29]. Based on recent studies, it has been confirmed that the massive production of ROS not only causes obvious oxidative stress but also upregulates the expression of PTEN to inhibit the PI3K/AKT pathway [30–32]. Under certain environmental stimuli, this is a common way for cells to undergo apoptosis. In conclusion, ROS, as a chemical messenger of cell signaling, can activate the PTEN/PI3K/AKT pathway and cause cell apoptosis [18, 33–35]. The ROS/PTEN/PI3K/AKT axis plays a significant role in elucidating the mechanisms of how a variety of toxicants damage cells [28, 36, 37]. It has been extensively shown that many toxicants induce apoptosis through this pathway, such as chlorpyrifos, which induces oxidative stress in L8824 cells, thereby inhibiting the PI3K/AKT pathway and regulating the mitochondrial apoptosis pathway [38]. Albicanol also has a significant antagonistic effect on profenofos-induced apoptosis in grass carp hepatocyte cells through the ROS/PTEN/PI3K/AKT axis [39].

Zinc (Zn) is an important trace element in the human body that not only nourishes the male reproductive organs, but also has antioxidant and anti-inflammatory effects [40]. Zn can influence the accumulation of heavy metals in tissues and the sensitivity of the organism to them. Appropriate amounts of Zn supplementation can reduce the damage caused by heavy metals [40–42], such as Pb. In plants, the toxicity of Pb to leaves is greatly weakened when in the cultivation medium of Pb and Zn [43]. Recent researches also shown that Zn counteracts oxidative stress and ameliorates sperm damage caused by Pb in mouse models. [44]. However, few studies have demonstrated the antagonistic effect

of Zn on germ cells exposed to Pb and elucidated its mechanism completely. Hence, we would like to know whether Zn had antagonism on germ cell exposed to Pb and explained the mechanism in detail. The pig, as a mammal, has several organs physiologically similar to the human body. Meanwhile, The ST cell, which is considered as an important type of germ cell, is widely used in lots of toxicology experiments [45, 46]. Therefore, ST cell is a good model for studying the reproductive toxicity of chemicals [45, 47, 48]. In our study, we determined the IC<sub>50</sub> of Zn on ST cells and the optimal inhibitory concentration of Zn on Pb, established 4 groups of corresponding cell models, and probed the cell apoptosis situation by CCK-8 method and flow cytometry. Then, we analyzed the Pb-induced oxidative stress and the alleviating effect of Zn by DCFH-DA staining and measurement of relevant oxidative stress indicators. Finally, we examined the mRNA and protein expression level of indicators related to the PTEN/PI3K/AKT axis and the apoptosis pathway by RT-PCR and Western blots, to explore the specific mechanism that Zn antagonizes the cytotoxicity of Pb. The purpose of our study was to assess the toxicity of Pb to the reproductive system and whether Zn could mitigate the effects of Pb on ST cells and to explore the mechanism of action.

## Materials and Methods

### Materials

Lead acetate (> 98% purity), Pb (CH<sub>3</sub>COO)<sub>2</sub>, and zinc acetate (> 98% purity), Zn (CH<sub>3</sub>COO)<sub>2</sub>, were supplied by Mingshuo Chemical Company (Tianjin, China). Zn and Pb ions were co-dissolved in PBS buffer (pH = 7.4) and filtered using a 0.22- $\mu$ M microporous filter.

### ST Cell Culture

Porcine testicular cell lines (ST, ATCC, USA) were cultured in modified DMEM basic (Gibco, USA) supplemented with 12% fetal bovine serum (Solarbio, China) and 2% penicillin-streptomycin (Solarbio, China). ST cells were inoculated at a density of  $5 \times 10^6$  cells/cm<sup>2</sup> in 25 cm<sup>2</sup> culture flasks. After at least three cell passages in a 37 °C, 5% CO<sub>2</sub> incubator, the recovered cells were used for the formal assay.

### Cell Viability Assay and Treatment

We seeded ST cells into 96-well culture plates. After the cells adhered to the sides of the wells, we treated them with different concentrations of Pb (200  $\mu$ M, 400  $\mu$ M, 600  $\mu$ M, 800  $\mu$ M, 1000  $\mu$ M, 1200  $\mu$ M) or Zn (10  $\mu$ M, 20  $\mu$ M, 30  $\mu$ M, 40  $\mu$ M, 50  $\mu$ M) for 24 h. Using CCK-8, a detection method that can quickly reflect the proportion of

living cells, we measured cell vitality and calculated cell viability of each group. IC50 (the half-maximal inhibitory concentration) indicates the concentration of poison that reduces 50% of cell viability, which can assess the toxicity of the poison in cytotoxicity. The optimal inhibitory concentration can represent the antagonist's effect in a poison exposure environment. Hence, we confirmed the half maximal inhibitory concentration of Pb on ST cells and the optimal antagonistic concentration of Zn. Cells were treated as follows: 0  $\mu\text{M}$  Pb and 0  $\mu\text{M}$  Zn (Control group), 100  $\mu\text{M}$  Pb and 0  $\mu\text{M}$  Zn (Pb group), 0  $\mu\text{M}$  Pb and 10  $\mu\text{M}$  Zn (Zn group), and 100  $\mu\text{M}$  Pb and 10  $\mu\text{M}$  Zn (Pb + Zn group).

