MOLECULAR DELINEATION, IMMUNE EXPRESSION ANALYSIS, AND ANTIOXIDANT FUNCTIONAL ASSESSMENT OF CATALASE (CAT) FROM CHUB MACKEREL (*Scomber japonicus*)

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INTRODUCTION

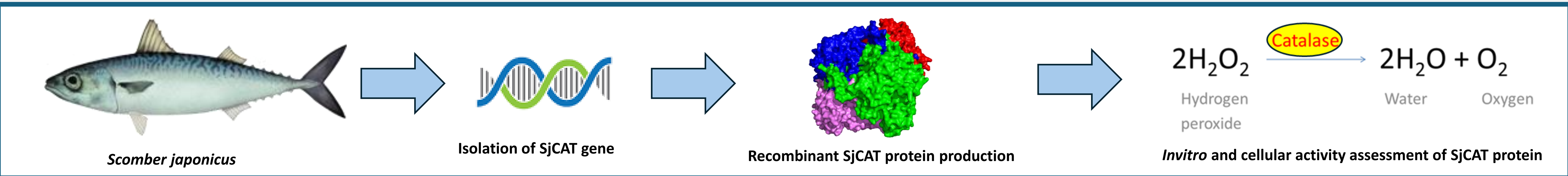
Catalase (CAT) is one of the crucial antioxidants and immunologically important enzymes that regulate host redox homeostasis through the breakdown of cellular H_2O_2 into water (H_2O) and oxygen (O_2). Intensive aquaculture practices of chub mackerel (*Scomber japonicus*) are always challenged by the oxidative and immunological stressors. The identification of multifunctional genes can be one of the promising ways to enhance their stress tolerance ability. Thus, the vital antioxidant and immunological functions of catalase from *S. japonicus* (SjCAT) were investigated under this study.

MATERIALS & METHODS

First, a group of healthy fish were reared, and their tissue samples were collected for the transcriptome establishment and the tissue specific expression analysis. Immune challenge experiment was performed to observe the expression of *SjCAT* upon the immunostimulants and the immune organs such as blood and spleen were collected. All the extracted tissues were subjected to RNA extraction and the cDNA was synthesized to observe the tissue-specific and temporal expression. Catalase from *S. japonicus* was identified from the transcriptome and subjected to the *in silco* analysis. The identified SjCAT coding sequence (CDS) was cloned into pMAL-c5X, pcDNA3.1$^{(+)}$ vector for the recombinant protein synthesis and cellular culture-based assays.

RESULTS & DISCUSSION

The CDS from *S. japonicus* was comprised of 1584 bp encoding 527 amino acids in length with a molecular weight of 59.99 kDa. The *in-silico* analysis revealed that the active site signature motif and the heme-binding ligand were highly conserved among the teleost fish and all the other identified vertebrate counterparts. The highest evolutionary identity (96%) and similarity (97.9%) relationship of SjCAT were indicated with *Thunnus maccoyii.* The highest *SjCAT* mRNA expression was observed in blood and followed by brain, heart, muscle, and liver. Furthermore, significant modulation of *SjCAT* expression was observed in blood and spleen upon the immunostimulants of Polyinosinic: polycytidylic acid (poly I: C), lipopolysaccharide (LPS), *Vibrio harveyi* (VH) and *Streptococcus iniae* (SI). The recombinant SjCAT (rSjCAT) protein functions were also characterized by catalase activity variation upon pH and temperature changes, ABTS radical scavenging assay, colony counting assay, and the catalase activity assay. In addition, the cell viability assay, and reduction of the metal cation generated ROS production and the NO scavenging assay further confirmed the antioxidant activity of SjCAT.



