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Research Article

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Omega-3 alleviates acetochlor-induced renal injury by targeting GRP78 to mitigate endoplasmic-reticulum stress

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Abstract: Acetochlor (ACT) is a widely used herbicide in agriculture and has become a global organic pollutant, posing significant environmental risks. As an aniline herbicide, ACT threatens kidney health through inflammatory responses. Omega-3 (Ω3) fatty acids play a crucial role in anti-inflammatory processes. This study investigates the protective effects of Ω3 against ACT-induced renal damage and the specific molecular mechanisms underlying these protective effects. Our findings indicate that exposure to ACT leads to renal inflammation and lipid-metabolism disorders, while Ω3 alleviates inflammation levels and improves lipid-metabolism abnormalities. Mechanistically, Ω3 mitigates the damage caused by ACT via the endoplasmic reticulum (ER) stress-NLRP3 inflammasome axis. Ω3 stabilizes the structure of GRP78, a key ER stress sensor, thereby preventing the activation of downstream pathways associated with ER stress, including the transcription factor CHOP. This stabilization subsequently reduces assembly of the downstream NLRP3 inflammasome complex, thereby alleviating kidney damage. These results provide new insights into the role of Ω3 in modulating ER stress and its anti-inflammatory mechanisms in an avian acetochlor-induced renal injury model.

Key words: NLRP3 Inflammasome; Endoplasmic reticulum stress; Inflammation; Omega-3; Acetochlor; Molecular dynamics

1. Introduction

Acetochlor (ACT), a vital pre-emergence herbicide, is widely utilised in agricultural production worldwide (Ye, 2003; Guzzella et al., 2006). Its tendency to form residue, high mobility, and poor degradability have led to extensive use in agricultural production, resulting in a gradual increase in the risk of exposure to ACT within ecosystems (Sun et al., 2011, Chen et al., 2024). While the European Commission has discontinued the registration of ACT and the US EPA has maintained only a conditional registration with strict restrictions and mandatory groundwater monitoring, the herbicide remains widely used in China, posing serious environmental residue concerns. ACT primarily accumulates in water bodies after nearby agricultural activity, and it can also persist on the surface of plants, posing potential harm to animals through their food sources (Lu et al., 2022). Consequently, ACT is a considerable threat to food safety for both humans and animals. This bioaccumulation and pollution may impact many species (Qin et al., 2020; Wang et al., 2024b). Numerous toxicological studies on ACT indicate that it exhibits significant toxicological characteristics, capable of causing damage to multiple organs (Wang et al., 2023b; Zhang et al., 2024, 2025a). In zebrafish, ACT covalently inhibits GPX4 activity via its chloroacetamide group, leading to accumulation of lipid peroxides, 4-HNE, and reactive oxygen species (ROS), and thereby inducing oxidative stress and lipid-metabolism disorders(Mesmar et al., 2024). In murine studies, ACT triggers oxidative stress, activates the p53 and MAPK/ERK

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pathways, modulates the Bax/Bcl-2 ratio, and induces caspase-3/9 activation, ultimately resulting in germ-cell apoptosis(Song et al., 2019). However, the nephrotoxicological mechanisms of ACT are still unclear. It is known that pesticides can accrue in the kidneys; researchers have detected them in the kidney tissue of frogs from the Pampas region (Brodeur et al., 2022). The kidney is a vital metabolic organ that primarily functions to eliminate toxins produced by intracellular and exogenous metabolism, regulating internal homeostasis (Levassort and Essig 2024). Research has demonstrated that exposure to ACT can lead to renal-cell apoptosis in bighead carp (Zhao et al., 2022), posing a non-negligible threat to their health. Further research is needed to elucidate the mechanism of and possible preventive measures for ACT-induced renal injury.

Endoplasmic reticulum (ER) stress is a cellular mechanism that responds to disruptions in protein homeostasis. When protein homeostasis is unbalanced, ER stress activates three ER UPR proteins, which are transmembrane proteins released via immunoglobulin heavy chain binding protein (GRP78): Activating Transcription Factor 6 (ATF6), Inositol requiring enzyme 1 (IRE1), and Protein kinase RNA–like endoplasmic reticulum kinase (PERK). These proteins initiate three distinct pathways of the ER stress response to maintain ER homeostasis (Hetz, 2012; Li et al., 2023). Research has shown that various compounds can induce ER stress in animals, and that it is closely related to nephrotoxicity (Ming et al., 2022; Li et al., 2024). Components of the immune signaling pathway form part of the interactive network of the ER stress response , which highlights its intimate connection with immune function (Di Conza and Ho, 2020). In many cases, organelle stress can switch on assembly of NLRP3 inflammatory vesicles, which is strongly associated with inflammation caused by external toxins (Dandekar et al., 2015; Han et al., 2025). Inflammasomes are multiprotein complexes assembled by cytosolic pattern-recognition receptors; they mediate innate immune defences and are closely linked to various diseases. The canonical NLRP3 inflammasome is assembled from the sensor NLRP3 and the adaptor Apoptosis-associated speck-like protein (ASC), which serves as a platform for the recruitment and activation of pro- cysteinyl aspartate specific proteinase 1 (Caspase-1).The NLRP3 inflammasome is a crucial component of the innate immune system, due to its role in responding to harmful signals (Song et al., 2021; Dong et al., 2022; Fu and Wu, 2023; Ren et al., 2024). However, aberrant activation of the this inflammasome is also closely associated with inflammatory disease (Blevins et al., 2022). Pyroptosis is induced when danger-associated molecular patterns come into contact with cells (Kelley et al. 2019).

Ω3, a group of indispensable polyunsaturated fatty acids that are often found in plants, seal oil, and deep-sea fish (Cholewski et al., 2018), plays an essential role in organismal health (Shahidi and Ambigaipalan, 2018). Ω3 not only serves as a necessary nutrient for the body of the organism but also exhibits anti-inflammatory properties. It is capable of reducing chronic inflammation, oxidative stress, and cell apoptosis (Ishihara et al., 2019; Wang et al., 2024a; Essawy et al., 2025). Our previous research has demonstrated the significant mitigating effects of Ω3 on copper-induced ileal toxicity in broiler chickens(Zhou et al., 2025). Recent studies have demonstrated that Ω3 exerts beneficial effects in renal fibrosis and chronic kidney disease (Yang et al., 2026), and is closely associated with the regulation of renal lipid metabolism (Qu et al., 2026). Moreover, Ω3 is reported to improve renal energy metabolism, thereby contributing to the attenuation of kidney-disease progression (Lee et al., 2026). The primary components of Ω3 include EPA and DHA. EPA specifically exhibits antioxidant activity. It can attenuate renal interstitial fibrosis in rats via the TGF-β signaling pathway and reduce lipid-induced renal toxicity by decreasing lipid autophagy (Shi et al., 2022). Previous studies have demonstrated that DHA exhibits anti-inflammatory properties and attenuates LPS-induced inflammation through activation of the cAMP/IκBα signaling pathway (Wu et al., 2025). Despite these findings, studies focusing on the renoprotective effects of DHA and EPA remain relatively limited, and the mechanisms by which Ω3 exerts beneficial effects in the kidney are still not fully understood.

In the present study, we established in vivo models of broiler chickens exposed to ACT, some of which received Ω3 supplementation, as well as in vitro models including ACT exposure, Ω3 supplementation, and groups treated with the ER stress inhibitor 4PBA and the NLRP3 inhibitor MCC950, to investigate the mechanisms of ACT-induced renal-tissue injury and Ω3 remission. These findings reveal significant roles for ER stress and pyroptosis in nephrotoxicity, suggesting that targeting ER stress and activating NLRP3 Inflammasomes could be an effective therapeutic strategy to alleviate ACT-induced kidney injury.

