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BPA and low-Se exacerbate apoptosis and mitophagy in chicken pancreatic cells by regulating the PTEN/PI3K/AKT/mTOR pathway

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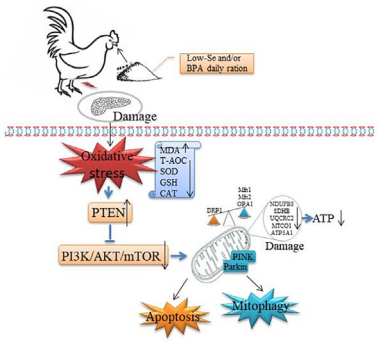
HIGHLIGHTS

- BPA and low-Se induced pancreatic tissue damage, apoptosis, and mitophagy.
- PTEN/PI3K/AKT/mTOR pathway was involved in apoptosis and mitophagy induced by BPA and low-Se.
- BPA and low-Se induced mitochondrial dysfunction and homeostasis imbalance.
- The co-exposure of BPA and low-Se exacerbated pancreatic tissue damage, apoptosis, and mitophagy.

GRAPHICAL ABSTRACT



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ABSTRACT

Introduction: Bisphenol A (BPA) is a widespread environmental pollutant which has serious toxic effects on organisms. One of the crucial trace elements is selenium (Se), whose shortage can harm biological tissues and enhance the toxicity of contaminants, in which apoptosis and autophagy are core events.
Objectives: An in vivo model was established to investigate the effects of BPA and low-Se on chicken pancreatic tissue, and identify the possible potential molecular mechanism.
Methods: A total of 80 1-day-old broiler chickens (Xinghua Chicken Farm, Harbin, China) were stochastically divided into 4 groups (n = 20/group): Control group, BPA group, low-Se group, and low-Se + BPA group. Pancreatic tissue was collected at day 42 to detect changes in markers.
Results: First, the data showed that BPA and low-Se exposure gave rose to structural abnormalities in pancreatic tissue, oxidative stress, mitochondrial dysfunction and homeostasis imbalance, apoptosis and mitophagy. In addition, the co-exposure of BPA and low-Se caused the most serious damage to pancreatic tissue. In terms of mechanism, it was found that apoptosis and mitophagy induced by BPA and low-Se were related to the activation of PTEN/PI3K/AKT/mTOR pathway.
Conclusion: In summary, the study found that BPA and low-Se exacerbated mitochondria damage, apoptosis and mitophagy by regulating the PTEN/PI3K/AKT/mTOR pathway.

Abbreviations: BPA, Bisphenol A; Se, selenium; PI3K, Phosphatidylinositol 3-kinase; AKT, Protein kinase B; mTOR, Mammalian target of rapamycin; AFB1, Aspergillus flavus B1; TEM, Transmission electron microscopy; H.E. staining, Hematoxylin-eosin staining; CAT, Catalase; SOD, Superoxide dismutase; MDA, Malondialdehyde; GSH, Glutathione; ATP, Adenosine triphosphate; T-AOC, Total antioxidant capacity; qRT-PCR, Real-time quantitative PCR; TUNEL staining, Terminal deoxynucleotidyl nick-end labeling staining; IF staining, Immunofluorescence staining; ROS, Reactive oxygen species; GSH-Px, Glutathione peroxidase; LPS, Lipopolysaccharide.

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Introduction

Bisphenol A (BPA) is a prevalent chemical contaminant that commonly applied in the production of various of industrial and everyday products [1]. Since the 1950 s, BPA has been widely used in the production of electrical equipment, bottles, beverage containers, etc., and can be ingested by humans and animals through leaching into food [2,3]. BPA poses a potential threat to ecosystems and organism health [4,5]. According to reports, from 2011 to 2012, the average daily intake of BPA by the general population in the United States was 25 ng/kg/d [6]. Based on global urinary concentration data from 2000 to 2016, the maximum daily intake of BPA for adults was 64.75 ng/kg/day [7]. However, in China, the maximum daily intake of BPA for adults can reach 106.77 ng/kg/day [8]. BPA is an endocrine disruptor and have estrogenic effects, or it can bind directly to androgen receptors to block endogenous androgenic effects [9,10]. BPA is also interacted with multiple physiological systems and organs, for example, the central nervous system, immune system, pancreas, and thyroid [11]. Several reports have shown that BPA exposure can lead to oxidative stress, apoptosis, mitophagy, metabolic disorders, and mitochondrial dysfunction [12–18].

Oxidative stress is induced by the imbalance of the body's oxidative/antioxidant system. However, BPA exposure could cause oxidative stress and further trigger multifarious signaling pathways, leading to cell apoptosis, and autophagy [19,20]. PTEN is a classic tumor suppressor factor that is crucial for cell energy metabolism and survival, mainly contained in regulating the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway [21]. The PI3K/AKT pathway is a classic signaling pathway, which is critical for maintaining cell development, basic functions, and stability [22]. Pollutants exposure can also induce autophagy and accelerate cell apoptosis in PC12 cells by mediating the PTEN/PI3K/AKT pathway [23]. Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that can respond to a variety of environmental signals. The PI3K/AKT pathway is an upstream regulatory factor of mTOR, which can regulate mTOR and further affect cell cycle and metabolism [24]. Zhou et al. discovered that Tan I caused apoptosis and autophagy in ovarian cancer cells by regulating the PI3K/AKT/mTOR pathway [25]. PTEN also protects the kidney from acute kidney injury by mediating PI3K/AKT/mTOR pathway to reduce apoptosis and promote autophagy [26]. In addition, it has been found in mouse experiments that BPA can disrupt pancreatic endocrine blood glucose homeostasis in mice [13].