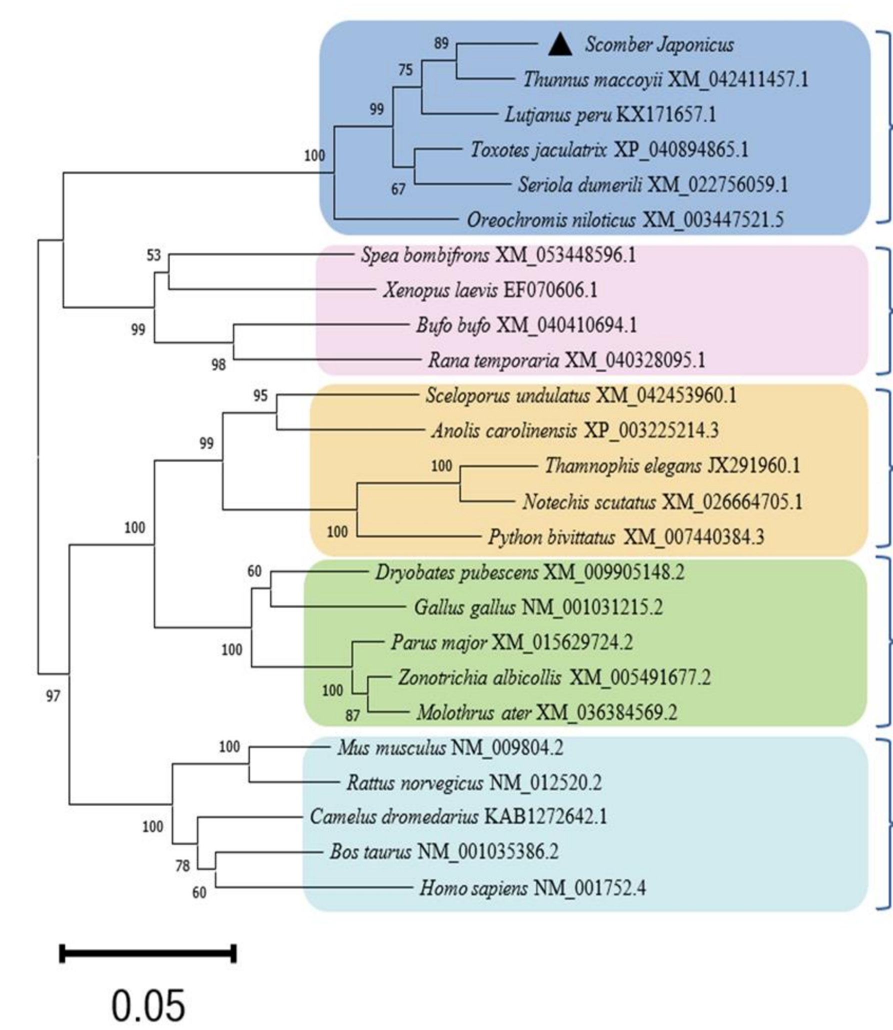


Fig 1: The reconstructed phylogenetic tree using MEGA11 software with 5000 bootstraps based on catalase orthologs from different species.