2. Materials and methods

2.1 Construction of the Animal Model

We obtained 40 one-day-old chicks and acclimatised them for 7 days in a laboratory animal centre. The sample size was determined based on previous studies which used similar animal models and experimental designs. After acclimatisation, the animals were randomly assigned to experimental groups using the random number table method. Equal numbers of male and female chickens were included in each experimental group to minimize potential sex-related bias. We used four groups: the control group (fed with basal diet), the ACT treatment group (fed with 500 mg/kg ACT in addition to basal diet), the Ω3 group (fed with 0.05 g/kg Ω3 in addition to basal diet), and the ACT+Ω3 combined treatment group (fed with 500 mg/kg ACT and 0.05 g/kg Ω3 in addition to basal diet). After 35 days of this treatment, the chicks were sacrificed, and their kidney tissue was preserved in a -80℃ refrigerator. The dose of 500 mg/kg ACT was selected based on our previous dose-setting study in chickens, whereas the high dose corresponded to approximately 1/10 of the wild bird LD50 value reported by the EPA (Zhang et al., 2025b).

The histopathological and biochemical measurements are fully described in the supplementary materials and methods.

3. Results

3.1 Ω3 alleviation of ACT-induced kidney injury

H&E staining revealed no inflammatory infiltration and only a few renal tubular epithelial vacuoles in the control (C) and Ω3 groups. In contrast, the ACT group exhibited significant inflammatory infiltration and an increased number of renal tubular epithelial vacuoles. However, the ACT+Ω3 group showed no inflammatory infiltration and a reduced number of renal tubular epithelial vacuoles (Fig. 1a). Electron microscopy results indicated normal ultrastructure in the C and Ω3 groups. However, the ACT group appeared to display loss of microvilli, renal tubular-cell swelling, and ER expansion. In the ACT+Ω3 group, the number of missing microvilli was lower, cell swelling was not as severe, and ER expansion was not affected (Fig. 1b). These findings suggested that Ω3 alleviated kidney inflammation and injury caused by ACT. Potential targets of ACT were enriched in processes related to fatty acid metabolism, lipid metabolism, inflammation, and ER (Figs. 1c and 1e). Interaction genes of Ω3 were enriched in biological processes such as inflammatory response, lipid metabolism, fatty acid metabolism, lipid transport, NLRP3 inflammasome assembly, and ER stress, as determined by GO enrichment analysis. Similarly, in KEGG pathway enrichment analysis, they were enriched in fatty acid metabolism and the PPAR signaling pathway (Figs. 1d, 1f, 1g and 1h).

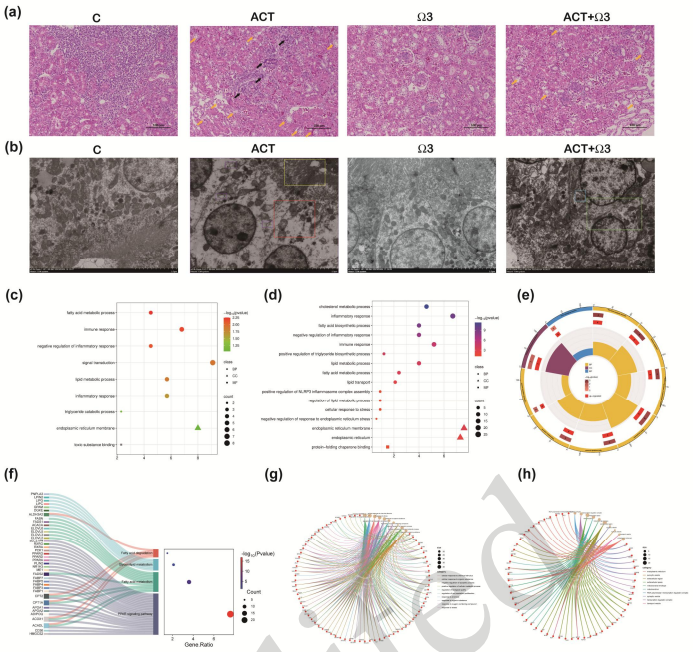

Fig.1 Ω3 alleviates kidney injury induced by ACT exposure

(a) H&E staining of kidney tissue (black arrows: inflammatory infiltration, orange arrows: renal tubular epithelial vacuolation). (b) Electron microscopy of kidney tissue. Red boxes are cell swelling, yellow boxes are microvilli absence and green boxes are cell swelling recovery, purple boxes are endoplasmic reticulum swelling, blue boxes are endoplasmic reticulum swelling recovery. (c) Bubble plot of GO enrichment analysis for potential acetochlor targets. (d) Bubble plot of GO enrichment analysis for Ω-3 interacting genes. (e) Circle plot of GO enrichment analysis for potential acetochlor targets. (f) Sankey bubble plot of KEGG enrichment analysis for Ω-3 interacting genes. (g) Network diagram of the GO Biological Process (BP) enrichment for Ω-3 interacting genes. (h) Network diagram of the GO Cellular Component (CC) enrichment for Ω-3 interacting genes. Data are presented as the mean ± SD

3.2 Ω3 mitigation of ACT-induced ER stress in the kidneys

Compared to the C group, the mRNA and protein levels of GRP78, ATF6, IRE1, and p-PERK mRNA were significantly increased in the ACT group (Figs. 2a, 2b, 2d, 2e, 2f, 2g and 2i), indicating the occurrence of ER stress. This in turn indicated the activation of all three pathways of the UPR. Additionally, the downstream targets of the PERK pathway in the UPR, ATF4 (Activating Transcription Factor 4), and CHOP (C/EBP-homologous protein), were significantly upregulated in the ACT group, along with an increase in mRNA levels of Eukaryotic translation initiation factor 2α (EIF2-α) (Figs. 2c, 2g and 2i). Comprehensive activation of ER stress in the kidneys was observed upon ACT exposure, although the difference was not statistically significant. Notably, the Ω3 treatment group showed a reduction in PERK mRNA levels compared to the ACT group (Fig. 2i). All other results were also significantly downregulated in the ACT+Ω3 group, indicating that Ω3 mitigated ER stress induced by ACT exposure.

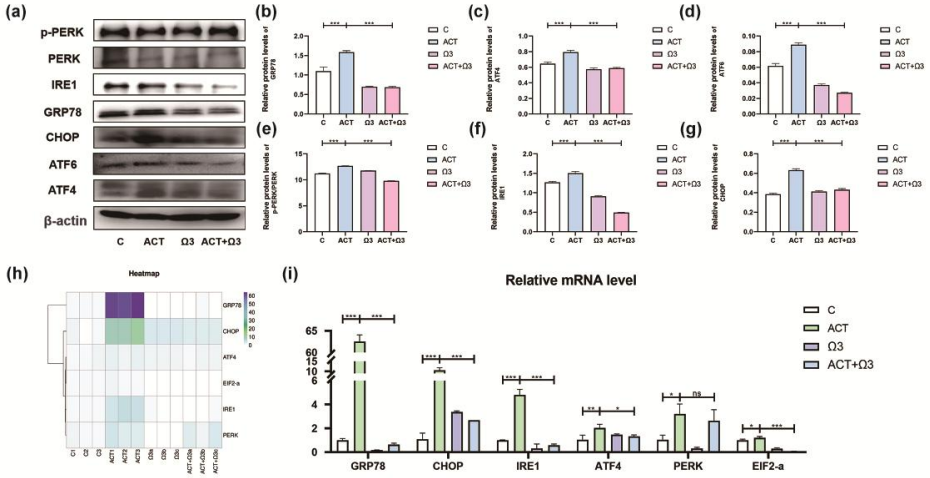

Fig.2 Ω3 mitigates ER stress in the kidneys induced by ACT exposure

(a-g) Protein expressions of p-PERK, PERK, IRE1, GRP78, CHOP, ATF6, and ATF. (h, i) Gene transcription levels of GRP78, CHOP, IRE1, ATF4, PERK, and EIF2-α. *P < 0.05, **P < 0.01, and ***P < 0.001. Data are shown as three independent samples (n=3). Data are presented as the mean ± SD

3.3 Ω3 attenuation of ACT-induced pyroptosis and inflammation

NLRP3 gene transcription and expression levels were significantly increased in the ACT group, and these increases were attenuated by Ω3 treatment (Figs. 3e, 3i and 3k). Similarly, ASC and Caspase-1 protein expression and mRNA levels were significantly elevated in the ACT group but reduced in the ACT+Ω3 group (Figs. 3a, 3b, 3c and 3f). This provided evidence that ACT induced assembly of the inflammasome, which Ω3 inhibits. Significant increases in IL-18 and Tumor Necrosis Factor-α (TNFα) mRNA levels in the ACT group and their subsequent reduction in the ACT+Ω3 group further supported the occurrence of pyroptosis and inflammation in the kidneys and their attenuation by Ω3 (Figs. 3d and 3j). Although the upregulation of Interleukin-1beta (IL-1β) and IL-8 in the ACT group and their attenuation by Ω3 were not significant, they still provided additional evidence for the mitigating effects of Ω3 on pyroptosis and inflammation induced by ACT exposure (Figs. 3g and 3h).

