Effects of CsA and Zn^{2+} Treatments on CN-Related Gene Expression and Oxidative Stress Indices in Gill and Kidney of Gymnocypris eckloni

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Introduction

This study revealed the regulatory mechanisms of calcineurin (CN) on the NFAT and NF-κB signaling pathways by detecting the mRNA expression levels of genes associated with these pathways (including *GeCNAα*, *GeCNB*, *GeNFAT*, *GeNF-κB*, *GeTNF-α*, *GeIL-1β*, *GeMT*, and *GeHsp90*) in the gills and kidneys of *G. eckloni* across different treatment groups subjected to CsA injection and Zn^{2+} stress. By investigating the changes in antioxidant enzyme activities (SOD, CAT, GSH-Px) and oxidative stress markers (MDA, ROS), this research explored the molecular mechanisms by which Zn^{2+} stress induces tissue damage through oxidative stress (ROS accumulation, MDA elevation) and imbalances in antioxidant enzyme activities (e.g., reduced SOD, CAT, GSH-Px activities). Additionally, it elucidated the regulatory role of CsA injection in restoring the oxidant-antioxidant balance via inhibition of calcineurin activity, thereby revealing the interaction patterns between heavy metal toxicity and drug intervention in the gills and kidneys of fish.

Methods

Cyclosporin A and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich Chemical Co. CsA was dissolved by DMSO to prepare 50 mg/L CsA mother liquor and diluted by PBS to 5 mg/L, 10 mg/L, and 15 mg/L, respectively. 100 μL of 5mg /L, 10 mg/L, and 15 mg /L CsA solutions were injected into the base of the ventral fin. After 0, 24, 48, and 72 h treatment, the gills and kidney were dissected immediately and flash frozen in liquid nitrogen for storage at -80 ℃ until use.

Following initial exposure to treatment with CsA (15 mg/L) for 48 h, the fish were subsequently subjected to Zn2+ (1 mg/L) for a duration of 12 h.