Selenium (Se) is a vital trace element in living organisms, playing a vital role in antioxidant, anti-inflammatory, detoxifying, and enhancing the body's immunity. Reports have shown that dietary low-Se caused pancreatic injury, apoptosis of pig intestinal epithelial cells, apoptosis of rat liver cells, and bursa autophagy [27–32]. Low-Se and toxicity of other environmental pollutants also have synergistic effects, for example, low-Se aggravates the immune toxicity of chicken spleen and liver induced by Aspergillus flavus B1 (AFB1) [33,34]. However, the mechanism of the toxic action of BPA and low-Se on the chicken pancreas and whether there is a synergistic effect of the two negative effects are unknown. Therefore, in this study, a series of animal models of BPA and low-Se were established. In this experiment, transmission electron microscopy (TEM), hematoxylin-eosin (H.E.) staining, kit detection, real-time quantitative PCR (qRT-PCR), Western blot analysis and other experimental techniques were used to investigate the mechanism of BPA and low-Se induced pancreatic toxicity. This study not only enriched the mechanisms of pathology, but also enriched our understanding of them.

Materials and methods

Ethics statement

The Northeast Agricultural University Ethics Committee (Approved Number: NEAUEC-2023–03111, China) gave its approval to all experimental methods.

Animal and experimental design

A total of 80 1-day-old broiler chickens (Xinghua Chicken Farm, Harbin, China) were stochastically divided into 4 groups (n = 20/group): Control group, BPA group, low-Se group, and low-Se + BPA group. The chickens in all groups had free access to food and water. The feed components of each group are shown in Table 1 (Supplementary material). The chickens were fed for 42 days. On the 42 day, each chick fasted for 24 h and were killed by heart puncture [35]. The collected pancreatic tissues were stored in 10 % formaldehyde or −80 °C.

Oxidative stress detection

Pancreatic oxidative stress indexes (Nanjing Jiancheng, China, A001-3 SOD assay kit, A006-1–1 GSH assay kit, A007-1–1 CAT assay kit, A003-1 MDA assay kit, A015-2–1 T-AOC assay kit) were detected with kit. Strictly follow the manufacturer's operating instructions. In brief, a 10 % normal saline homogenate with 0.1 g pancreas was prepared, centrifuged (7500 r/min, 10 min), the supernatant was collected, and the OD value was measured according to the manufacturer's guidelines. The activity or content of SOD, GSH, CAT, MDA and T-AOC were calculated according to the formula (Supplementary material).

ATP content determination

The content of ATP in pancreatic tissue was detected by kit (Nanjing Jiancheng, China, A095–1–1 ATP content assay kit). Strictly follow the manufacturer's operating instructions. In brief, a 10 % normal saline homogenate with 0.1 g pancreas was prepared, centrifuged (7500 r/min, 10 min), the supernatant was collected, and the OD value was measured according to the manufacturer's guidelines. The content of ATP was calculated according to the formula (Supplementary material).

H.E. staining

Pancreatic tissues were fixed for 24 h (10 % formaldehyde), embedded in paraffin, and sliced (0.5 μm), stained with hematoxylin and eosin. Image observation was done with a light microscope.

TEM observation

The steps were as follows: (1) Each group of pancreatic tissue were fixed with 2.5 % glutaraldehyde; (2) 1 % osmium tetroxide were used for fixation; (3) Analytical pure-grade ethanol was used for dehydration; (4) The samples were embedded in alardate and cut into thin slices; (5) Images were captured using an H-9500 TEM after the slices had been dyed with lead citrate and uranium acetate.

Immunofluorescence (IF) staining

According to Wu et al. 's research[36], specific test operations were carried out. (1) Pancreatic tissue was fixed with 10 % formaldehyde; (2) Embedded in paraffin; (3) Gradient alcohol dewaxing; (4) Antigen repair; (5) Seal the sections with serum (5 % FBS-TBST) for 30 min and incubate overnight with the p62 and LC3B antibodies (p62, 1:200, Bioss, China; LC3B, 1:200, ABclonal, China); (6)the next day, incubate the Dylight 488 goat anti-rabbit IgG and Dylight 594 goat anti-rabbit IgG antibodies (Dylight 488 goat anti-rabbit IgG, 1:1000, Biodragon, China; Dylight 594 goat anti-rabbit IgG, 1:1000, Biodragon, China) for 30 min; (7) After sealing, observe and take photos using a fluorescence microscope.