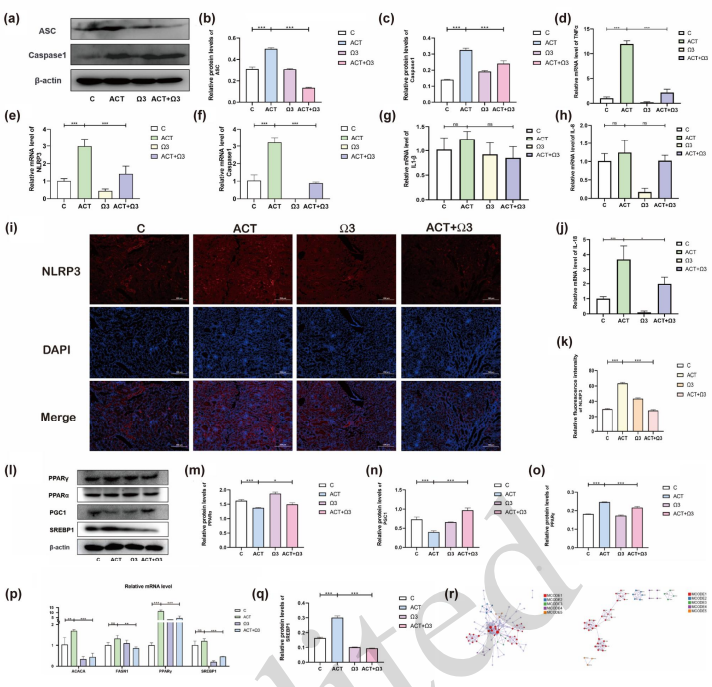

Fig.3 Ω3 attenuation of ACT-induced pyroptosis and inflammation and Ω3 amelioration of ACT-induced lipid metabolism disorders

(a-c) Protein expression of ASC and Caspase1. (d-h, j) Gene transcription levels of TNF-α, NLRP3, Caspase1, IL-1β, IL-8, and IL-18. (k) Immunofluorescence and fluorescence intensity quantification of NLRP3. *P < 0.05, **P < 0.01, and ***P < 0.001. (l-o,q) Protein expression of PPARγ, PPARα, PGC1, and SREBP1. (p) Gene transcription levels of ACACA, FASN1, PPARγ, and SREBP1. *P < 0.05, **P < 0.01, and ***P < 0.001. Data are shown as three independent samples (n=3). Data are presented as the mean ± SD

3.4 Ω3 amelioration of ACT-induced lipid metabolism disorders

The protein–protein interaction (PPI) enrichment analysis showed that potential target genes of EPA were enriched in the fatty acid metabolism pathway, with a P-value of 10^-24.3 (Fig. 3r). The experimental results aligned with this analysis. Peroxisome proliferators-activated receptor γcoactivator 1 (PGC1), a protein involved in fatty acid oxidation, exhibited a significant reduction in the ACT group (Fig. 3n). Meanwhile, peroxisome proliferator activated receptor alpha (PPARα) levels declined significantly in the ACT group (Fig. 3m). Significant upregulation of peroxisome proliferator activated receptor gamma (PPARγ) protein and mRNA levels was observed in the ACT group. (Figs. 3o, p). At the mRNA level, ACACA and FASN1 were significantly upregulated in the ACT group (Fig. 3p). Both the mRNA and protein levels of Sterol regulatory element-binding protein 1 (SREBP1) were found to be elevated in the ACT group (Figs. 3p and 3q). The ACT+Ω3 group exhibited corresponding alleviation results for the indicators.

3.5 Ω3 alleviation of ACT-induced cell pyroptosis via the ER stress pathway

ER-Tracker red staining showed a decrease in ER fluorescence intensity in the ACT group, which was less than that in the ACT+Ω3 and ACT+4PBA groups (the latter is an ER stress inhibitor) (Figs. 4a and 4f). Furthermore, there was no significant difference between the ACT and ACT+MCC950 groups (MCC950 is an NLRP3 inhibitor) (Figs. 4a and 4f). Comparing the ACT+4PBA and ACT+Ω3 groups, IF and WB analyses indicated that GRP78 gene-expression levels were analogous, suggesting similar mechanisms of action for Ω3 and 4PBA (Figs. 4c, 4g, 4e, 4h). The results of PERK demonstrated that PERK gene-expression levels in the ACT+Ω3 and ACT+4PBA groups were similar but lower than those in the ACT group (Figs. 4b and 4d). Although PERK mRNA levels were reduced in the ACT+MCC950 group compared to the ACT group, they were still higher than those in the ACT+Ω3 and ACT+4PBA groups (Fig. 4l). The IF results of CHOP and the protein results of ATF4 were reduced in the ACT+Ω3 and ACT+4PBA groups compared to the ACT group, and there was no significant difference between the ACT and ACT+MCC950 groups (Figs. 4g, 4i, 4j, 4n and 4m). Despite a reduction in CHOP mRNA levels in the

ACT+MCC950 group compared to the ACT group, they remained higher than those in the ACT+Ω3 and ACT+4PBA groups (Fig. 4k). These findings suggested that the regulatory effect of Ω3 on ER stress did not take place not downstream of NLRP3 inflammasome assembly but was directly mediated through ER stress.

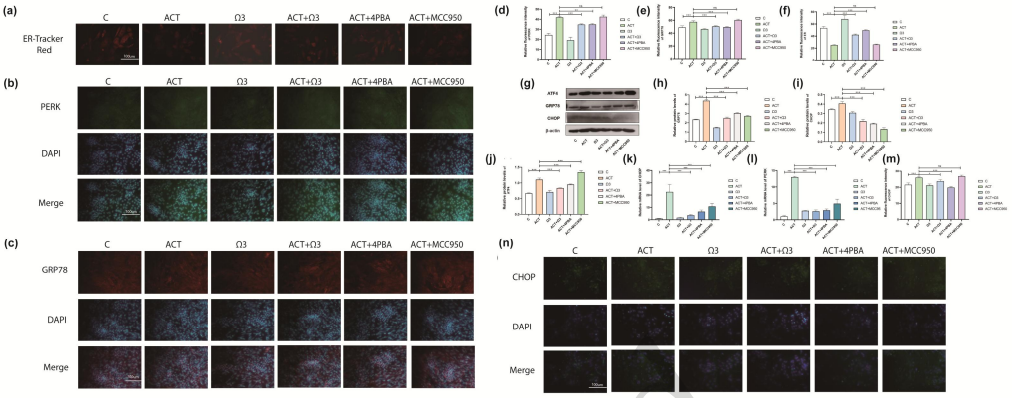

Fig.4 Ω3 alleviates cell pyroptosis induced by ACT via the ER stress pathway

(a, f) Red fluorescent probe for endoplasmic reticulum and fluorescence intensity quantification. (b, c, d, e) Immunofluorescence and fluorescence intensity quantification of PERK and GRP78. (m, n) Immunofluorescence and fluorescence intensity quantification of CHOP. (g-j) Protein expression of ATF4, GRP78, and CHOP. (k, l) Gene transcription levels of CHOP and PERK. *P < 0.05, **P < 0.01, and ***P < 0.001. Data are shown as three independent samples (n=3). Data are presented as the mean ± SD

3.6 Ω3 mitigation of ACT-induced cell pyroptosis via NLRP3 inflammasome inhibition