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Results

GeCNAα and *GeCNB* mRNA expression after CsA treatment

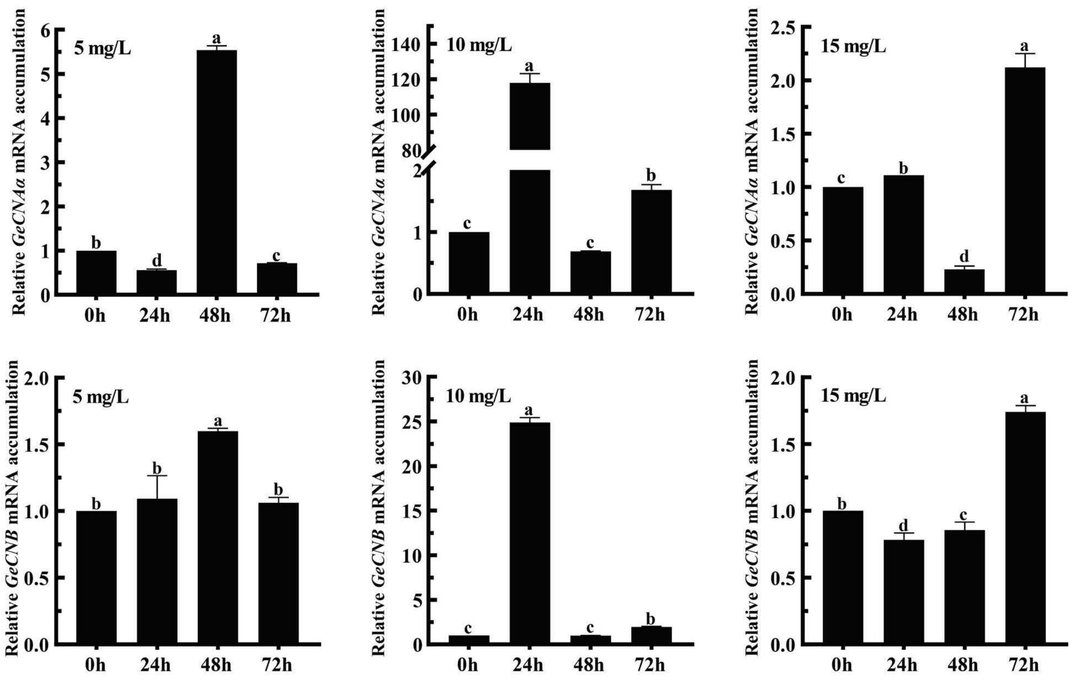

Figure 1. The expression of *GeCNAα* and *GeCNB* was detected in the mixed samples of gill and kidney after 100 μL of 5, 10, and 15 mg/L CsA treated for 0 h, 24 h, 48 h, and 72 h.

The expression levels of GeCNs and other related genes after 15 mg/L CsA treatment

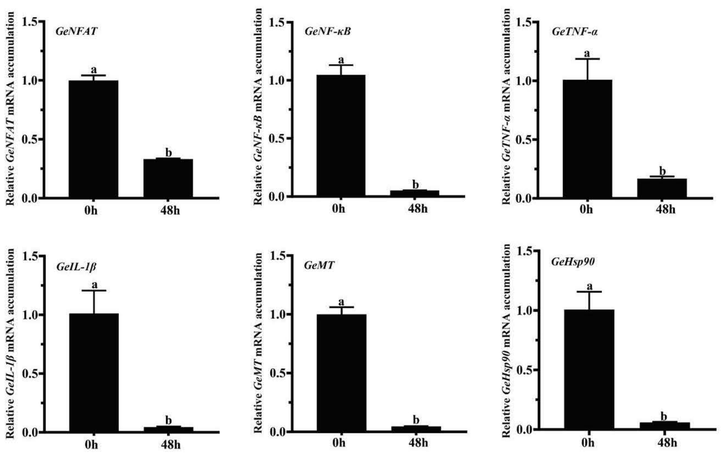

Figure 2. The expression of GeNFAT, GeNF-κB, GeTNF-α, GeIL-1β, GeMT, and GeHsp90 in the gills of G. eckloni after 15 mg/L CsA treatment.

Results

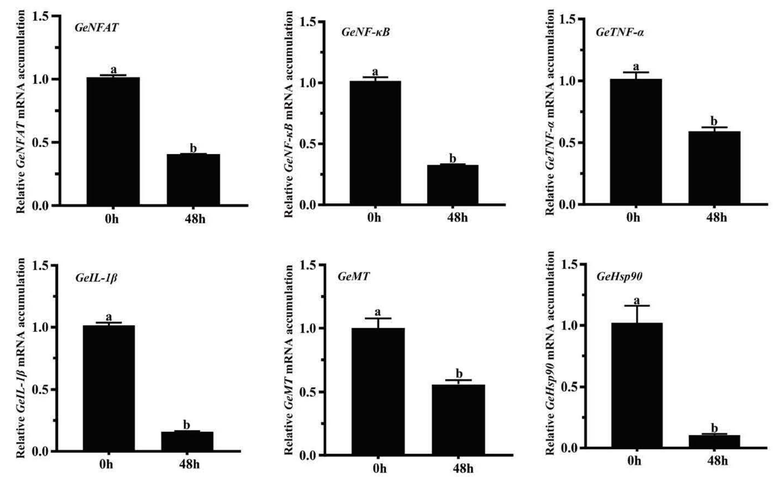

Figure 3. The expression of GeNFAT, GeNF-κB, GeTNF-α, GeIL-1β, GeMT, and GeHsp90 in the kidney of G. eckloni after 15 mg/L CsA treatment.