### Flow Cytometry

Flow cytometry is a biological technology used to count and sort tiny particles suspended in fluid [49]. We collected  $1 \times 10^5$  cells from each of the four different groups and washed them twice with PBS. Annexin V-FITC/PI was then added, and the percentage and type of dead cells in each group were quantified using flow cytometry. Cells were classified into four categories: q1 representing necrotic cells, q2 representing early apoptotic cells, q3 representing late apoptotic cells, and q4 representing live cells. The results were analyzed with FlowJo software.

### DCFH-DA Staining

DCFH-DA staining can detect the generation of reactive oxygen species in cells. DCFH-DA, a non-fluorescent compound, can penetrate the cell membrane and be converted to DCFH by hydrolase. ROS in cells can oxidize non-fluorescent DCFH to produce fluorescent DCF. The cells from the four groups were washed twice with PBS and incubated with the fluorescent probe DCFH-DA for 15 min. Finally, the results were observed using a fluorescence microscope and analyzed with Image J software.

### Determination of Enzymes Related to Antioxidant Stress System

ST cells from the four groups were collected and resuspended in 100  $\mu\text{L}$  PBS. The activity of malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GPX), and superoxide dismutase (SOD) was measured using test kits (A001-1-2 SOD test kit, A003-1-2 MDA test kit, A007-1-1 CAT test kit, A005-1-2 GSH-Px test kit, from Jiancheng Biological Research Institute, Nanjing, China).

### RNA Extraction and Quantitative Real-Time PCR

We extracted total RNA from ST cells in four groups using TRIzol (Invitrogen, China) and synthesized cDNA from RNA using a cDNA reverse transcription kit (Bioer, Hangzhou China). We added 10  $\mu\text{L}$  of mixed reactants to each well of a 96-well plate, including 1  $\mu\text{L}$  of diluted cDNA, 5  $\mu\text{L}$  of  $2 \times$  SYBR Green PCR Master, 3.4  $\mu\text{L}$  of PCR-grade water, 0.3  $\mu\text{L}$  of upstream primers, and 0.3  $\mu\text{L}$  of downstream primers (as listed in Table 1). The mRNA expression level of the target gene was evaluated using the LineGene 9600 system. We used  $\beta$ -actin as the internal reference and calculated the mRNA expression of the target gene using the  $2^{-\Delta\Delta\text{Ct}}$  method.

### Western Blot Analysis

The cells treated with cell lysis buffer and PMSF were thoroughly mixed on ice and then centrifuged at 12,000 rpm at 4  $^{\circ}\text{C}$ . Next, we pipetted the supernatant into a new conical centrifuge tube, added SDS, and boiled the sample for 10 min. The total protein in the sample was separated by SDS-PAGE and transferred onto a nitrocellulose membrane in a transfer tank containing Tris-glycine electrophoresis buffer. The membrane was then sealed with 5% skimmed milk at 37  $^{\circ}\text{C}$  for 3 h and incubated overnight with the primary antibody. Information

**Table 1** Gene primers used in quantitative real-time PCR

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
$\beta$ -actin	AGAGAGGCATCCTGACCCT	CCATCTCCTGCTCGAAGTCC
PTEN	TGCTATGGGGTTTCCTGCAG	CGTGTGGGTCTGAATTGGA
PI3K	GGGTTAACCATGCAGCTCCT	CAGGGGCTTCTTCTGGAGG
AKT	GAGGAGATGGAGGTGTCCCT	TTGATGCCCTCCTGCAGAG
BCL-2	CGCTGGGAGAACAGGGTATG	CATCCCAGCCTCCGTTATCC
Bax	CATGAAGACAGGGGCCCTTT	CTCCAGATGGTGAGCGAGG
Cyt-c	AGAAGTTGACGGGAGCATGG	TCAAGCCGGTCTGAATCTG
Caspase-3	ACCGAATGGCATGTGATC	CTGTTTCAGCGCTGCACAAA
Caspase-9	ACCCCTGACATGATCGAGGA	AAATGCAGCAAGTGGAAGCC

**Table 2** Information on antibodies used for Western blot

Antibodies	Dilution ratio	Company
$\beta$ -actin	1:10000	Sigma-Aldrich
PTEN	1:1500	Wanleibio
PI3K	1:1000	Wanleibio
P-AKT	1:1000	Wanleibio
BCL-2	1:500	Wanleibio
Bax	1:500	Wanleibio
Cyt-c	1:300	Wanleibio
Caspase-3	1:1000	Wanleibio
Caspase-9	1:500	Lab Homemade

and dilution ratios of the different antibodies used in this experiment are shown in Table 2. Following this, we washed the membrane three times with TBST, incubated it with the secondary antibody for 2 h at 25 °C, and washed it again with TBST. Finally, the enhanced chemiluminescence system was used to measure the protein bands. We used  $\beta$ -actin protein as the internal reference and calculated the relative protein expression level using Image J.