Flow cytometry results using PI staining showed similar cell-death counts in the ACT+Ω3, ACT+4PBA, and ACT+MCC950 groups, all of which exhibited attenuating effects compared to the ACT group (Fig. 5m). Protein levels of NLRP3 and ASC, two components of the NLRP3 inflammasome structure, were decreased in these three groups (Figs. 5d and 5f). This pointed to a similar effect of ER stress inhibitors, NLRP3 inflammasome inhibitors, and Ω3 in attenuating the upregulation of NLRP3 and ASC induced by ACT (Figs. 5d and 5f). However, protein levels of Caspase-1 and key proteins involved in pyroptosis (C-terminal of GSDMD and pro-IL-1β) were lower in the ACT+MCC950 group compared to the ACT+4PBA group (Figs. 5c, 5e, 5g). IF results showed similar levels of Interleukin-2 (IL-2) and Gasdermin D (GSDMD) in the ACT+MCC950 and ACT+Ω3 groups, which were slightly higher than those in the ACT+4PBA group (Figs. 5a, 5i, 5k and 5l). TNFα protein expression was slightly lower in the ACT+Ω3 group compared to the ACT+4PBA and ACT+MCC950 groups (Fig. 5j). Nuclear factor kappa-B (NF-κB) protein levels were similar in the ACT+Ω3 and ACT+4PBA groups, slightly lower than those in the ACT+MCC950 group (Fig. 5h). These findings indicated that ER stress regulated assembly of the NLRP3 inflammasome upstream of its activation, contributing to the attenuating effects of Ω3 on ACT-induced cell damage.

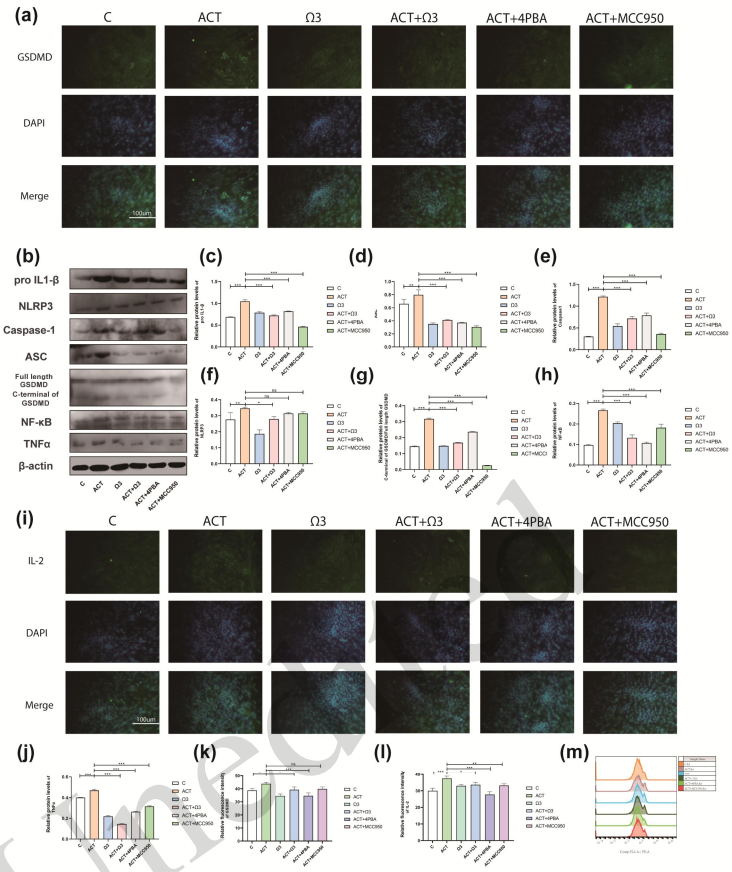

Fig.5 Ω3 mitigates cell pyroptosis induced by ACT by inhibiting NLRP3 inflammasome release

(a, k) Immunofluorescence and fluorescence intensity quantification of GSDMD. (b-h, j) Protein expression of pro-IL-1β, NLRP3, Caspase1, ASC, GSDMD, NF-kB, and TNF-α. (i, l) Immunofluorescence and fluorescence intensity quantification of IL-2. (m) Flow cytometry PI results and means fluorescence intensity quantification. *P < 0.05, **P < 0.01, and ***P < 0.001. Data are shown as three independent samples (n=3). Data are presented as the mean ± SD

3.7 Interaction of ACT and Ω3 with ER stress chaperone GRP78

Global homology alignment of chicken-derived GRP78 and human GRP78 revealed a 98.93% amino acid sequence identity, indicating a high degree of conservation at the level if primary protein structure (Table. S3). Since DHA exhibits weaker binding energy to GRP78 than EPA, the potential docking mechanism was investigated using EPA (Fig. S3). Given the current lack of a reliable, experimentally validated protein sequence and of functional annotation for chicken GRP78 (HSPA5), we adopted the highly conserved human GRP78 in this study as a surrogate reference sequence for inference of function and key binding sites. To investigate the potential mechanism by which ACT induces ER stress and the molecular basis for the mitigating effect of Ω3 fatty acids via GRP78, we performed molecular docking analyses for ACT and Ω3 with GRP78. Twenty docking poses were obtained for both ACT and Ω3(Figs. 6a and 6c). The results indicated that the average binding energy of Ω3 with GRP78 was superior to that of ACT, suggesting a stronger binding affinity of Ω3 for GRP78 (Fig. 6B3, D3). Further analysis revealed that in poses C1–C5, the binding site of Ω3 on GRP78 overlapped significantly with the known ATP-binding pocket. Based on the spatial distribution characteristics of the small molecule–protein binding pockets, molecular dynamics (MD) simulations of 100 ns were performed for the pose with the optimal binding energy within each category. The MD results demonstrated that in the ACT–GRP78 complex, only pose A1 exhibited good structural stability, with its root-mean-square deviation (RMSD) consistently remaining below 5 Å and stabilizing around a mean value (Fig. 6B2), while fluctuations in solvent-accessible surface area (SASA) and radius of gyration (Rg) were minimal (Figs. 6B1 and 6B9). The free-energy landscape (FEL) exhibited only one distinct energy basin (Figs. 6B4). In the

Ω3–GRP78 complexes, poses C1, C11, C16, and C20 all demonstrated high stability. The RMSD values for poses C1, C16, and C20 stabilised below 5 Å, while the RMSD for pose C11 stabilised around approximately 10 Å (Figs. 6D2). The SASA and Rg values for these poses fluctuated slightly within their respective mean ranges (Figs. 6D1, 6D9, 6D11-13). FEL analysis similarly revealed a single energy basin, indicating that overall, the complexes were structurally stable (Figs. 6D4, 6D6-8).

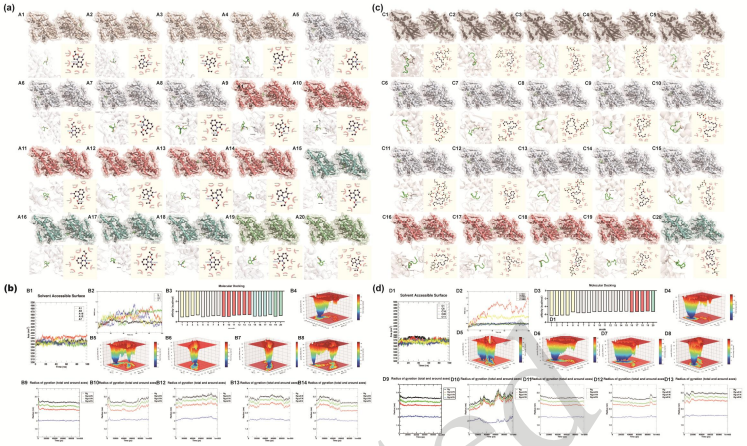

Fig.6 Interaction between EPA, ACT and GRP78

(a) Molecular Docking of ACT and GRP78. (b) Molecular Dynamics Simulation of ACT and GRP78: (B1) SASA (Solvent Accessible Surface Area) Results, (B2) RMSD (Root Mean Square Deviation) results (B3) Binding Energy of Molecular Docking, (B4-B8) Free Energy Landscape, (B9-B14) Rg(radius of gyration) results. (c) Molecular Docking of EPA and GRP78. (d) Molecular Dynamics Simulation of EPA and GRP78: (D1) SASA (Solvent Accessible Surface Area) Results, (D2) RMSD (Root Mean Square Deviation) results (D3) Binding Energy of Molecular Docking, (D4-D8) Free Energy Landscape, (D9-D14) Rg(radius of gyration) results. Data are presented as the mean ± SD

3.8 Ω3 amelioration of ACT-induced lipid metabolism disorders via NLRP3-GRP78 crosstalk

Oil Red O staining demonstrated that ACT induced lipid accumulation in renal cells; this was significantly improved in the ACT+Ω3, ACT+4PBA, and ACT+MCC950 groups. Downregulation of PPARα expression was observed in the ACT+4PBA and ACT+MCC950 groups, with expression levels similar to those in the Ω3 treatment group (Figs. 7c and 7d). Trends in SREBP1 mRNA levels and PPARγ mRNA levels were also similar, suggesting that inhibition of both ER stress and the NLRP3 inflammasome pathway attenuates lipid metabolism disorders induced by ACT (Figs. 7a and 7b). As mentioned earlier, Ω3 fatty acids attenuate these disorders by alleviating ER stress.