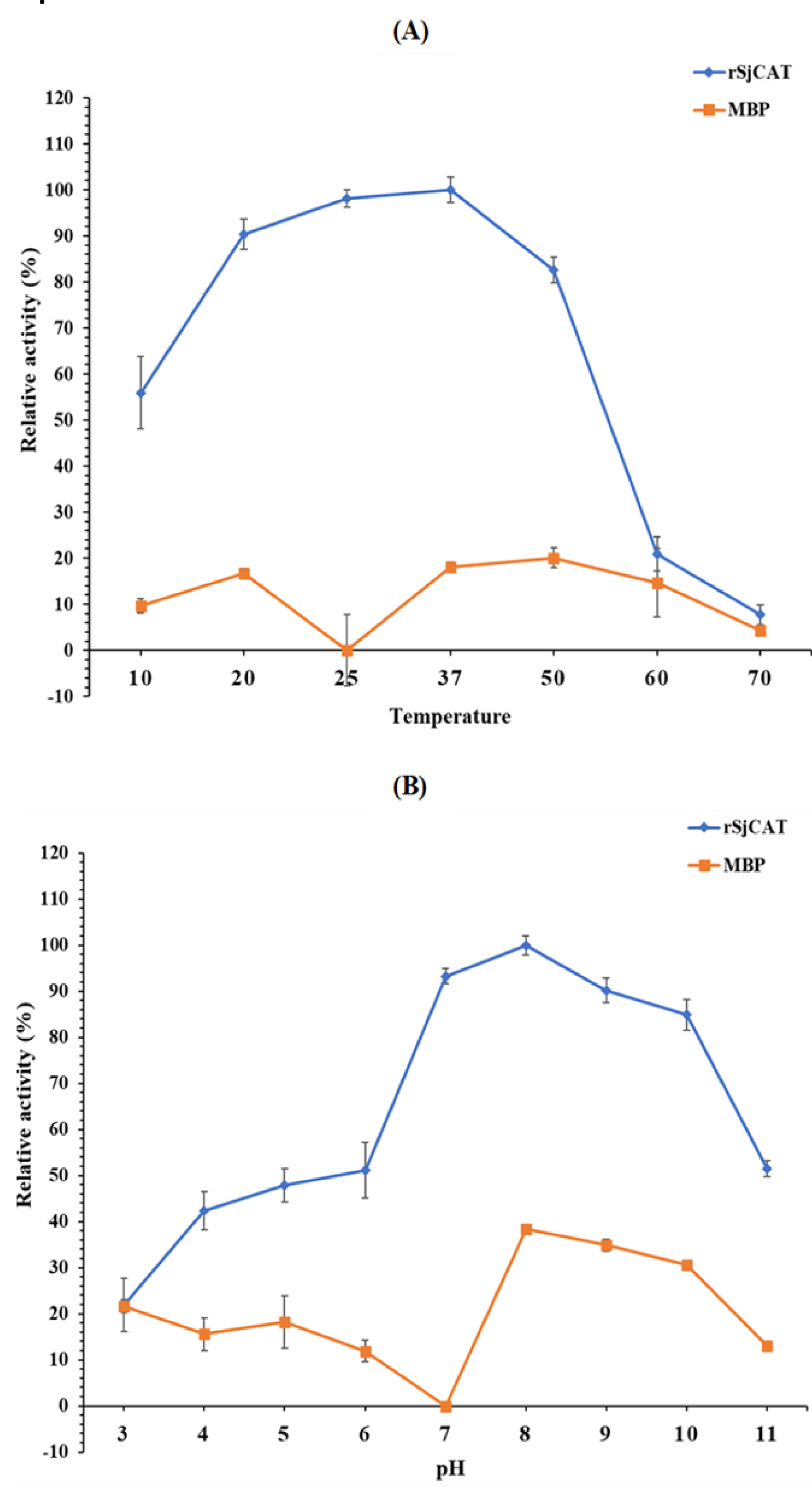


Fig 6: The catalase activity upon the effect of (A) temperature and (B) pH.

CONCLUSION

The results of the current study suggest that SjCAT is a potent antioxidant and immunologically important gene for host survival during redox unstable environments and critical pathogenic interventions.