Changes in oxidative stress indicators

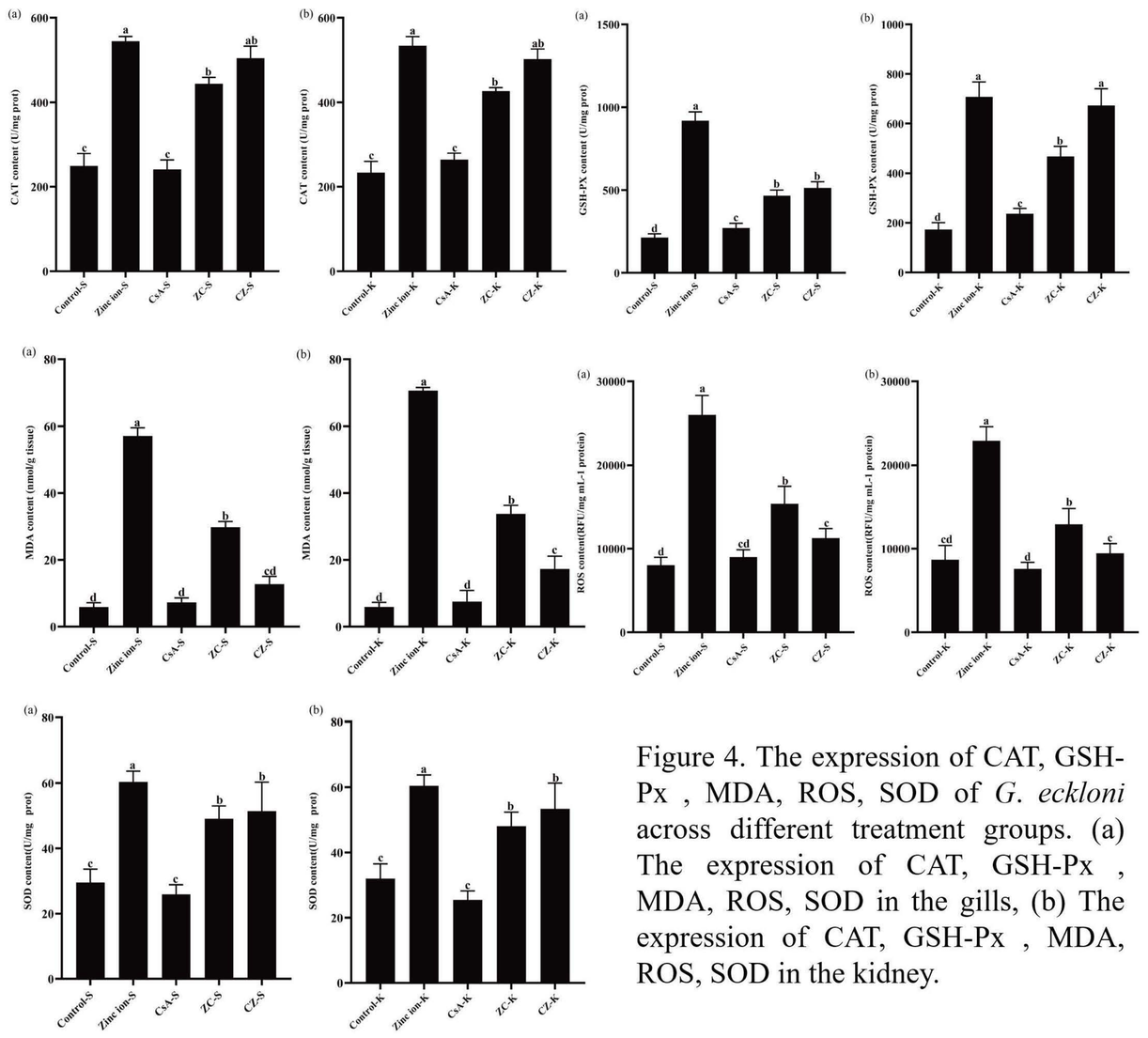

Figure 4. The expression of CAT, GSH-Px , MDA, ROS, SOD of *G. eckloni* across different treatment groups. (a) The expression of CAT, GSH-Px , MDA, ROS, SOD in the gills, (b) The expression of CAT, GSH-Px , MDA, ROS, SOD in the kidney.