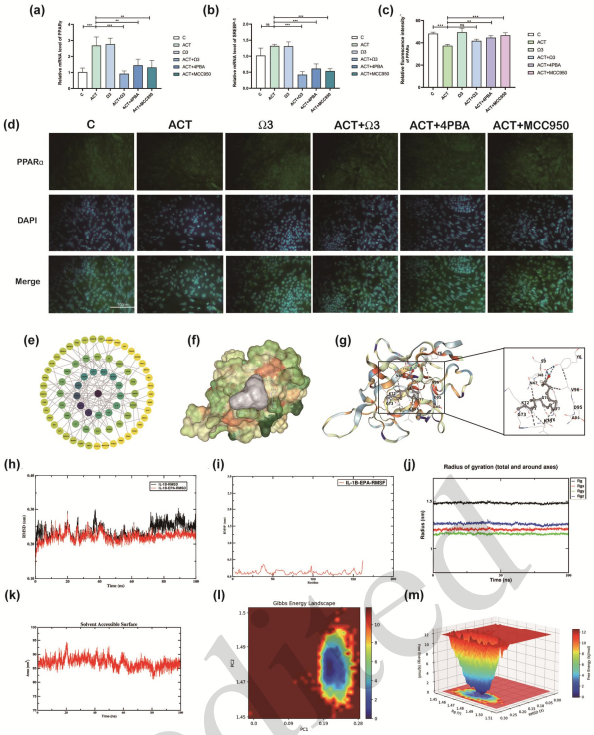

Fig.7 Ω3 ameliorates ACT-induced lipid metabolism disorders via NLRP3-GRP78 crosstalk

(a, b) Gene transcription levels of PPARγ and SREBP1. (c, d) Immunofluorescence and fluorescence intensity quantification of PPARα. (e) Enrichment of protein-protein interactions at potential EPA binding sites. (f, g) Molecular docking analysis between EPA and IL-1β. (h–m) Molecular dynamics simulations of the EPA and IL-1β complex. *P < 0.05, **P < 0.01, and ***P < 0.001. Data are shown as three independent samples (n=3). Data are presented as the mean ± SD

3.9 Stable binding of EPA to IL-1β: a potential mechanism for Ω3 mitigation of ACT toxicity

To elucidate the pharmacological mechanism by which Ω3 mitigates ACT-induced toxicity, we performed PPI analysis, which indicated a strong potential for interaction between EPA and IL-1β (Fig. 7e). Molecular docking confirmed this interaction, revealing a binding energy of –4.9 kcal/mol (Figs. 7f and 7g). During molecular-dynamics (MD) simulation, the EPA–IL-1β complex reached a stable equilibrium, achieving convergence at the 70 ns time point. The continuous RMSD trajectory suggested stable binding of the ligand within the protein pocket (Fig. 7h). Most segments of the complex had RMSF values under 0.5 nm, denoting high residual stability. Additionally, an Rg value below 2 nm implied a tightly packed molecular architecture, consistent with a folded or stably folded secondary structure (Figs. 7i and 7j). The stability of the complex was further supported by a consistent SASA (82.5–95 nm²) (Fig. 7k). A single energy basin was observed in the complex, indicating highly dynamic stability and a uniform conformational profile (Fig. 7l and 7m).

4. Discussion

ACT is widely distributed globally, with detectable residues in both soil and water. The environmental stress is undeniable (Wang et al., 2023a). Numerous studies have demonstrated the toxic effects of ACT on organs including the cardiovascular system, liver, nervous system, and ovaries (Wang et al., 2019, 2023b, 2024c; Zhang et al., 2020). Previous research has indicated that exposure to ACT can lead to apoptosis and necrosis of renal cells through oxidative stress (Zhao et al., 2022). However, the toxicological mechanisms of ACT on the kidneys remain unexplored. Our study demonstrates that ACT induces inflammatory injury and disrupts lipid metabolism in renal tissue, resulting in significant toxicological damage to the kidneys. Given the scarcity of research on the

detoxification mechanisms of ACT, we decided to investigate the potential protective effects of the dietary supplement Ω3 in ACT-induced kidney damage. Ω3 can be used as a supplement in the treatment of chronic kidney-disease patients (Fassett et al., 2010; Hu et al., 2017), exerting positive effects on inflammation in the kidneys (Lin et al., 2022). We analysed the potential binding site of action of Ω3 on the relevant genes involved in the action of ACT. Gene-set analysis of the potential targets of Ω3 suggests its involvement in lipid metabolism and inflammation. Our results indicate the potential of Ω3 fatty acids to modulate renal lipid metabolism and attenuate inflammatory responses. Fatty acids, as an essential component of lipids, can be deposited in various renal cells under pathological conditions and participate in the onset and progression of acute kidney injury (AKI) or chronic kidney disease (CKD). Fatty-acid metabolism disorder is a potential mode of kidney damage. We hypothesize that ACT plays an important role in renal toxicity. Our findings suggest that the expression of genes involved in fatty-acid oxidation, transport, synthesis, and storage in this model is abnormal. In the ACT-exposed group, fatty-acid synthesis was increased, oxidation and transport were blocked, fatty-acid accumulation was increased, and lipid-metabolism disorders appeared in the kidneys. Meanwhile, Ω3 significantly mitigated this abnormal expression.

Studies have shown that ER stress can induce inflammation in chicken lungs and lipid-metabolism disorders in the mouse pancreas (Lu et al., 2024; Cho et al., 2025). In addition, the ERS response promotes renal tubular epithelial cell injury in mice, exacerbating diabetic nephropathy (Jh et al., 2025). In our results, ACT is associated with inflammation and lipid metabolism in the kidney, and the Ω3 treatment group showed a reduction in ACT-induced renal tubular-cell vacuolation. Based on these findings, we hypothesized that the renal inflammation and lipid-metabolism disorders caused by ACT, as well as the mitigating effects of Ω3, occur through ER stress. Interestingly, our results support this hypothesis. We found that ER function declined in the ACT group, while Ω3 effectively alleviated this decline. By examining relevant indicators of ER stress, we concluded that Ω3 significantly reduces ACT-induced ER stress in the kidneys. We found that the mitigating effect of Ω3 on ACT-induced GRP78 upregulation was similar to that of the ER stress-inhibitor group in measurements related to GRP78 expression. The molecular docking results indicate strong binding energy between Ω3 and GRP78, suggesting that Ω3 exerts its protective effects by binding to GRP78 and alleviating ER stress, thereby improving lipid metabolism and reducing inflammation. GRP78 is a dimeric molecular chaperone possessing an "ATP-binding domain" and a "substrate-binding domain." In a resting state, the substrate-binding domain tightly binds to PERK, ATF6, and IRE1(Yang et al., 2015). When the accumulation of unfolded proteins exceeds a threshold, GRP78 releases these binding pockets to bind unfolded proteins, thereby activating downstream signaling pathways associated with ER stress (Ibrahim et al. 2019). Our study demonstrates that both EPA and ACT exhibit potential for binding to GRP78. In its interaction with GRP78, the stable conformation of ACT is located at the front of the ATP-binding pocket, which may interfere with ATP binding and affect its function. Concurrently, the multiple binding conformations of ACT with GRP78 may lead to instability of the complex, triggering ER stress. EPA may contribute to the maintenance of GRP78 conformational and functional homeostasis under ACT-induced ER stress. Rather than constitutively locking GRP78 in a UPR sensor-bound state, EPA binding near the ATP-binding domain/dimer interface may reduce ACT-induced structural disturbance and help preserve GRP78 chaperone activity. Under these conditions, excessive dissociation or activation of PERK, ATF6, and IRE1 may be attenuated, thereby limiting downstream PERK–ATF4–CHOP signaling and NLRP3 inflammasome activation. Our findings confirm that ACT-induced toxicity is closely associated with cellular pyroptosis, and also reveal that Ω3 mitigates inflammasome-mediated kidney damage through the pyroptosis pathway.