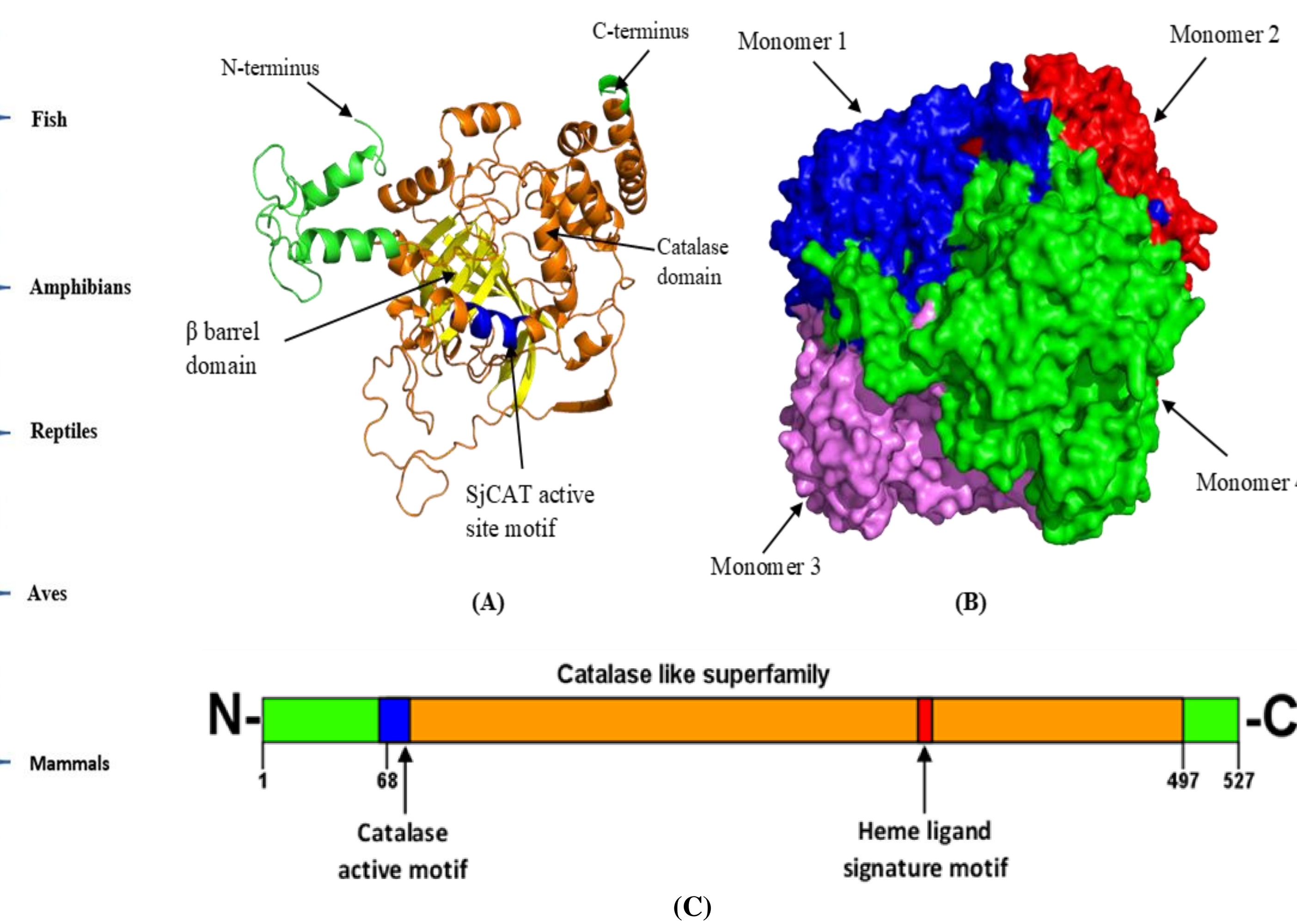


Fig 2: (A) Three-dimensional structure of the SjCAT protein monomer and, (B) tetrameric surface structure of SjCAT were predicted using PyMOL software. (C) Domain and active motif organization of SjCAT.