### Statistical Analysis

We repeated all the experiments for three times and showed the experimental results through GraphPad Prism. The experimental data was analyzed using analysis of variance (ANOVA) and expressed in the form of mean  $\pm$  standard deviation (SD). The different number of \* between the two groups represents their significant difference.

## Results

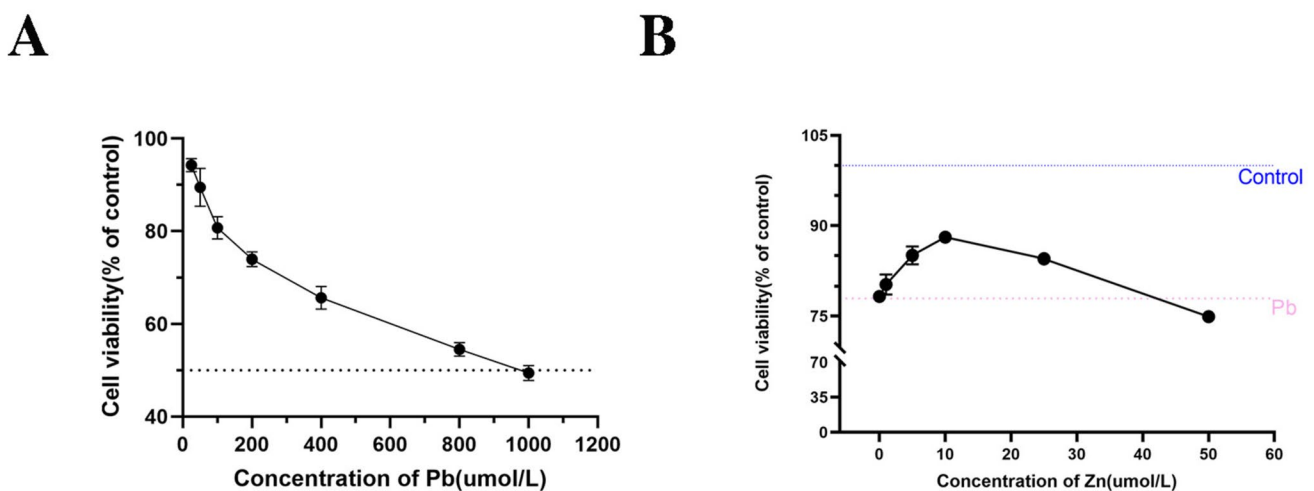
### Pb and Zn Exposure Effect on ST Cell Viability

Cell viability exposed to different concentrations of Pb was measured using the CCK-8 method. As depicted in Fig. 1A, ST cell activity decreases proportionally with increasing Pb concentration. Upon calculation, we confirmed that the IC50 of Pb was 994.4  $\mu$ M.