The NLRP3 inflammasome is associated with inflammatory and autoimmune disease. Hyperuricemia causes kidney damage by promoting NLRP3-mediated inflammation in rats (Wu et al., 2021a). ACT may induce systemic inflammation and immune response. Notably, Ω3 has potential effects on inflammation and the assembly of the NLRP3 inflammasome. We then measured the cellular response to inflammation; the NLRP3 inflammasome is often closely associated with inflammation (Mangan et al., 2018). We examined relevant indicators of the NLRP3 inflammasome in our model and found that ACT exposure upregulated NLRP3 inflammasome-related markers, triggering pyroptosis in the kidneys. Importantly, a significant mitigating effect of Ω3 was observed, as evidenced by reduced assembly of the NLRP3 inflammasome and decreased occurrence of pyroptosis in renal tissue. Furthermore, assessment of inflammation-related markers in the kidney indicated that Ω3 effectively alleviated renal inflammation induced by ACT.

ER stress can activate assembly of the NLRP3 inflammasome in mouse hearts and stimulate ROS production in

mouse corneas, leading to NLRP3 inflammasome activation (Tan et al., 2025). Meanwhile , ER stress can mediate pyroptosis in the chicken thymus (Wang et al. 2025). In our investigation, both ER stress and the inflammasome showed upregulated expression under ACT exposure. Therefore, we infer that ACT exposure regulates NLRP3 activation through ER stress, while Ω3 inhibits release of the NLRP3 inflammasome by alleviating ER stress (Li et al., 2020; Pereira et al., 2022). In vitro results revealed that the MCC950 group, which is an NLRP3 inflammasome inhibitor, did not significantly inhibit ER stress-related markers. However, the ER stress-inhibitor 4PBA group demonstrated inhibitory effects on NLRP3-related proteins. This preliminary evidence supports our hypothesis that Ω3 inhibits assembly of the NLRP3 inflammasome by alleviating ACT-induced ER stress. Based on previous research as well as our findings, we postulate that Ω3 restores GRP78 levels, leading to a reduction in PERK activation, which in turn decreases ATF4 expression and then reduces CHOP transcription, ultimately alleviating inflammasome activation and pyroptosis. Analysis of the potential pharmacological effects of Ω-3 indicates that Ω-3 may form a stable bond with IL-1β, which could mediate its mitigating action. This suggests that, in addition to suppressing IL-1β production via canonical upstream signaling pathways, Ω3 may also directly neutralize its activity, further blocking downstream inflammatory signaling.

The ER is a crucial organelle for intracellular lipid metabolism (Schwarz and Blower, 2016), and studies have shown that ER stress can induce lipotoxicity in renal cells (Çeker et al., 2022). The NLRP3 inflammasome also contributes to lipid accumulation in the kidneys of mice with diabetic nephropathy (Wu et al., 2021b). Both in vivo and in vitro experiments in this study confirmed that Ω3 can alleviate ACT-induced abnormalities in the PPAR pathway. Furthermore, in vitro experiments examining lipid metabolism and inflammation-related markers revealed that both the ER-stress-inhibitor and NLRP3-inhibitor groups exhibited corresponding mitigating effects on lipid-metabolism disorders. Based on these experimental results, we hypothesize that this process is associated with the PPAR pathway. Both inhibitor groups also showed corresponding mitigating effects on inflammation. Therefore, we conclude that Ω3 reduces ACT-induced lipid metabolism and inflammation by mitigating ER stress and inhibiting assembly of the NLRP3 inflammasome (Lebeaupin et al., 2015).

In summary, the kidney is a crucial metabolic organ that plays a central role in the body's circulatory system. ACT causes stress in renal cells and triggers pyroptosis, inflammation, and disorders of lipid metabolism. Ω3 mitigates kidney damage by alleviating this stress induced by ACT deposition. Ω3 can stabilize the structure of GRP78, inhibiting the release of downstream ATF6 and IRE1 and the phosphorylation of PERK in the ER stress pathway. This leads to suppression of ATF4 translation and inhibition of CHOP nuclear translocation, preventing cell death. Simultaneously, the process of ER stress recovery reduces expression of Caspase-1 and NLRP3, significantly inhibiting the protein ASC, which is required for NLRP3 inflammasome assembly. As a result, the NLRP3 inflammasome cannot assemble, reducing cleavage of IL-1β and GSDMD by Caspase-1 and alleviating pyroptosis and inflammation. In addition, abnormalities in lipid metabolism related to the PPAR pathway are mitigated, restoring proper renal lipid metabolism.

This study has several limitations. First, the 500 mg/kg ACT exposure level we used is higher than concentrations generally reported in environmental matrices or food chains; therefore, the current model should be regarded as a high-dose toxicological model for exploring ACT-induced renal injury and the protective effects of Ω3, rather than a direct representation of typical exposure. Second, although the in vivo intervention used a mixed Ω3 preparation containing both EPA and DHA, the in vitro mechanistic experiments mainly focused on EPA as a representative active component. Thus, the individual contributions of EPA and DHA were not fully distinguished. Future studies using environmentally relevant, chronic low-dose ACT exposure models and purified EPA/DHA interventions, alone or in combination, are needed to validate these findings under more realistic exposure conditions and clarify the respective roles of EPA and DHA in regulating GRP78-mediated ER stress and NLRP3 inflammasome activation.

5. Conclusions

In this study, we found that Ω3 could alleviate pyroptosis, inflammation, and lipid-metabolism disorders induced by the ER stress-NLRP3 axis caused by ACT exposure in the kidneys. We also propose that Ω3 has the potential to bind to IL-1β and thus inhibit pyroptosis. Meanwhile, Ω3 can stabilize the structure of GRP78, thereby alleviating ER

stress. Our study provides mechanistic evidence for the protective potential of Ω3 against pesticide-induced renal injury in broiler chickens, while further studies in mammalian models are required to evaluate its broader translational relevance. These results provide new insights into the role of Ω3 in modulating ER stress and its anti-inflammatory mechanisms: Ω3 alleviates pesticide-induced renal injury through multi-target mechanisms.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Data availability statement

The data will be made available upon request.

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Author contributions

Yuze Dong: Conceptualization, Data curation, Software, Formal analysis, Writing - Original Draft, Writing - Review & Editing. **Hongmin Lu**: Conceptualization, Software, Formal analysis, Investigation, Visualization. **Ruoqi Wang**: Software, Investigation, Data Curation, Methodology. **Yunfan Zhang:** Data Curation. X**in Zhang**: Validation. **Tiantian Guo:** Visualization. **Hao Liu:** Data Curation. **Qi Wang:** Investigation. **Mingwei Xing**: Data curation, Validation, Investigation, Project, Funding acquisition.

Compliance with ethics guidelines

Yuze DONG, Hongmin LU, Ruoqi WANG, Yunfan ZHANG, Xin ZHANG, Tiantian GUO, Qi WANG, Hao LIU and Mingwei XING declare that they have no conflicts of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed. NEFU's Animal Care, Use, and Ethics Committee approved creation of animal models and conduct of animal experiments in this study (Approval No. 2023059).

Declaration on the use of generative AI tools

No generative AI tools were used in the preparation of this manuscript.