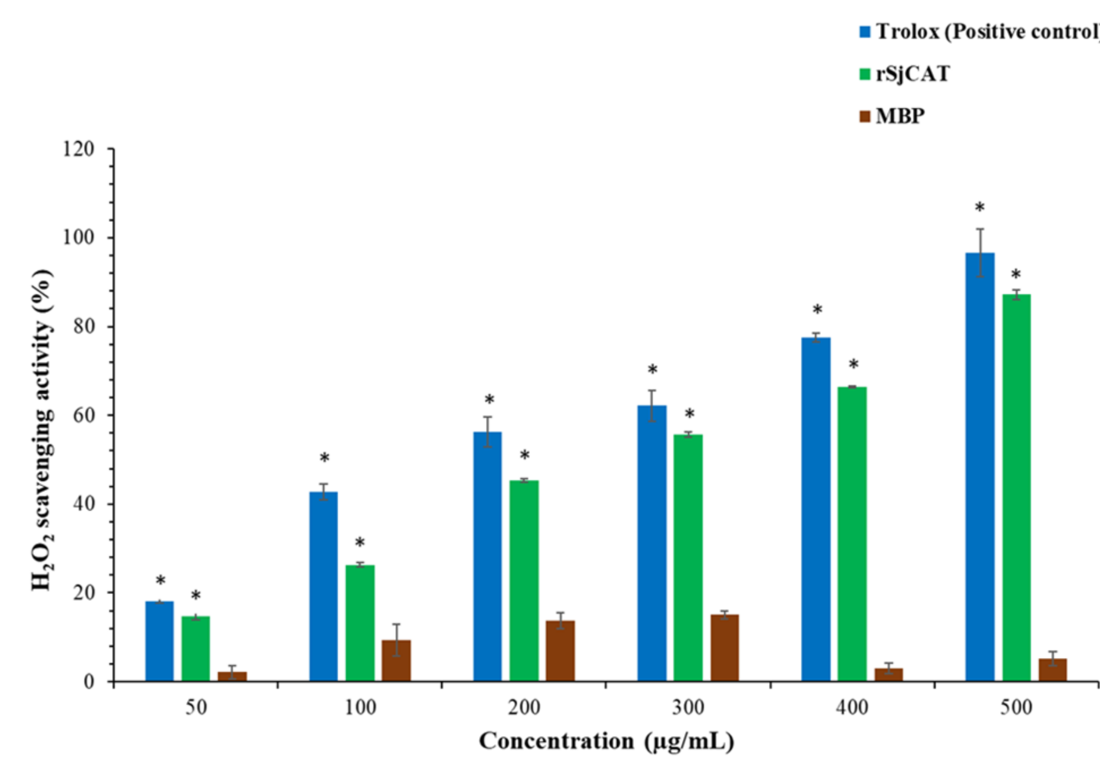


Fig 7: H_2O_2 scavenging activity of rSjCAT at different concentrations (50, 100, 200, 300, 400, and 500 µg/mL)