Terminal deoxynucleotidyl nick-end labeling (TUNEL) staining

The TUNEL kit (Shanghai Biyuntian, China) was applied to detect the number of apoptotic cells in pancreatic samples, and optical microscope (Olympus BX63, Japan) was used to observe and took photos of apoptotic cells. After fixation, dehydration, paraffin embedding, and sectioning (5 μm), the pancreatic was stained. The nucleus of TUNEL stained positive cells were brownish brown, while the normal cells were blue.

qRT-PCR analysis

Total RNA were separated from pancreatic tissue using the Trizol method [37]. Detailed procedures and contents were provided in the supplementary materials. The primer sequences are shown in Table 2 (supplementary materials).

Western blot analysis

The tissue lysates (PMSF + IP = 100:1, Beyotime, China) were used to process pancreatic tissue to extract total protein for subsequent Western blot analysis [38]. Detailed steps can be found in supplementary materials. The dilution concentration of the antibodies was shown in Table 3 (supplementary materials).

Statistical analysis

All test data were normal distribution. One-way ANOVA and Tukey's method were used to compare the differences between groups for statistical significance. Every piece of data was shown as mean ± standard deviation. GraphPad Prism was applied to statistically analyze all the data. Different superscripts indicate significant differences ($P < 0.05$), while containing the same superscripts indicates no significant differences ($P \geq 0.05$).

Results analysis

Effects of BPA and low-Se on pancreatic pathological damage

Light microscopy was used to conduct histopathological examination of the chicken pancreatic in all groups. Hematoxylin dyes can stain the nucleus and cytoplasmic ribosomes blue, and eosin dyes can stain the cytoplasmic and extracellular matrix components red, so the histological cells state can be judged according to different colors and their distribution. The results of Fig. 1 A indicated that the Control group didn't show any pathological changes, and the acini were arranged neatly with complete structure. The glia in the acinar cavity was uniform and the contours were visible. The BPA group and low-Se group showed significant pathological changes in the pancreas, with some acinar epithelial cells showing atrophy, degeneration, or loss, disordered acinar arrangement, some nuclear pyknosis or dissolution disappearance, and slight vacuolar degeneration. However, the pathological changes in the low-Se + BPA group were more pronounced, with a loose acinar arrangement and widened interlobular spaces.

In addition, the TEM method was used to further observe the ultrastructure of the pancreas. The results of Fig. 1 B indicated that the cellular structure of pancreas tissue in the Control group was clear and the organelle structure was complete. Autophagy bodies (Red arrow), mitochondrial ridge breaks, and chromatin agglutination were found in pancreatic cells of the BPA group and low-Se group. The same phenomenon was observed in the pancreatic cells in the low-Se + BPA group, and the ultrastructural damage of pancreatic cells in the co-exposure group was significantly increased compared with that in the bisphenol a group and the low-Se group.

Effects of BPA and low-Se on pancreatic cells apoptosis and autophagy

Firstly, the apoptosis of pancreatic cells apoptosis and autophagy were detected by IF staining. Compared to the Control group, the TUNEL staining showed that a significant increase in the number of apoptotic cells which induced by BPA and/or low-Se exposure (Fig. 2 A, B). The IF staining results of pancreatic tissues showed that compared with the Control group, the fluorescence intensity of autophagy index LC3B was significantly enhanced, and the fluorescence intensity of autophagy index p62 was significantly decreased in all treatment groups ($P < 0.05$) (Fig. 2 C, D). In addition, the number of autophagy and apoptotic cells in the co-exposure group was higher than that in the single treatment group. This indicated that both BPA and low-Se induced apoptosis and autophagy, and their co-exposure exacerbated cell apoptosis and autophagy.

Effects of BPA and low-Se on pancreatic oxidative stress

Oxidative stress is often the trigger of various diseases or pathological lesions, so the oxidative stress indicators (T-AOC, MDA CAT, GSH, SOD) were measured using commercial kits. Compared with the Control group, the MDA contents in the BPA and/or low-Se groups were increased, and the activities or levels of CAT, SOD, GSH, T-AOC were reduced ($P < 0.05$). In particular, the oxidative stress induced by combined exposure was stronger than that of the single treatment group (Fig. 3). This indicates that both BPA and low-Se resulted in oxidative stress in the chicken pancreas, and the co-exposure group had more severe oxidative stress.

Effects of BPA and low-Se on the PTEN/PI3K/AKT/mTOR pathway in pancreatic cells

The effects of apoptosis and autophagy induced by BPA and low-Se exposure may be related to the PTEN/PI3K/AKT/mTOR pathway. The results were shown in Fig. 4, compared with the Control group, the expressions and protein levels of PTEN in the BPA and/or low-Se groups were obviously upregulated, and the mRNA expressions of PI3K, AKT, and mTOR, as well as the protein levels of p-PI3K and p-AKT were significantly downregulated ($P < 0.05$). The co-exposure group showed significant changes in PTEN, PI3K, AKT, mTOR, p-PI3K, and p-AKT compared to the BPA or low-Se groups ($P < 0.05$). This indicated that BPA and/or low-Se caused apoptosis and mitophagy in chicken pancreatic cells via regulating the PTEN/PI3K/AKT/mTOR pathway. Similarly, there was a certain synergistic effect between BPA and low-Se.