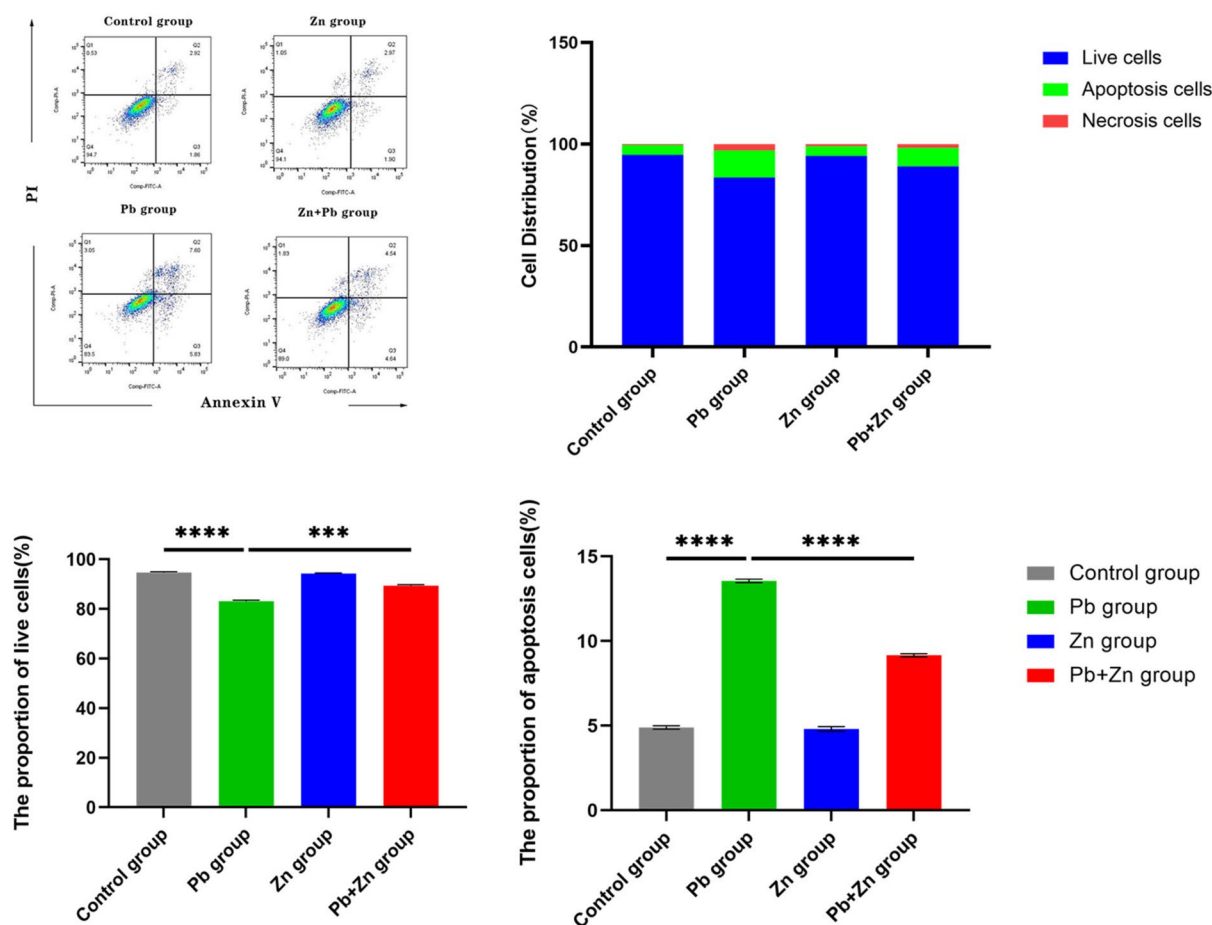
We selected 100  $\mu$ M lead concentration as the Pb group, and cell viability was observed to be 77% under this condition. Based on this, ST cells were then exposed to varying concentrations of Zn. Results show that cell viability in the Pb group was significantly improved by lower doses of zinc, with the optimal inhibitory concentration of Zn being 10  $\mu$ M (Fig. 1B). However, with the increase of Zn concentration, cell activity gradually decreased, indicating a combined toxicity to ST cells. We established four cell models, each treated as follows: 0  $\mu$ M Pb and 0  $\mu$ M Zn (control group), 100  $\mu$ M Pb and 0  $\mu$ M Zn (Pb group), 0  $\mu$ M Pb and 10  $\mu$ M Zn (Zn group), and 100  $\mu$ M Pb and 10  $\mu$ M Zn (Pb + Zn group).

### Zn Alleviates Pb-Induced ST Cells Apoptosis

As shown in Fig. 2, the proportion of apoptotic cells in the Pb group increases significantly ( $p < 0.05$ ) compared to the control group. However, the addition of Zn to the Pb-treated cells reduced the proportion of apoptotic cells from 13.43 to 9.18%. Moreover, there was no significant difference in the proportion of live and apoptotic cells between the control and Zn groups ( $p > 0.05$ ). These results demonstrated that Pb induced apoptosis of ST cells, while Zn could effectively counteract its cytotoxic effects.



**Fig. 1** The effect of Pb on the viability of ST cells (A). ST cells were treated with different concentrations of Pb. And then we pretreated ST cells with 100uM Pb followed by administering with different concentrations of Zn. Each value represents the mean  $\pm$  SD of 3 individuals (B)



**Fig. 2** The effect of Zn and/or Pb on the death patterns of ST cells. The proportion of apoptosis cells and living cells was detected by the flow cytometry (q1 region is necrotic cell, q2 region is early apopto-

sis cell, q3 region is late apoptosis cell, and q4 region is living cell). Each value represents the mean  $\pm$  SD of 3 individuals, \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$