Transmission electron microscopy (TEM)

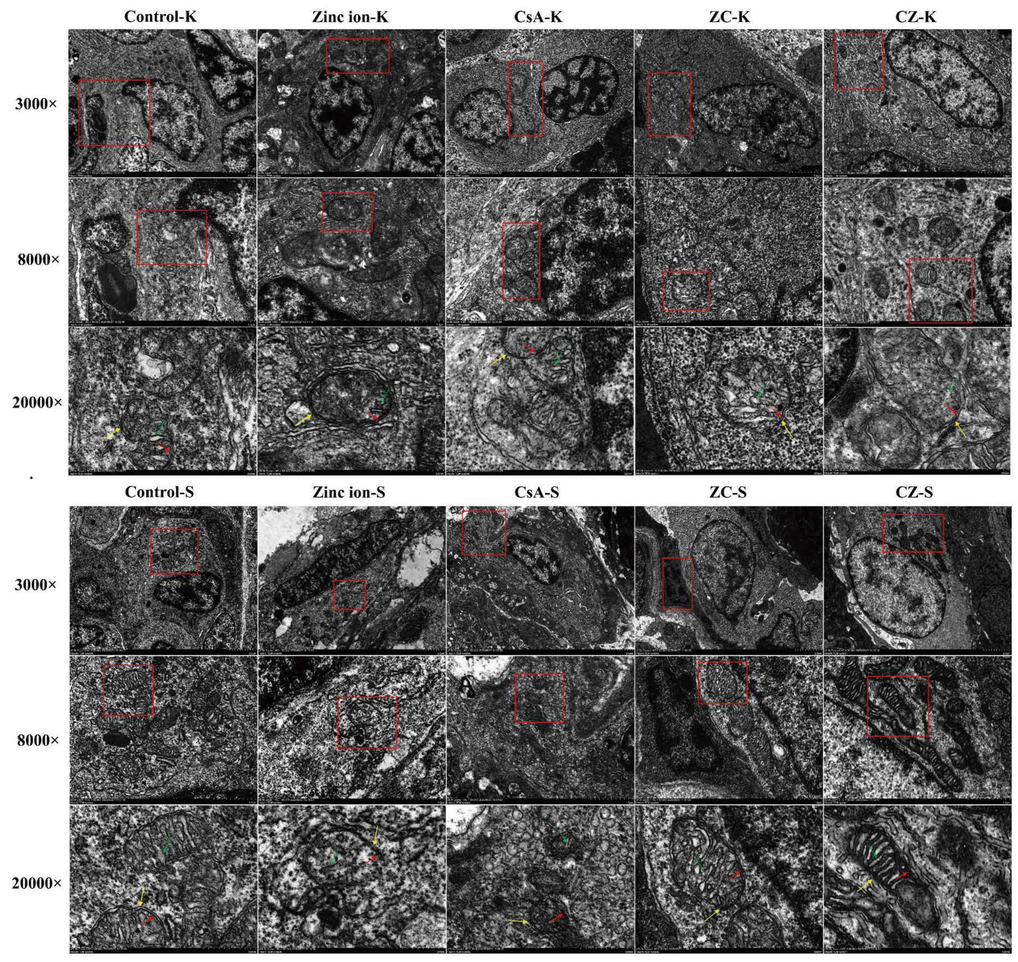

Figure 5. Transmission electron micrographs of kidneys and gills in G. eckloni from different treatment groups of CsA and Zn2+.The red box indicates structural changes in mitochondria, the yellow arrows point to the outer mitochondrial membrane, the red arrows denote the inner mitochondrial membrane, and the green arrows mark the mitochondrial cristae.

Conclusion

CsA has the ability to greatly reduce the transcription levels of CN and its downstream immune target genes in the gills and kidney of G. eckloni. This suggests that CsA can regulate the NFAT and NF-κB signaling pathways through CN, leading to a direct or indirect reduction in the expression of TNF-α, IL-1β, MT, and Hsp90. Following Zn^{2+} treatment, the activities of the antioxidant enzymes SOD, CAT, and GSH-Px, as well as the levels of ROS and MDA, were elevated. In gill tissues, mitochondrial structures appeared fragmented, with partial rupture and disappearance of inner and outer membranes, and extensive loss of mitochondrial cristae; in renal tissues, mitochondrial structures remained intact with clearly visible inner and outer membranes, yet significant cristae loss was observed.