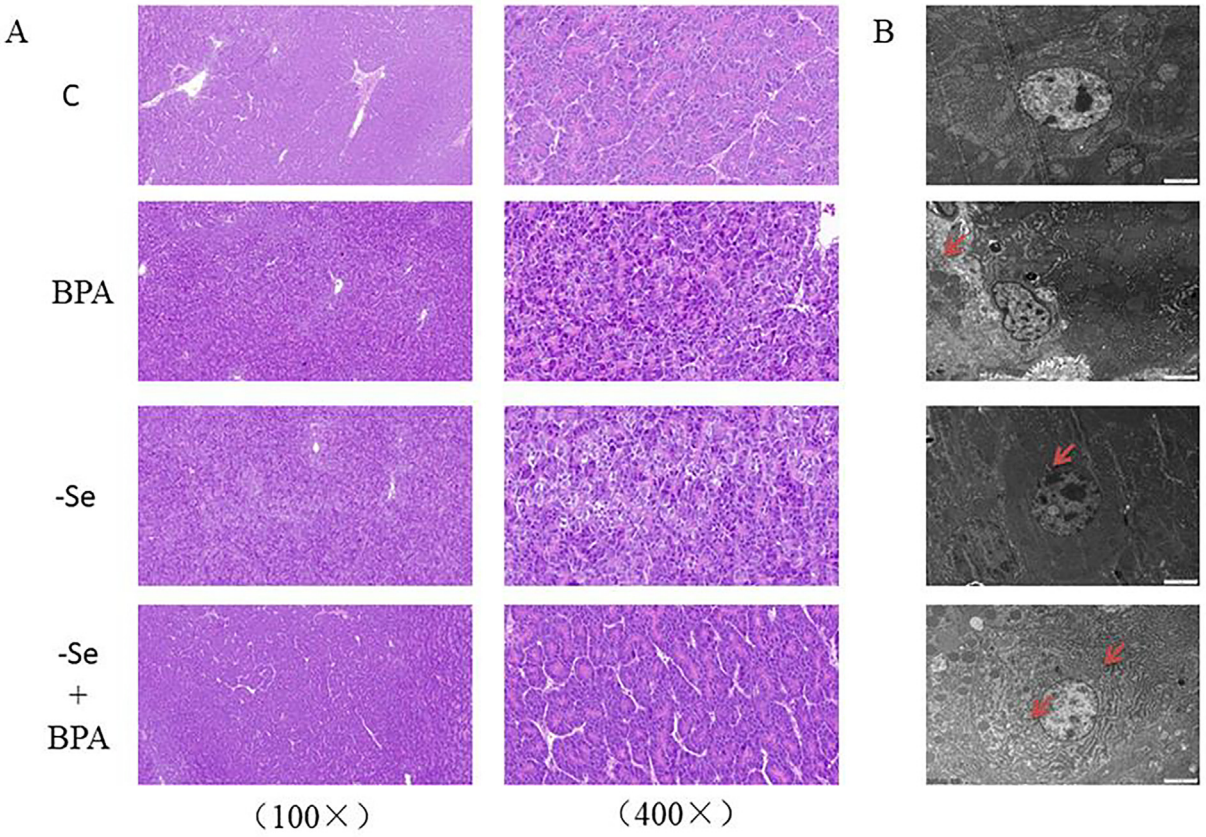

Fig. 1. Effects of BPA and low-Se on pancreatic pathological damage (n = 3). A: H.E. staining results; B: Transmission electron microscopy results.