### Zn Improves Pb-Induced Oxidative Stress of ST Cells

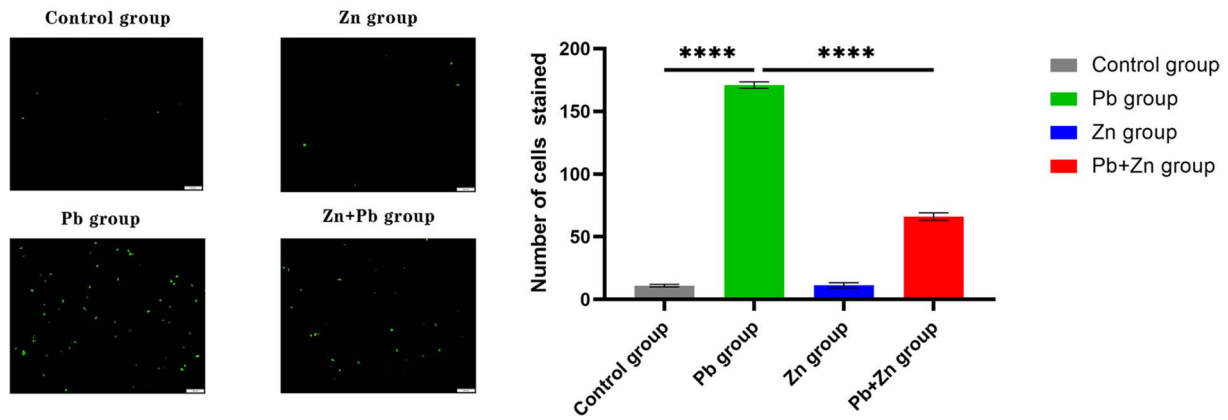
To confirm the ability of Zn to mitigate Pb-induced oxidative stress in ST cells, we used the DCFH-DA staining method to detect the four groups of ST cells. As shown in Fig. 3, the Pb group exhibits a stronger green fluorescence compared to the control group, indicating increased ROS generation due to Pb exposure. However, the green fluorescence in the Pb+Zn group significantly decreased, suggesting that Zn can alleviate Pb-induced oxidative stress. We quantified the results using Image J software and found a significant difference between the groups ( $p < 0.05$ ).

Furthermore, we measured relevant oxidative stress indicators (Fig. 4) to comprehensively clarify the antagonistic effect of Zn on Pb-induced oxidative stress. We observed a decrease in the activity of SOD, CAT, and GPX, as well as an increase in MDA levels in the Pb+Zn group compared to the Pb group ( $p < 0.05$ ). There was no significant difference between the Zn and control groups

( $p > 0.05$ ). These results suggested that Zn could effectively improve Pb-induced oxidative stress in ST cells.

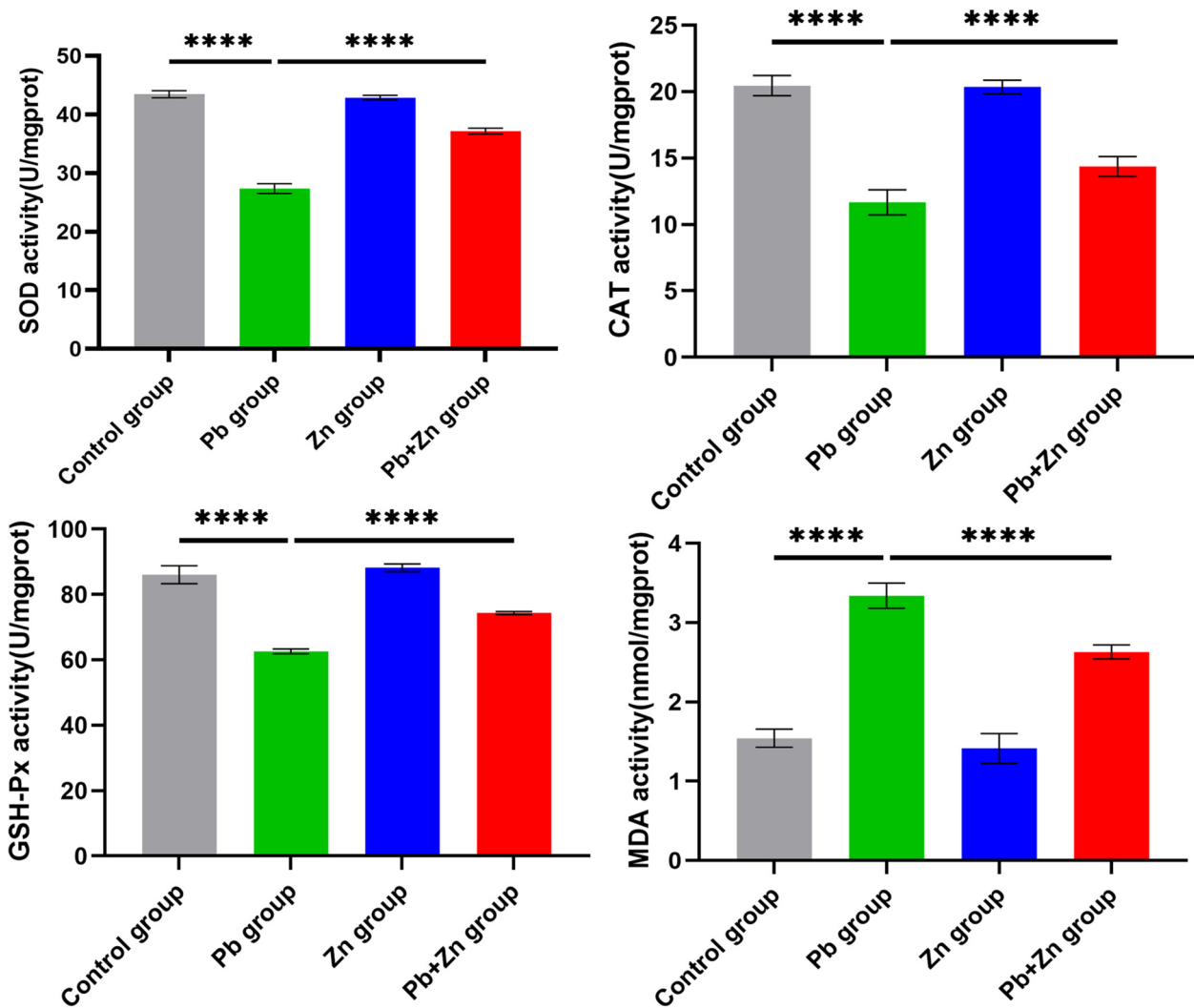
### Zn Regulates the Expression of PTEN/PI3K/AKT Pathway of ST Cells Exposed to Pb

