Effectiveness of thioacetamide in an induced liver fibrosis model in zebrafish larvae

Nguyen Huynh Mai Anh[1,3], Le Van Trinh[1,2], Minh-Dung Truong[3], Truong Hai Nhung[1,2*] and Vu Thanh Nguyen[3,4*]
1. Laboratory of Regenerative Biomedicine, Faculty of Biology and Biotechnology, University of Science, Vietnam National University, Ho Chi Minh City, VIETNAM
2. Vietnam National University, Ho Chi Minh City, VIETNAM
3. Biotechnology Center of Ho Chi Minh City, 2374 Highway 1, Quarter 2, Trung My Tay Ward, District 12, Ho Chi Minh City, VIETNAM
4. Department of Biotechnology, HUTECH Institute of Applied Sciences, HUTECH University, 475A Dien Bien Phu Street, Ward 25, Binh Thanh District, Ho Chi Minh City, VIETNAM
*thnhung@hcmus.edu.vn; nt.vu@hutech.edu.vn

Abstract

Liver fibrosis, a major global health concern, necessitates effective research models for its understanding and treatment. This study aimed to evaluate the potential of zebrafish larvae as a model for liver fibrosis by inducing this condition with thioacetamide (TAA). Zebrafish larvae at four days postfertilization (dpf) were exposed to 0.05% TAA for 72 or 120 hours. The effects of TAA were assessed through survival rate analysis, histological examination of liver tissue and gene expression analysis of markers related to cell death, fibrosis development and inflammation. Liver fibrosis was quantified by staining collagen fibers with Sirius Red and analyzing the stained area in liver sections.

TAA-exposed zebrafish larvae presented a high survival rate and clear signs of liver damage and fibrosis. This finding was supported by increased expression of a gene involved in promoting fibrosis. Quantitative analysis of Sirius Red-stained samples confirmed increased collagen deposition in TAA-treated larvae. This study successfully demonstrated that TAA effectively induces liver fibrosis in zebrafish larvae, validating its use as a valuable model for investigating this disease. The transparency, rapid development, high survival rate and quantifiable fibrosis of zebrafish larvae make them particularly suitable for liver fibrosis research, offering potential for future studies on disease mechanisms and therapeutic interventions.

Keywords: Collagen deposition, Gene expression, Liver fibrosis, Zebrafish larvae, Thioacetamide.

Introduction

Liver fibrosis, a pathological hallmark of chronic liver diseases, arises from excessive accumulation of extracellular matrix (ECM) proteins, predominantly collagen, within the liver parenchyma. This progressive scarring process disrupts the intricate architecture of the liver and impairs its vital functions, potentially culminating in cirrhosis-related liver failure and hepatocellular carcinoma[13,20]. The global burden of liver fibrosis is substantial, underscoring the urgent need for effective therapeutic interventions[14]. However, the development of such therapies has been hampered by the complex and multifaceted nature of fibrogenesis[22] which involves the dynamic interplay of various cell types, signaling pathways and molecular mediators.

Animal models play pivotal roles in revealing the intricate mechanisms underlying liver fibrosis and evaluating potential therapeutic strategies. While rodent models have traditionally been the mainstay of preclinical research, their inherent limitations including ethical concerns, high costs and relatively long experimental durations[11,21] have spurred the exploration of alternative model organisms. In this context, zebrafish (*Danio rerio*) has emerged as a promising model system for studying liver diseases including fibrosis. The zebrafish offers several distinct advantages including high fecundity, rapid development, optical transparency during the embryonic and larval stages and a remarkable degree of conservation in liver biology with mammals[3,5,10].

Recent advances in single-cell RNA sequencing have revealed striking similarities between zebrafish and human hepatic stellate cells (HSCs), the key effector cells involved in liver fibrosis, at the transcriptional and molecular levels[17]. These findings underscore the translational relevance of zebrafish models in liver fibrosis research, offering a unique opportunity to bridge the gap between basic research and clinical applications. Thioacetamide (TAA) has been extensively employed as a hepatotoxic agent to induce liver fibrosis in various animal models[7,23,28], including zebrafish[26].