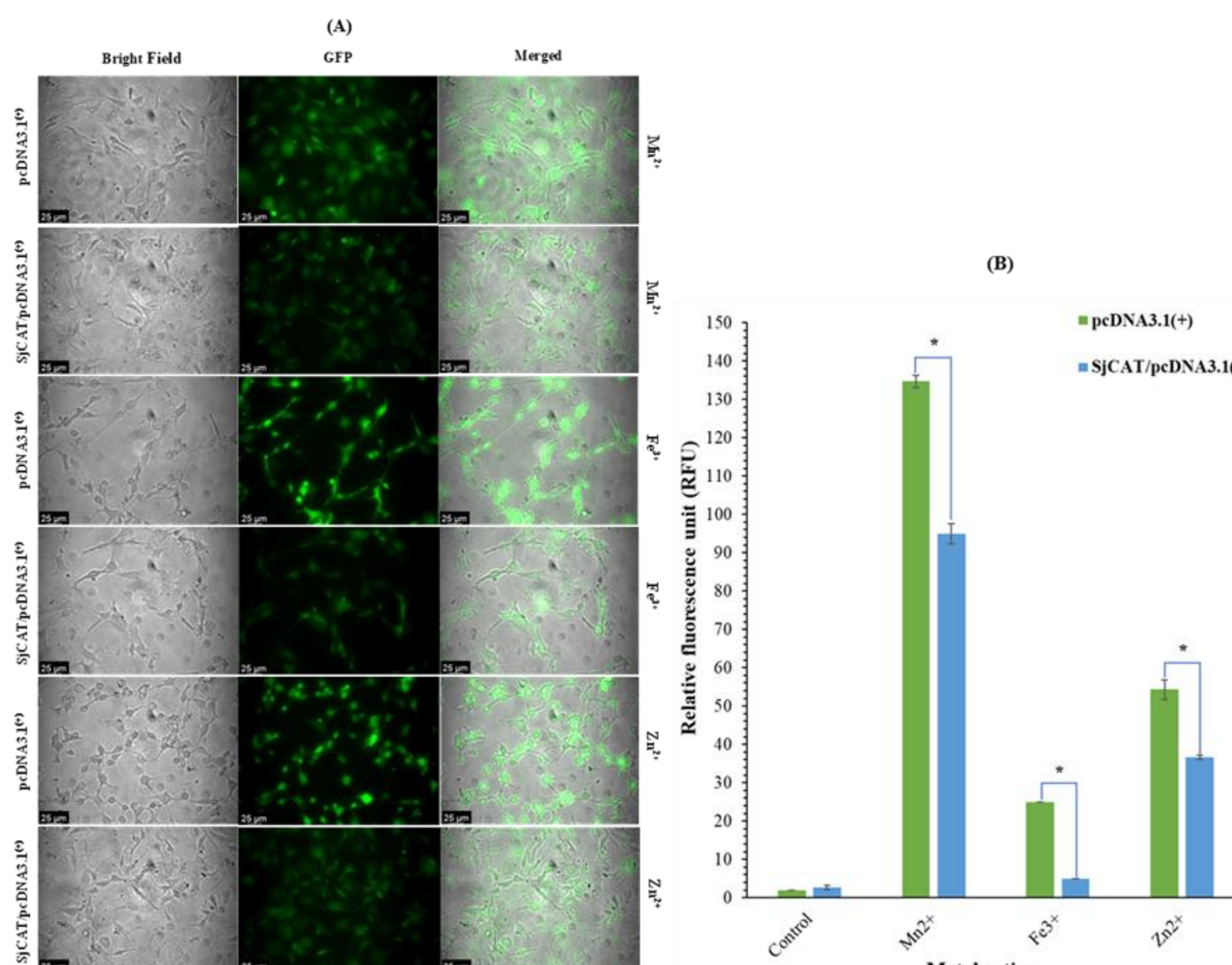


Fig 8: (A) Metal cation triggered ROS production in fathead minnow (FHM) cells. The cells were stained with DCFH-DA and visualized under the bright field (BF), and green, fluorescent light. (B) Relative fluorescent units of SjCAT/pcDNA3.1$^{(+)}$ transfected cell and empty pcDNA3.1$^{(+)}$ treated cells upon the metal cation-induced ROS production were statistically compared in triplicates.