To clarify the specific mechanism of Zn's inhibition against Pb toxicity, we measured the mRNA and protein expression level of indicators related to the AKT/PI3K/PTEN pathway. Recent studies have shown that a large number of ROS can activate PTEN and inhibit the PI3K/AKT pathway. As shown in Fig. 5, we observe a notable increase in the expression of PTEN in the Pb group, while the expression of PTEN in the Zn+Pb group decreases compared to the Pb group ( $p < 0.05$ ). Moreover, the expression of PI3K and AKT decreased in the Pb group, but increased significantly in the Zn+Pb group compared to the Pb group ( $p < 0.05$ ). The results indicated that Zn could regulate the PTEN/PI3K/AKT pathway in Pb-exposed ST cells.

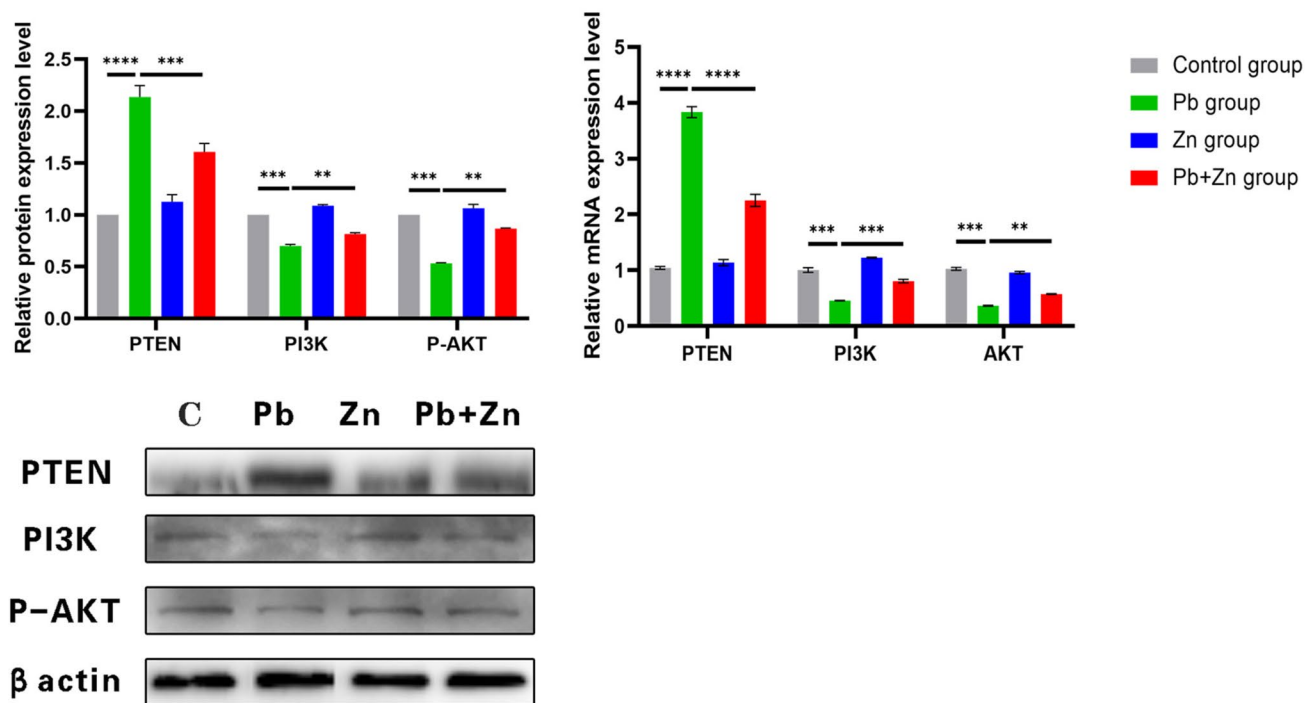


**Fig. 3** The effect of Zn and/or Pb on the production of reactive oxygen species in ST cells was observed by DCFH-DA fluorescence staining. And calculating the number of cells with green fluorescence

from three randomly selected visual fields. Each value represents the mean  $\pm$  SD of 3 individuals, \*\*\*\* $p < 0.0001$