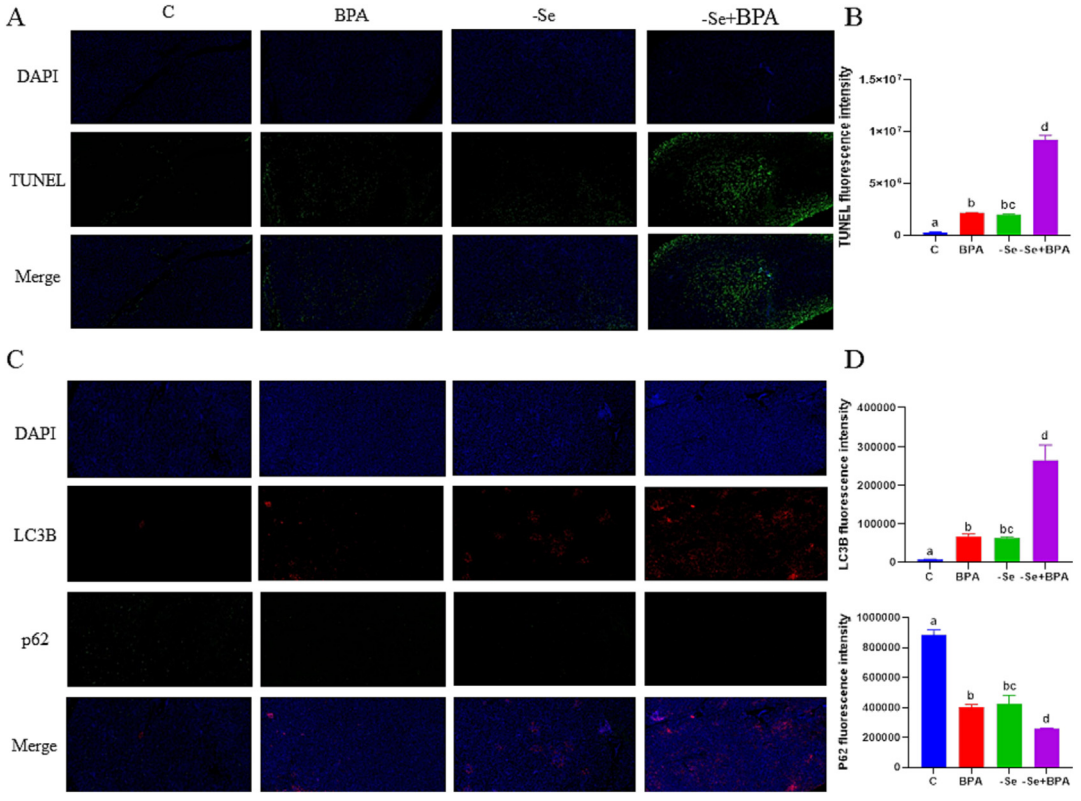

Fig. 2. Effects of BPA and low-Se on pancreatic cell apoptosis and autophagy (n = 3). A: TUNEL staining results (100 ×); B: TUNEL staining column analysis diagram; C: LC3B, p62 IF staining results (100 ×); D: LC3B, p62 IF staining column analysis diagram. Different superscripts indicate significant differences ($P < 0.05$), while containing the same superscripts indicates no significant differences ($P \geq 0.05$). The entire text used the same annotation method.

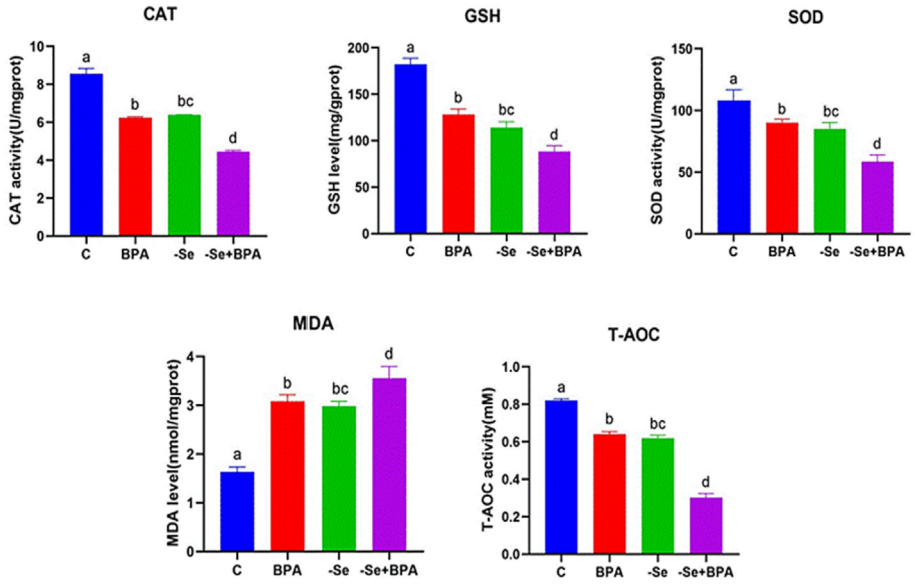

Fig. 3. Effects of BPA and low-Se on pancreatic oxidative stress indicators (CAT, GSH, SOD, MDA, T-AOC) (n = 3).

Effects of BPA and low-Se on mitochondrial function and homeostasis in pancreatic cells

BPA and low-Se exposure caused mitochondrial ridge breaks. The commercially kits, qRT-PCR and Western blot analysis was used to detect the associated indicators of mitochondrial function. The study showed that compared with the Control group, the ATP contents in the BPA and/or the low-Se groups were decreased signally (Fig. 5 A), and the mitochondrial homeostasis was unbalanced, namely, the expressions and protein levels of Mfn1, Mfn2, OPA1 were decreased signally, and the expressions and protein levels of DRP1 were increased signally (Fig. 5 B, C). The levels of mitochondrial oxidative phosphorylase were decreased (Fig. 5 D) ($P < 0.05$). This indicated that exposure to BPA and low-Se lead to mitochondrial dysfunction and homeostasis imbalance in pancreatic cells, and the low-Se + BPA group showed more obvious changes.