TAA administration triggers a cascade of events within the liver including the generation of toxic metabolites, oxidative stress, hepatocyte death and subsequent activation of HSCs, ultimately leading to fibrogenesis[7,12]. The pathological features observed in TAA-induced liver fibrosis closely recapitulate those observed in human liver fibrosis, making TAA a valuable tool for preclinical investigations[4,15]. While previous studies have demonstrated the utility of TAA in inducing liver damage including steatosis and apoptosis, in zebrafish embryos[26], the potential of zebrafish larvae as a model for studying the dynamic process of liver fibrosis remains underexplored. In this study, we sought to establish and comprehensively characterize a liver fibrosis model in zebrafish larvae via TAA exposure. By leveraging the unique advantages of the zebrafish model and the well-established

fibrogenic properties of TAA, we aimed to gain novel insights into the progression of temporal dynamics and to quantify collagen deposition via histological analysis. We hypothesized that TAA exposure would induce a robust fibrotic response in zebrafish larvae, recapitulating key pathological features observed in mammalian models and human liver disease. The findings of this study have the potential to contribute to a deeper understanding of the molecular mechanisms underlying liver fibrosis and to facilitate the development of innovative therapeutic strategies for this debilitating condition.

Material and Methods

Zebrafish Husbandry and TAA Exposure: Wild-type (AB) zebrafish were maintained at the Biotechnology Center of Ho Chi Minh City, Vietnam, under standard laboratory conditions (25–28°C on a 14-h light/10-h dark cycle). The zebrafish breeding and husbandry protocols followed those described in Nguyen et al[18,19]. At 4 days postfertilization (dpf), the larvae were randomly assigned to two groups: the control group (n = 65) and the TAA-exposed group (n = 70). The TAA-exposed group was placed in embryo medium containing 0.05% thioacetamide (TAA; Merck, Germany) for 72 or 120 hours. The control group was maintained in regular embryo medium without TAA. Larval mortality was monitored every 12 hours throughout the exposure period.

Histological analysis and collagen quantification: At 72 and 120 hpf, larvae (n = 4 per group per time point) were randomly selected from each group (TAA-exposed and control) and fixed in 4% paraformaldehyde (PFA) at 4°C overnight. The fixed larvae were then washed with phosphate-buffered saline (PBS) and embedded in 3% agarose (Bioline, UK) to facilitate subsequent processing steps. The agarose-embedded larvae were dehydrated through a graded series of ethanol (70%, 80%, 90%, 95% and 100%), cleared with xylene and embedded in paraffin wax (Sakura, UK). Serial sections (5 µm thick) were cut via a microtome and mounted on glass slides.

Histological Staining and Imaging: For histological analysis, deparaffinized sections were stained with hematoxylin and eosin (H&E) or Picrosirius Red. H and E staining was performed via standard protocols with hematoxylin solution (Merck, Germany) for 1 minute, followed by incubation with eosin Y (Yunsei, Japan) for 1 minute. Picrosirius red staining was performed with 0.1% Sirius Red F3BA in saturated picric acid solution (Sigma–Aldrich, USA) for 80 minutes at room temperature. The stained sections were then dehydrated, cleared and mounted with a coverslip using permanent mounting medium. Brightfield images of both H and E and Picrosirius Red-stained sections were captured via a light microscope (Olympus BX51, Japan) equipped with a digital camera at 40X magnification.

Morphological assessment and collagen quantification: H and E stained sections were examined for morphological changes including hepatocyte necrosis, inflammatory cell infiltration and tissue disorganization, via Image Focus Plus software (Olympus, Japan). Picrosirius Red-stained sections were used to quantify the deposition of collagen, a key indicator of fibrosis. For each larva, 16 nonoverlapping fields within the liver region were randomly selected and imaged. ImageJ software (National Institutes of Health, USA) was used to apply color thresholding to isolate the red-stained collagen fibers from the background. The area occupied by collagen fibers was then measured and expressed as a percentage of the total area of each field. The mean percentage of collagen area across all 16 fields was calculated for each larva and was used for statistical comparisons between the control and TAA-exposed groups.

RNA isolation, cDNA synthesis and quantitative real-time PCR (qPCR): At 72 and 120 hpf, all surviving larvae (n = 30 per group per time point) from each experimental group were collected for gene expression analysis. Total RNA was extracted from pooled larvae via TRIzol reagent (Bioline, UK) according to the manufacturer's instructions. The RNA concentration and purity were assessed via a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA).