**Fig. 4** The effect of Zn and/or Pb on the antioxidant stress system of ST cells was detected by the test kit of SOD, CAT, MDA, and GSH-Px. Each value represents the mean  $\pm$  SD of 3 individuals, \*\*\*\* $p < 0.0001$



**Fig. 5** The effect of Pb and/or Zn on the expression of the genes related to the PTEN/PI3K/AKT pathway in ST cells. The mRNA and protein expression levels of the PTEN/PI3K/AKT pathway in the four treatments (control group, Pb group, Zn group, Pb+ Zn group) were

detected by RT-PCR and Western blot analysis. Each value represents the mean  $\pm$  SD of 3 individuals, \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$

### Zn Alleviates the Expression of Apoptosis Pathway of ST Cells Exposed to Pb

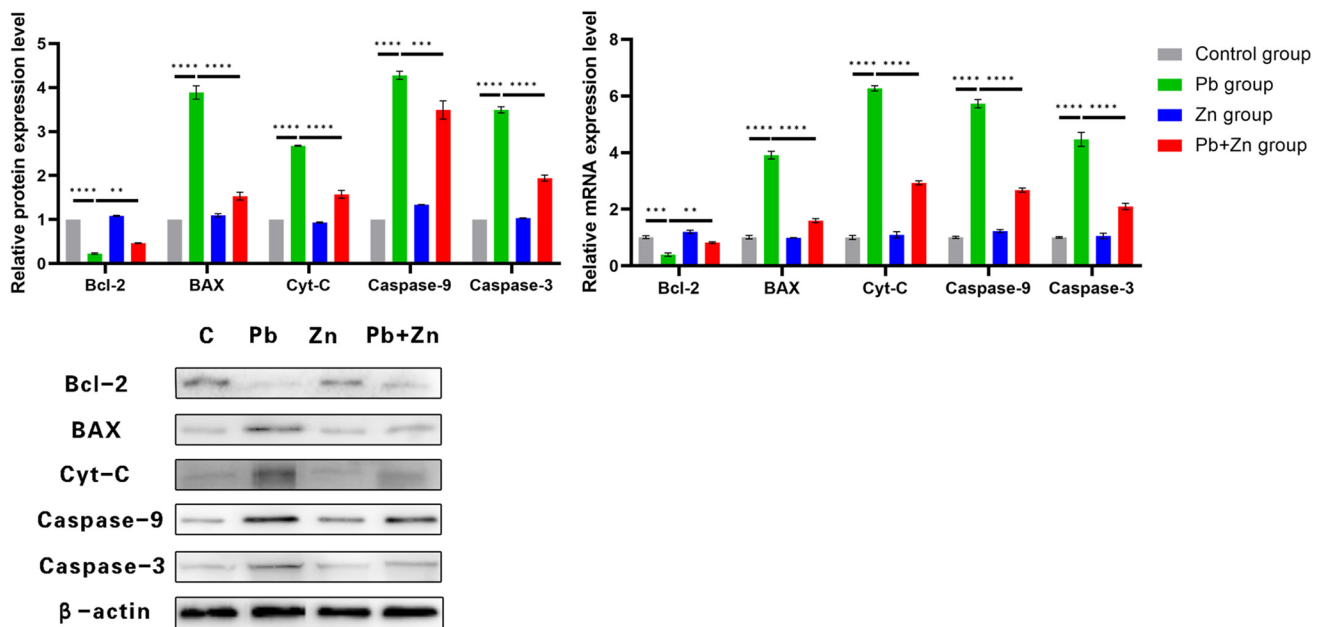
To verify the activated apoptosis pathway exposed to Pb and the antagonism of Zn, we detected the mRNA and protein expression level of indicators related to the apoptosis pathway. As shown in Fig. 6, we observe a notable increase in the expression of Caspase 3, Caspase 9, BAX, and Cyt-c in the Pb group, while the expression of these genes decreases significantly in the Zn+Pb group compared to the Pb group ( $p < 0.05$ ). More than this, the expression of Bcl-2 decreased in the Pb group, but increased clearly in the Zn+Pb group compared to the Pb group ( $p < 0.05$ ). These results suggested Zn could alleviate the expression of the apoptosis pathway in Pb-exposed ST cells.