Effects of BPA and low-Se on pancreatic apoptosis and mitophagy

BPA and low-Se damaged the pancreatic tissue, induced apoptosis and mitochondrial autophagy. This was further confirmed using Western blot analysis and qRT-PCR methods. The results were displayed in Fig. 6, compared with the Control group, the expressions and protein levels of BAX, Caspase-3, and Caspase-9 were obvious up-regulation in the BPA and/or low-Se groups, while Bcl-2 was lower. Especially in the low-Se + BPA group, the expression and protein levels of BAX, Bcl-2, Caspase-3, and Caspase-9 showed higher changes compared to the individual exposure groups ($P < 0.05$). Similarly, the expressions and levels of LC3-II/I, ATG5,

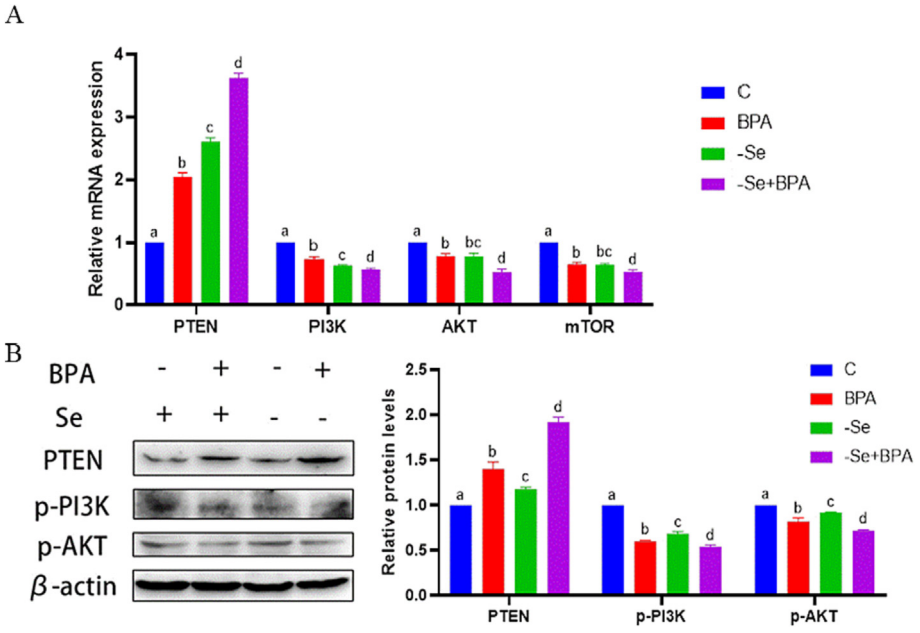

Fig. 4. Effects of BPA and low-Se on the PTEN/PI3K/AKT/mTOR pathway in pancreatic cells (n = 3). A: The mRNA expression of signaling pathway; B: The protein banding and columnar analysis of the signaling pathway.

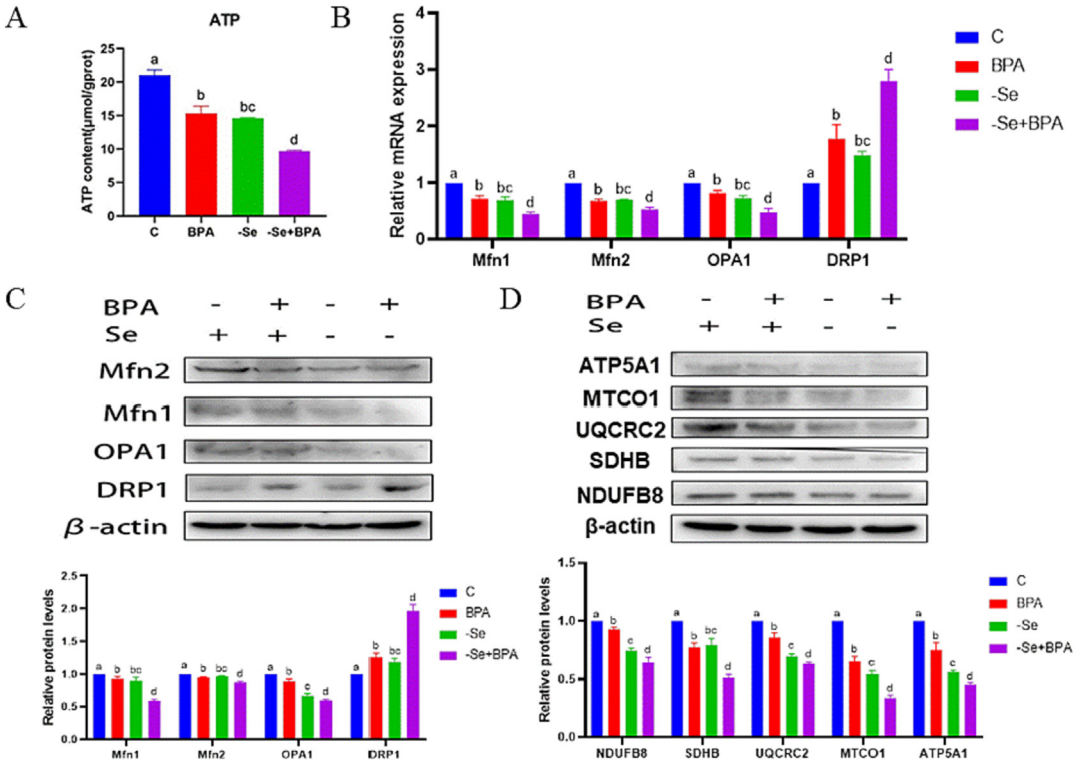

Fig. 5. Effects of BPA and low-Se on mitochondrial function and homeostasis in pancreatic cells (n = 3). A: Changes in ATP content; B: The mRNA expression of Mfn1, Mfn2, OPA1, DRP1; C: The protein banding and columnar analysis of Mfn1, Mfn2, OPA1, DRP1; D: The protein banding and columnar analysis of mitochondrial oxidative phosphorylase (NDUFB8, SDHB, UQCRC2, MTCO1, ATP5A1).