References

Blevins HM, Xu Y, Biby S, Zhang S (2022) The NLRP3 inflammasome pathway: A review of mechanisms and inhibitors for the treatment of inflammatory diseases. Front Aging Neurosci 14:879021. https://doi.org/10.3389/fnagi.2022.879021

Brodeur JC, Damonte MJ, Rojas DE, et al (2022) Concentration of current-use pesticides in frogs from the pampa region and correlation of a mixture toxicity index with biological effects. Environ Res 204:112354. https://doi.org/10.1016/j.envres.2021.112354

Çeker T, Yılmaz Ç, Kırımlıoglu E, Aslan M (2022) Endoplasmic-reticulum-stress-induced lipotoxicity in human kidney epithelial cells. Toxicol Res 11:683–695. https://doi.org/10.1093/toxres/tfac041

Chen W-J, Chen S-F, Song H, et al (2024) Current insights into environmental acetochlor toxicity and remediation strategies. Environ Geochem Health 46:356. https://doi.org/10.1007/s10653-024-02136-7

Cho W, Choi SW, Lim DS, et al (2025) Donepezil alleviates hepatic steatosis by mitigating ER stress via the AMPK/autophagy pathway. Mol Cell Endocrinol 601:112523. https://doi.org/10.1016/j.mce.2025.112523

Cholewski M, Tomczykowa M, Tomczyk M (2018) A comprehensive review of chemistry, sources and bioavailability of omega-3 fatty acids. Nutrients 10:1662. https://doi.org/10.3390/nu10111662

Dandekar A, Mendez R, Zhang K (2015) Cross talk between ER stress, oxidative stress, and inflammation in health and disease. Methods Mol Biol Clifton NJ 1292:205–214. https://doi.org/10.1007/978-1-4939-2522-3_15

Di Conza G, Ho P-C (2020) ER stress responses: An emerging modulator for innate immunity. Cells 9:695. https://doi.org/10.3390/cells9030695

Dong P-F, Li Z-F, Lian C-Y, et al (2022) Inhibited transcription factor EB function induces reactive oxygen species overproduction to promote pyroptosis in cadmium-exposed renal tubular epithelial cells. Chem Biol Interact 368:110249. https://doi.org/10.1016/j.cbi.2022.110249

Essawy AE, Jimmiey EM, Abdel-Wahab WM, et al (2025) The protective efficacy of omega-3 polyunsaturated fatty acids on oxidative stress, inflammation, neurotransmitter perturbations, and apoptosis induced by monosodium glutamate in the brain of male rats. Metab Brain Dis 40:114. https://doi.org/10.1007/s11011-025-01539-4

Fassett RG, Gobe GC, Peake JM, Coombes JS (2010) Omega-3 polyunsaturated fatty acids in the treatment of kidney disease. Am J Kidney Dis Off J Natl Kidney Found 56:728–742. https://doi.org/10.1053/j.ajkd.2010.03.009

Fu J, Wu H (2023) Structural mechanisms of NLRP3 inflammasome assembly and activation. Annu Rev Immunol 41:301–316. https://doi.org/10.1146/annurev-immunol-081022-021207

Guzzella L, Pozzoni F, Giuliano G (2006) Herbicide contamination of surficial groundwater in northern italy. Environ Pollut 142:344–353. https://doi.org/10.1016/j.envpol.2005.10.037

Han M, Fu J, Suonan Z, et al (2025) Ershiwuwei shanhu pills alleviates cerebral ischemia injury in rats by regulating endoplasmic reticulum stress through GRP78/XBP1/CHOP pathway. Phytomedicine Int J Phytother Phytopharm 145:156969. https://doi.org/10.1016/j.phymed.2025.156969

Hetz C (2012) The unfolded protein response: Controlling cell fate decisions under ER stress and beyond. Nat Rev Mol Cell Biol 13:89–102. https://doi.org/10.1038/nrm3270

Hu J, Liu Z, Zhang H (2017) Omega-3 fatty acid supplementation as an adjunctive therapy in the treatment of chronic kidney disease: A meta-analysis. Clin Sao Paulo Braz 72:58–64. https://doi.org/10.6061/clinics/2017(01)10

Ibrahim IM, Abdelmalek DH, Elfiky AA (2019) GRP78: A cell's response to stress. Life Sci 226:156–163. https://doi.org/10.1016/j.lfs.2019.04.022

Ishihara T, Yoshida M, Arita M (2019) Omega-3 fatty acid-derived mediators that control inflammation and tissue homeostasis. Int Immunol 31:559–567. https://doi.org/10.1093/intimm/dxz001

Jh B, Pf L, J T, et al (2025) Endoplasmic reticulum stress as a driver and therapeutic target for kidney disease. Nat Rev Nephrol. https://doi.org/10.1038/s41581-025-00938-1

Kelley N, Jeltema D, Duan Y, He Y (2019) The NLRP3 inflammasome: An overview of mechanisms of activation and regulation. Int J Mol Sci 20:3328. https://doi.org/10.3390/ijms20133328

Lebeaupin C, Proics E, de Bieville CHD, et al (2015) ER stress induces NLRP3 inflammasome activation and hepatocyte death. Cell Death Dis 6:e1879–e1879. https://doi.org/10.1038/cddis.2015.248

Lee SM, Jeong YI, Jung S, et al (2026) Omega-3 Fatty Acids Attenuate Renal Myostatin Expression and Mitochondrial Alterations Under Uremic Conditions. Int J Mol Sci 27:4030. https://doi.org/10.3390/ijms27094030

Levassort H, Essig M (2024) [the kidney, its anatomy and main functions]. Soins Gerontol 29:10–20. https://doi.org/10.1016/j.sger.2023.12.003

Li D, Zhang K, Xu C, et al (2023) Cypermethrin induces apoptosis, autophagy and inflammation via ERS-ROS-NF-κB axis in hepatocytes of carp (cyprinus carpio). Pestic Biochem Physiol 196:105625. https://doi.org/10.1016/j.pestbp.2023.105625

Li W, Cao T, Luo C, et al (2020) Crosstalk between ER stress, NLRP3 inflammasome, and inflammation. Appl Microbiol Biotechnol 104:6129–6140. https://doi.org/10.1007/s00253-020-10614-y

Li X, Wu Q, Chen D, et al (2024) Environment-relevant concentrations of cadmium induces necroptosis and inflammation; baicalein maintains gill homeostasis through suppressing ROS/ER stress signaling in common carps (Cyprinus carpio L.). Environ Pollut 340:122805. https://doi.org/10.1016/j.envpol.2023.122805

Lin Y-L, Wang C-L, Liu K-L, et al (2022) Omega-3 fatty acids improve chronic kidney disease-associated pruritus and inflammation. Med Kaunas Lith 58:796. https://doi.org/10.3390/medicina58060796

Lu H, Guo T, Zhang Y, et al (2024) Endoplasmic reticulum stress-induced NLRP3 inflammasome activation as a novel mechanism of polystyrene microplastics (PS-MPs)-induced pulmonary inflammation in chickens. J Zhejiang Univ Sci B 25:233–243. https://doi.org/10.1631/jzus.B2300409

Lu Q, Shen Z, Zheng K, et al (2022) Estimating the bioavailability of acetochlor to wheat using in situ pore water and passive sampling. Sci Total Environ 833:155239. https://doi.org/10.1016/j.scitotenv.2022.155239

Mangan MSJ, Olhava EJ, Roush WR, et al (2018) Targeting the NLRP3 inflammasome in inflammatory diseases. Nat Rev Drug Discov 17:588–606. https://doi.org/10.1038/nrd.2018.97

Mesmar F, Muhsen M, Mirchandani R, et al (2024) The herbicide acetochlor causes lipid peroxidation by inhibition of glutathione peroxidase activity. Toxicol Sci Off J Soc Toxicol 202:302–313. https://doi.org/10.1093/toxsci/kfae113

Ming S, Tian J, Ma K, et al (2022) Oxalate-induced apoptosis through ERS-ROS-NF-κB signalling pathway in renal tubular epithelial cell. Mol Med Camb Mass 28:88. https://doi.org/10.1186/s10020-022-00494-5