### Discussion

Lead (Pb) is a prevalent environmental pollutant in nature that has been shown to cause male reproductive dysfunction and organic lesions, which reduces the proliferation of male reproductive cells by inducing apoptosis signal transduction [3, 44]. Zinc (Zn) is an essential trace element in the human body with antioxidant and anti-inflammatory effects. It can nourish male

reproductive organs and antagonize the effects of various heavy metals, such as cadmium [16, 38, 47]. In this study, we aim to confirm the effect of Pb on ST cells and explain how Zn alleviates the damage caused by Pb. Our research indicated that Pb exposure induced ST cells to ROS, increased PTEN expression, decreased PI3K and AKT expression, and ultimately led to cell apoptosis. Furthermore, we found that Zn improved the oxidative stress of ST cells exposed to Pb by reducing ROS production, hence alleviating the destruction of the PTEN/PI3K/AKT pathway and ultimately decreasing cell apoptosis. Our study proved that Zn inhibited Pb-induced apoptosis and oxidative stress of swine testicular cells through the ROS/PTEN/PI3K/AKT axis.

ROS is a natural by-product of normal oxygen metabolism and plays a crucial role in cell signal transduction [47]. Under environmental pressure, ROS levels can increase sharply, causing oxidative stress to cell structures [48]. In our study, we found that a large amount of ROS was produced in ST cells exposed to Pb. Moreover, the content of several important antioxidant stress kinases, including SOD, CAT, and GPX, decreased significantly. At the same time, the amount of malondialdehyde (MDA), which is one of the conjugates formed by the reaction of lipid and oxygen free radicals [48], significantly increased. These experiments fully demonstrated



**Fig. 6** The effect of Pb and/or Zn on the expression of the genes related to the PTEN/PI3K/AKT pathway in ST cells. The mRNA and protein expression levels of the PTEN/PI3K/AKT pathway in the four treatments (control group, Pb group, Zn group, Pb+ Zn group) were

detected by RT-PCR and Western blot analysis. Each value represents the mean  $\pm$  SD of 3 individuals, \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$

that Pb caused severe oxidative stress in cells. However, after the co-culture of Zn and Pb, the oxidative stress of cells significantly alleviated, and the relevant indicators recovered to nearly 50%. This showed that Zn had the effect of antagonizing oxidative stress in cells exposed to Pb. The excessive production of ROS can damage lipids, proteins, and DNA, leading to cell death. We conducted flow cytometry to prove this point and found that the proportion of dead cells exposed to Pb and Zn was significantly lower than that exposed to Pb alone, which confirmed the antagonism of Zn to Pb toxicity. It has been reported that massive ROS can activate PTEN, thereby inhibiting the PI3K/AKT pathway to initiate the apoptosis program of cells [29]. Therefore, we detected the expression of relevant mRNA and protein. Our results demonstrated that excessive ROS can activate the expression of PTEN, thus inhibiting the expression of PI3K/AKT pathway in ST cells exposed to Pb. Furthermore, Zn could reduce the oxidative stress of cells, improved the destruction of PTEN/PI3K/AKT pathway, and maintained the stability of cell function.

The PI3K/AKT pathway, which is related to phosphatidylinositol, plays an essential role in cell survival and regulates apoptosis through various mechanisms. In this study, we aimed to investigate the mechanism of Pb-induced apoptosis and the protective effects of Zn by examining the expression of genes related to apoptosis. The Bcl-2 protein family, which regulates mitochondrial

membrane potential, is known to be involved in apoptosis. Among these, BAX and Bcl-2 are particularly important. Previous studies have shown that increased BAX expression can initiate programmed cell death, while increased Bcl-2 expression can protect cells by forming Bcl-2/BAX dimers. Additionally, phosphorylated AKT can prevent the secondary destruction of the mitochondrial membrane, leading to the inhibition of the apoptosis pathway. Therefore, we measured the mRNA and protein expression levels of these genes to understand their involvement in Pb-induced apoptosis. Our results showed that Pb activated the apoptosis pathway in ST cells, leading to programmed cell death. However, Zn was found to reduce the damage to mitochondria by inhibiting ROS production, and it also decreased apoptosis by alleviating the destruction of the PTEN/PI3K/AKT pathway. We measured the mRNA and protein expression levels of the aforementioned genes to verify this.

In summary, our study demonstrated that Zn could significantly counteract the toxicity induced by Pb in ST cells through the ROS/PTEN/AKT/PI3K axis. Our research expanded the toxicological knowledge of Pb, providing valuable insights into the underlying mechanism of its reproductive toxicity and the antagonistic effects of Zn. Although the complex environmental problem of Pb pollution is still being explored, our findings suggest that Zn may serve as a potential antidote to Pb.



**Author Contribution** Haoyu Zhang was in charge of conceptualization, methodology, formal analysis, data curation, and writing the main manuscript text.

Kexin Sun was in charge of resources, visualization, and also writing part of the manuscript text.

Meichen Gao was in charge of validation and investigation.

Shiwen Xu was in charge of review and editing the manuscript text.

**Data Availability** Data and materials are available on request from the authors.

## Declarations

**Competing Interests** The authors declare no competing interests.

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