PINK1, and Parkin were obviously increased in the BPA and low-Se groups, while p62 was reduced ($P < 0.05$). Moreover, the expressions and levels of LC3-II/I, ATG5, PINK1, and Parkin in the low-Se + BPA group were obviously higher than those in the BPA and low-Se groups, while p62 was reduced ($P < 0.05$). These data indicated that both BPA and low-Se caused apoptosis and mitophagy, and the co-exposure of BPA and low-Se lead to more severe apoptosis and mitophagy.

Discussion

A large number of reports have shown that BPA exposure was related to obesity, diabetes, and cancer [39–41]. Besides, BPA exposure induces apoptosis and autophagy [42]. The pancreas is also one of the target organs for BPA, which can damage the pancreas [14,43]. Low concentration BPA exposure induces Beta-TC-6 cells to oxidative stress, apoptosis and mitochondrial dysfunction [15]. Oxidative stress regulates multiple signaling pathways and promotes cellular toxicological processes [44]. Exposure to environmental-related doses of BPA can also induce Caspase-3 activation and apoptosis in mouse pancreatic α cells [45]. Similarly, low-Se leads to a decrease in the number of functional acinar mitochondria and impaired mitochondrial integrity, oxidative stress, pancreatic underdevelopment and atrophy [46–49]. Besides, report has shown that the lack of Se had a synergistic influence on the toxicity of pollutants [50]. Consistent with previous research findings, the study suggested that both BPA and low-Se gave rise to oxidative stress, mitochondrial dysfunction and homeostasis imbalance, apoptosis, and mitophagy, PTEN expression was enhanced, while PI3K/AKT/mTOR expressions were inhibited. Moreover, the co-exposure of BPA and low-Se had a stronger toxicity, indicating a synergistic effect between low-Se and the toxicity caused by BPA exposure.

Oxidative stress is concerned with regulating a variety of cell processes, for instance apoptosis, autophagy, and pyroptosis. Previous studies have confirmed that BPA can induce oxidative stress, increase ROS levels, lead to decreased SOD and GSH activities, and show a dose-dependent effect [51,52]. In addition, low-Se can also significantly induce oxidative stress, resulting in decreased activities of glutathione peroxidase (GSH-Px) and CAT, and increased MDA content [27]. These results suggested that both BPA and low-Se can cause oxidative stress. Similarly, our data showed that the activities of SOD, GSH, and CAT were significantly decreased, and MDA levels were increased. T-AOC represents total antioxidant capacity, which refers to the defense ability of the antioxidant system and is an effective indicator of oxidative stress [53]. Our results found that BPA and low-Se leaded to a decrease in T-AOC levels, and the co-exposure of BPA and low-Se exacerbated the decrease in T-AOC, indicating that BPA and low-Se generated oxidative stress and leaded to oxidative damage.

It is worth noting that the emergence of oxidative stress can induce upregulation of PTEN expression, inhibits the PI3K/AKT pathway, and eventually lead to cell apoptosis [54]. Chlorpyrifos exposure induces oxidative stress in grass carp hepatocytes, increases PTEN expression, inhibits PI3K/AKT expression, and promotes apoptosis and necrosis of hepatocytes [55]. The above studies all indicate an opposite correlation between PTEN and the PI3K/AKT pathway. mTOR is a highly conserved protein kinase closely related to apoptosis, growth, autophagy. The activation of the PI3K/AKT/mTOR pathway has an anti-apoptosis effect, capsaicin regulates PI3K/AKT/mTOR pathways to reduce acute lung injury induced by Lipopolysaccharide (LPS) [56]. Artemisinin inhibites cartilage PI3K/AKT/mTOR pathway to further activates mitophagy and alleviates osteoarthritis [57]. In addition, polystyrene microplastics induces autophagy and apoptosis in birds' lungs cells by activating PTEN/PI3K/AKT/mTOR pathway [58]. When