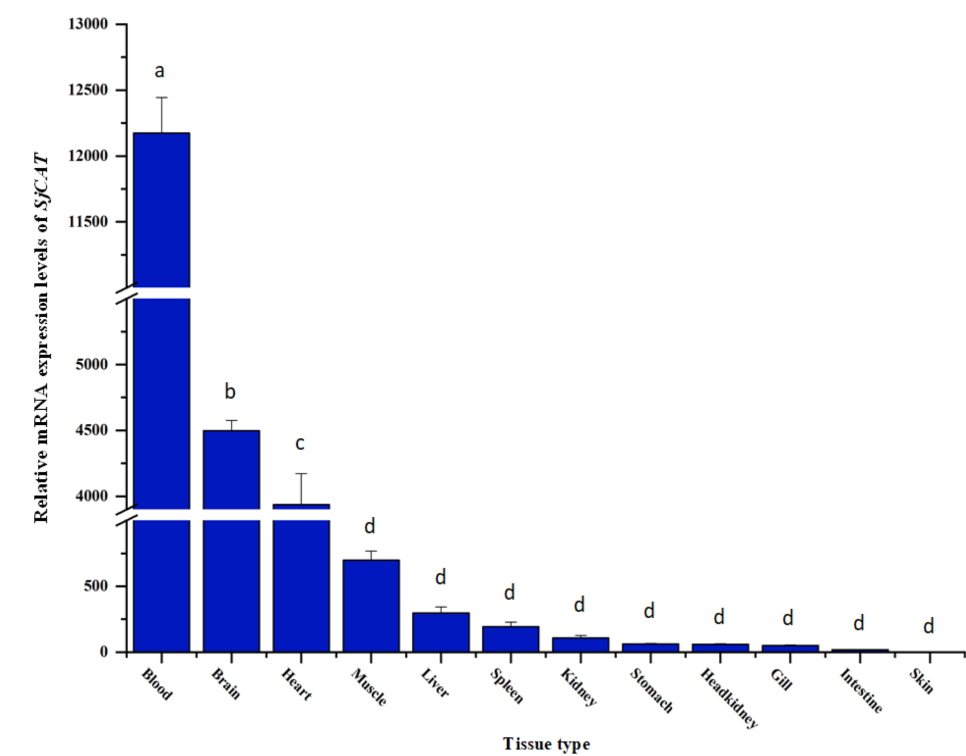


Fig 4: Tissue-specific relative mRNA expression of *SjCAT*. Spatial expression was detected by quantitative real-time PCR (qPCR) and evaluated using the Livak $2^{-\Delta\Delta CT}$ method.