Pereira AC, De Pascale J, Resende R, et al (2022) ER-mitochondria communication is involved in NLRP3 inflammasome activation under stress conditions in the innate immune system. Cell Mol Life Sci CMLS 79:213. https://doi.org/10.1007/s00018-022-04211-7

Qin X, Jiang Y, Wang Z, et al (2020) The migration of acetochlor from feed to milk. RSC Adv 10:44344–44351. https://doi.org/10.1039/D0RA06895K

Qu Z, Li Z, Wang Y, et al (2026) Calycosin attenuates renal fibrosis by modulating lipid metabolism via the PCK1/TWIST1/CPT1α axis. Life Sci 399:124423. https://doi.org/10.1016/j.lfs.2026.124423

Ren M, Lv X, Xu T, et al (2024) Effects of atrazine and curcumin exposure on TCMK-1 cells: Oxidative damage, pyroptosis and cell cycle arrest. Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc 185:114483. https://doi.org/10.1016/j.fct.2024.114483

Schwarz DS, Blower MD (2016) The endoplasmic reticulum: Structure, function and response to cellular signaling. Cell Mol Life Sci CMLS 73:79–94. https://doi.org/10.1007/s00018-015-2052-6

Shahidi F, Ambigaipalan P (2018) Omega-3 polyunsaturated fatty acids and their health benefits. Annu Rev Food Sci Technol 9:345–381. https://doi.org/10.1146/annurev-food-111317-095850

Shi H-H, Zhang L-Y, Chen L-P, et al (2022) EPA-enriched phospholipids alleviate renal interstitial fibrosis in spontaneously hypertensive rats by regulating TGF-β signaling pathways. Mar Drugs 20:152. https://doi.org/10.3390/md20020152

Song N, Li X, Cui Y, et al (2021) Hydrogen sulfide exposure induces pyroptosis in the trachea of broilers via the regulatory effect of circRNA-17828/miR-6631-5p/DUSP6 crosstalk on ROS production. J Hazard Mater 418:126172. https://doi.org/10.1016/j.jhazmat.2021.126172

Song X, Zhang F, Chen D, et al (2019) Study on systemic and reproductive toxicity of acetochlor in male mice. Toxicol Res 8:77–89. https://doi.org/10.1039/C8TX00178B

Sun X, Zhou Q, Ren W, et al (2011) Spatial and temporal distribution of acetochlor in sediments and riparian soils of the songhua river basin in northeastern China. J Environ Sci 23:1684–1690. https://doi.org/10.1016/S1001-0742(10)60595-5

Tan W-L, Yu X, Jia J, et al (2025) Alpha-lipoamide prevents acute kidney injury in mouse by inhibiting renal tubular epithelial cell pyroptosis. Biochem Pharmacol 116942. https://doi.org/10.1016/j.bcp.2025.116942

Wang H, Meng Z, Zhou L, et al (2019) Effects of acetochlor on neurogenesis and behaviour in zebrafish at early developmental stages. Chemosphere 220:954–964. https://doi.org/10.1016/j.chemosphere.2018.12.199

Wang K, Shi X, Lin H, et al (2025) Selenium deficiency exacerbates ROS/ER stress mediated pyroptosis and ferroptosis induced by bisphenol a in chickens thymus. J Environ Sci China 148:13–26. https://doi.org/10.1016/j.jes.2024.01.002

Wang R, Hou L, Lu H, et al (2024a) Unveiling the interplay of MAPK/NF-κB/MLKL axis in brain health: Omega-3 as a promising candidates against copper neurotoxicity. J Environ Manage 370:122791. https://doi.org/10.1016/j.jenvman.2024.122791

Wang W, Man Y, Xie J, et al (2023a) Occurrence and risk assessment of three chloroamide herbicides in water and soil environment in northeastern, eastern and southern China. Environ Res 219:115104. https://doi.org/10.1016/j.envres.2022.115104

Wang W, Wang D, Liu Q, et al (2024b) Distribution characteristics and risk assessment of 57 pesticides in farmland soil and the surrounding water. Toxics 12:85. https://doi.org/10.3390/toxics12010085

Wang X, Chen F, Lu J, et al (2023b) Developmental and cardiovascular toxicities of acetochlor and its chiral isomers in zebrafish embryos through oxidative stress. Sci Total Environ 896:165296. https://doi.org/10.1016/j.scitotenv.2023.165296

Wang X, Peng B, Zhang C, et al (2024c) Hepatic effects of acetochlor chiral isomers in zebrafish and L02 cells. Sci Total Environ 913:169781. https://doi.org/10.1016/j.scitotenv.2023.169781

Wu C, Li Q, Zhao Z, et al (2025) FFAR4 Functions as a DHA Receptor to Attenuate LPS-Induced Inflammation via the cAMP/IκBα Pathway in Large Yellow Croaker (Larimichthys crocea). FASEB J Off Publ Fed Am Soc Exp Biol 39:e71068. https://doi.org/10.1096/fj.202501468R

Wu M, Ma Y, Chen X, et al (2021a) Hyperuricemia causes kidney damage by promoting autophagy and NLRP3-mediated inflammation in rats with urate oxidase deficiency. Dis Model Mech 14:dmm048041. https://doi.org/10.1242/dmm.048041

Wu M, Ma Y, Chen X, et al (2021b) Hyperuricemia causes kidney damage by promoting autophagy and NLRP3-mediated inflammation in rats with urate oxidase deficiency. Dis Model Mech 14:dmm048041. https://doi.org/10.1242/dmm.048041

Yang J, Nune M, Zong Y, et al (2015) Close and Allosteric Opening of the Polypeptide-Binding Site in a Human Hsp70 Chaperone Structure 23:2191–2203. https://doi.org/10.1016/j.str.2015.10.012

Yang L, Tang L, Li J, et al (2026) Tubular Omega-3 Fatty Acid Receptor FFAR4 Deficiency Aggravated Renal Aging and Chronic Kidney Disease. Aging Cell 25:e70546. https://doi.org/10.1111/acel.70546

Ye C (2003) Environmental behavior of the herbicide acetochlor in soil. Bull Environ Contam Toxicol 71:919–923. https://doi.org/10.1007/s00128-003-0217-8

Zhang Y, Lu H, Hou L, et al (2025a) GPR120 exacerbates the immune-inflammatory response in chicken liver by mediating acetochlor induced macrophage M1 polarization. J Hazard Mater 485:136928. https://doi.org/10.1016/j.jhazmat.2024.136928

Zhang Y, Lu H, Hou L, et al (2025b) GPR120 exacerbates the immune-inflammatory response in chicken liver by mediating acetochlor induced macrophage M1 polarization. J Hazard Mater 485:136928. https://doi.org/10.1016/j.jhazmat.2024.136928

Zhang Y, Xue W, Long R, et al (2020) Acetochlor affects zebrafish ovarian development by producing estrogen effects and inducing oxidative stress. Environ Sci Pollut Res 27:27688–27696. https://doi.org/10.1007/s11356-020-09050-2

Zhang Y, Zhang E, Hou L, et al (2024) Assessing and mitigating foodborne acetochlor exposure induced ileum toxicity in broiler chicks: The role of omega-3 polyunsaturated fatty acids supplementation and molecular pathways analysis. Pestic Biochem Physiol 199:105761. https://doi.org/10.1016/j.pestbp.2023.105761

Zhao X, Shi X, Liu Q, Li X (2022) Tea polyphenols alleviates acetochlor-induced apoptosis and necroptosis via ROS/MAPK/NF-κB signaling in ctenopharyngodon idellus kidney cells. Aquat Toxicol Amst Neth 246:106153. https://doi.org/10.1016/j.aquatox.2022.106153

Zhou B, Cao Y, Zhang Y, et al (2025) Unveiling excessive feed-sources copper-induced ileitis in chickens: Insights into tight junction damage and ROS/NLRP3/pyroptosis axis. Comp Biochem Physiol Toxicol Pharmacol CBP 299:110357. https://doi.org/10.1016/j.cbpc.2025.110357

Supplementary information
Sample: Figs. S1, S2, S3; Materials and methods; Tables S1 S2and S3.