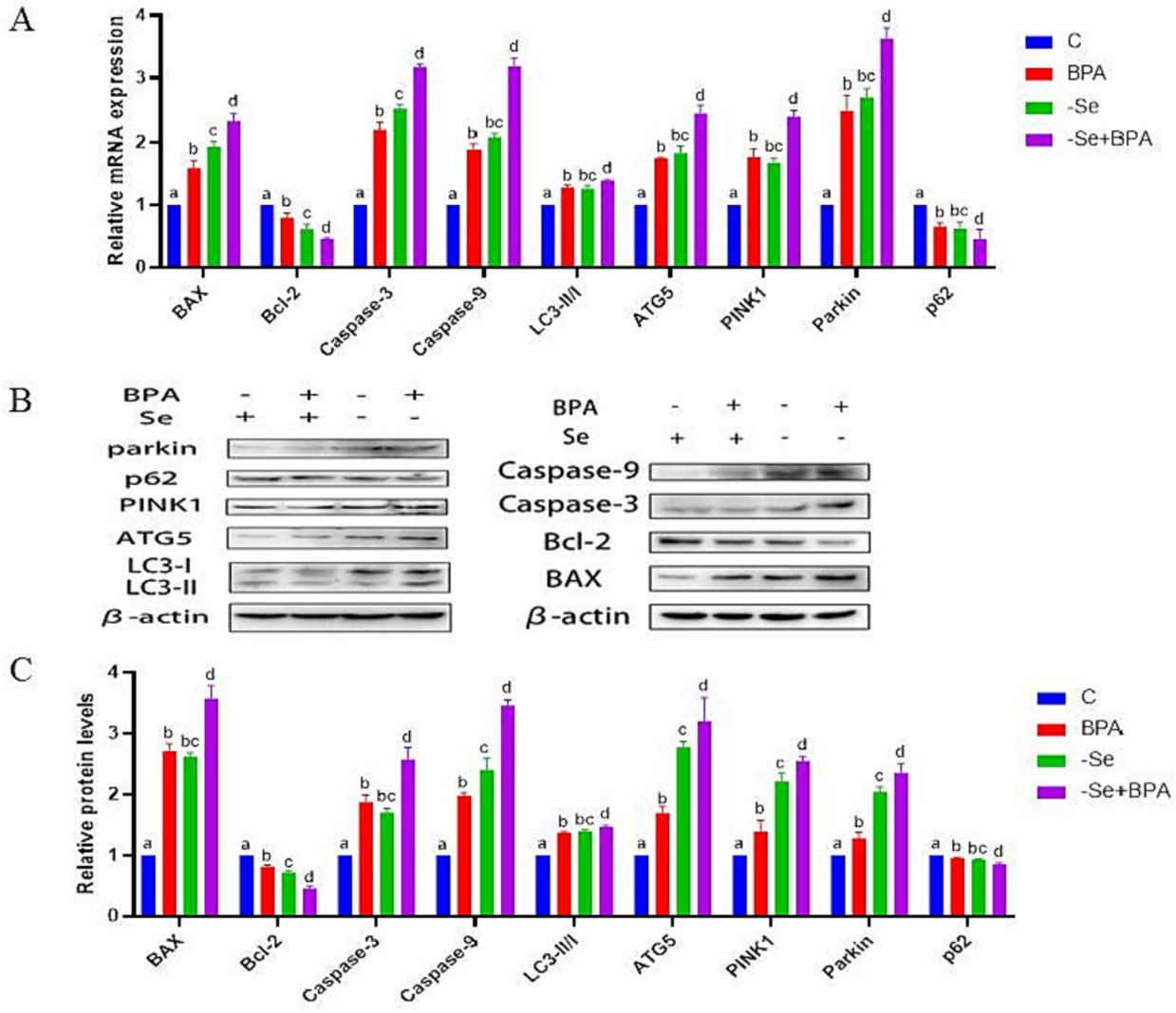

Fig. 6. Effects of BPA and low-Se on pancreatic apoptosis and mitophagy (n = 3). A: The mRNA expression of indicators related to cell apoptosis and mitophagy; B: Protein bands related to indicators of cell apoptosis and mitophagy; C: Protein column analysis of indicators related to cell apoptosis and mitophagy.

melatonin and sirolimus are combined to treat HNSCC cells, the AKT/mTOR pathway is inhibited, regulating mitochondrial function, and activating mitophagy and apoptosis [59]. In our study, BPA and low-Se induced pancreatic tissue damage in chickens, leading to mitochondrial dysfunction via activation of the PTEN/PI3K/AKT/mTOR pathway, further inducing cell apoptosis and mitophagy. The toxicity of BPA and low-Se had a certain synergistic effect.

Conclusion

In conclusion, the possible mechanism of the activation of PTEN/PI3K/AKT/mTOR pathway contributed to BPA and low-Se-induced pancreatic injury is expounded. PTEN/PI3K/AKT/mTOR pathway activation caused mitochondrial dysfunction and mitochondrial homeostasis imbalance to trigger apoptosis and mitophagy, contributing to BPA and/or low-Se-triggered pancreatic toxicity. These results shed light on the crosstalk among PTEN/PI3K/AKT/mTOR pathway, apoptosis and mitophagy in BPA and/or low-Se-induced pancreatic injury. This study not only enriched the mechanism of toxic of BPA and low-Se, but also provided a new understanding of their co-exposure, and provided valuable new insights into pancreatic injury.

Statement of professional ethics requirements

The Northeast Agricultural University Ethics Committee (Approved Number: NEAUEC-2023-03111, China) gave its approval to all experimental methods.

CRediT authorship contribution statement

Wenying Sun: Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Yutian Lei:** Software, Investigation. **Zhihui Jiang:** Conceptualization, Supervision, Validation. **Kun Wang:** Software, Investigation, Visualization. **Huanyi Liu:** Software, Investigation, Visualization. **Tong Xu:** Conceptualization, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2024.01.029.

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