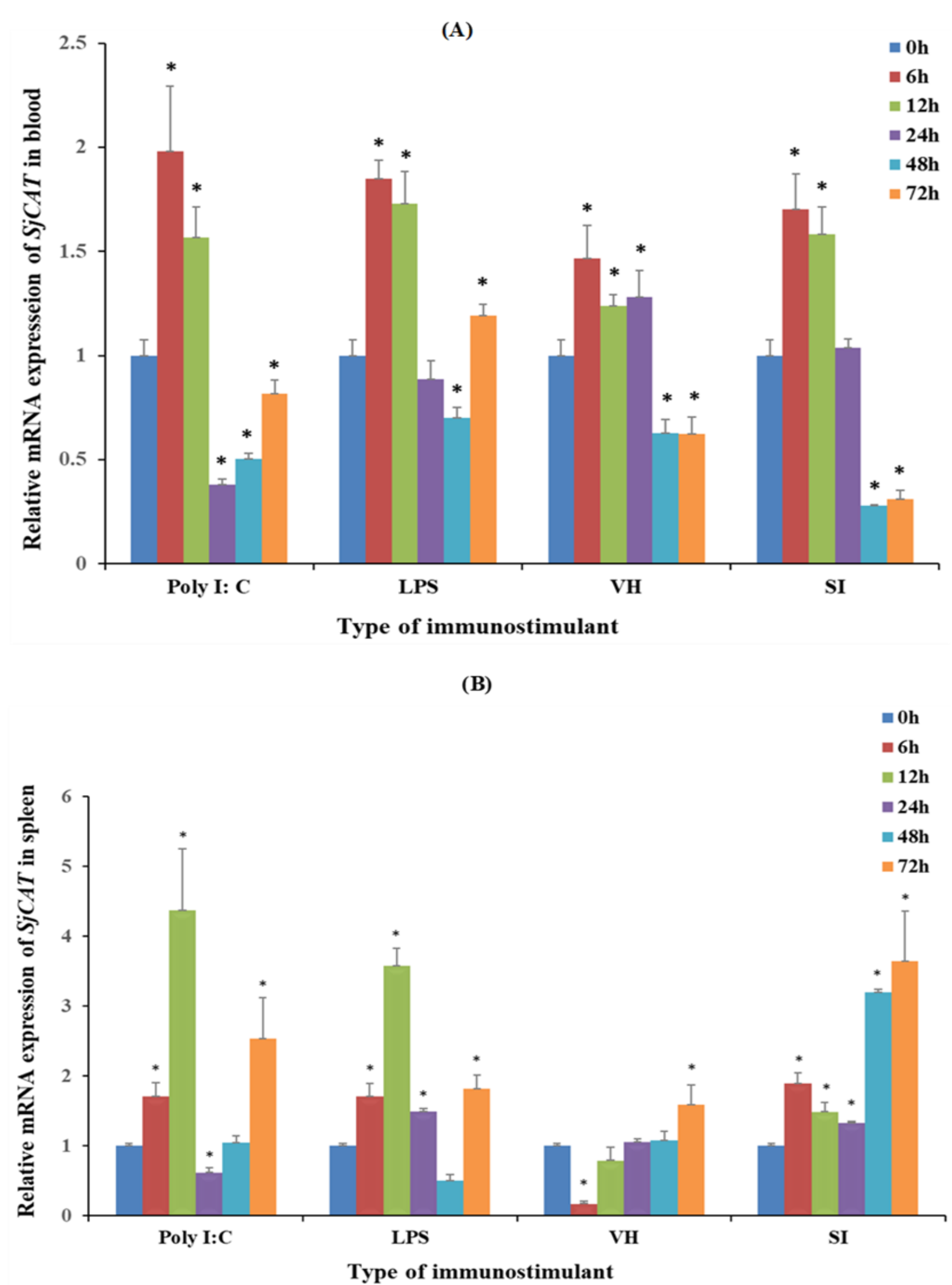


Fig 5: Relative expression of *SjCAT* in (A) blood and (B) spleen tissues upon the stimulation of polyinosinic: polycytidylic acid (Poly I: C), lipopolysaccharides (LPS), *Vibrio harveyi* (VH) and *Streptococcus iniae* (SI).

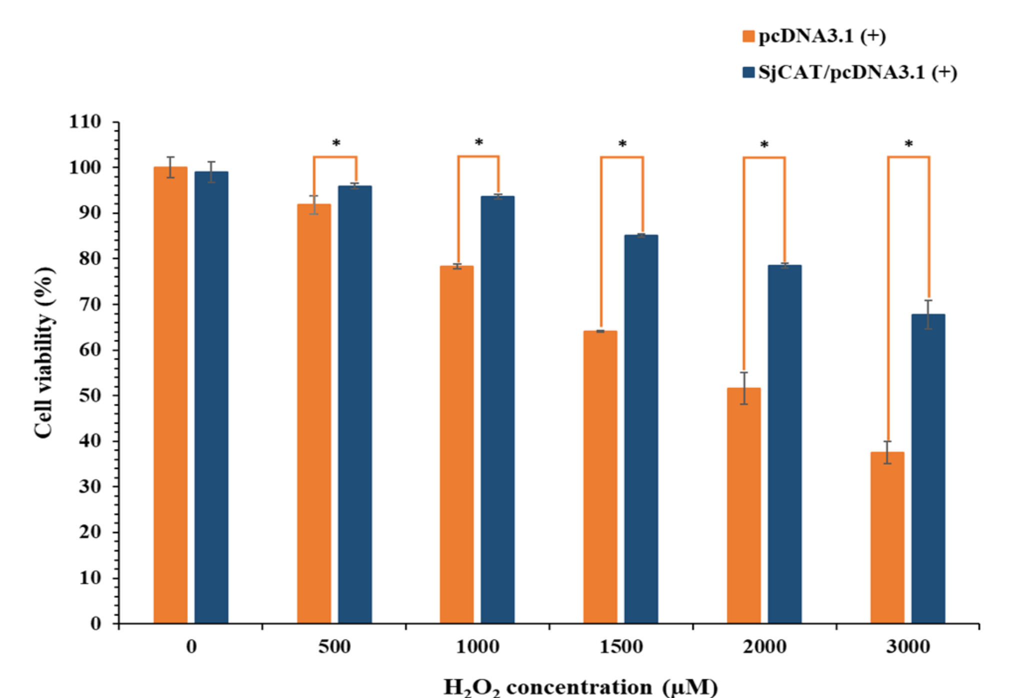


Fig 9: Cell viability assay using the MTT reagent in response to the H_2O_2-stimulated apoptosis in FHM cells.