Complementary DNA (cDNA) was synthesized from 1 µg of total RNA via the SensiFAST cDNA Synthesis Kit (Bioline, UK). qPCR was performed via 2x SensiFAST SYBR No-ROX Mix (Bioline, UK) on a LightCycler 96 Real-time PCR System (Roche, USA). The following cycling conditions were used: initial denaturation at 95°C for 5 seconds and annealing/extension at 60°C for 10 seconds. The gene-specific primers used are listed in table 1 and were synthesized by Phusa Genomics (Phusa, Vietnam). Relative gene expression levels were calculated via the $2^{-\Delta\Delta Ct}$ method, with *ef1α* used as the reference gene.

Statistical analysis: Survival data were analyzed via the Kaplan–Meier method and survival curves were compared via the log-rank test. All other data are expressed as the mean ± standard error of the mean (SEM). Statistical comparisons between the control and TAA-treated groups were performed via Student's t test or one-way ANOVA followed by Tukey's post hoc test, as appropriate. Differences were considered statistically significant at $p \leq 0.05$. All the statistical analyses were performed via GraphPad Prism 9.4.0 software (GraphPad Software, USA).

Results

TAA exposure and larval survival: To assess the impact of TAA on zebrafish larval survival, larvae were exposed to 0.05% TAA at 4 dpf. The survival rate was monitored every 12 hours. The results revealed that TAA exposure did not significantly affect larval survival during the first 72 h, with a survival rate of 93.3% in the TAA-exposed group compared with 100% in the control group (Fig. 1). However, mortality increased in both groups after 96 hours, leading to the selection of 72 hpf and 120 hpf as the experimental

endpoints.

Histopathological changes and fibrosis: Histological analysis of H and E stained liver sections revealed significant morphological alterations in TAA-exposed larvae compared with control larvae at both 72 and 120 hpf. The TAA-exposed larvae exhibited notable features of liver injury including hepatocyte necrosis, loss of cellular integrity and disrupted tissue architecture (Fig. 2). These observations indicate that TAA exposure effectively induced liver damage in zebrafish larvae.

To further assess the extent of liver fibrosis, Sirius Red staining was performed to visualize and quantify collagen deposition. These findings further confirmed the development of fibrosis in TAA-exposed larvae. Fig. 3 showed the impact of TAA exposure on liver fibrosis in zebrafish larvae via Sirius Red staining. Compared with untreated control larvae, TAA-treated larvae (0.05%) presented a marked increase in collagen deposition (red staining) at 72 hours postexposure (hpe) (Fig. 3A, B). This observation was further supported by quantification which revealed a statistically significant increase in the percentage of liver fibrosis in the TAA-treated group (Fig. 3C, ***$p \leq 0.001$).

Similarly, at 120 hpe, TAA-treated larvae continued to display greater collagen deposition than control larvae did (Fig. 3D, E), with quantification confirming a significant increase in liver fibrosis (Fig. 3F, ***$p \leq 0.001$). These findings collectively indicate that TAA exposure induces time-dependent progression of liver fibrosis in zebrafish larvae.

Table 1
Primers for real-time PCR

Gene	Sequence	Functions
ef1α[1,18]	5'-CGTGGTAATGTGGCTGGAGA-3	*House keeping gene*
	5'-CTGAGCGTTGAAGTTGGCAG-3'	
col1α1[26]	5′-CTTTTGCTCACAGGGCCTTT-3'	*Collagen deposition and scar tissue formation*
	5′-AAGACTGCATGCATCACAGC-3'	
acta-2[26]	5'- TTGTGCTGGACTCTGGTGAT-3'	*Producing extracellular matrix proteins*
	5′-GGCCAAGTCCAAACGCATAA-3'	
TGF-β[29]	5′-GAACTCGCTTTGTCTCCA-3'	*Profibrogenic cytokine*
	5′-TACAGTCGCAGTATAACCTCA-3'	
caspase-3[29]	5′-CCGCTGCCCATCACTA-3'	*Enzyme involved in apoptosis*
	5′-ATCCTTTCACGACCATCT-3'	
bax[29]	5′-GGCTATTTCAACCAGGGTTCC-3'	*Pro-apoptotic protein*
	5′-TGCGAATCACCAATGCTGT-3'	
SOD1[29]	5′-GGCCAACCGATAGTGTTGA-3′	*Antioxidant enzyme*
	5′-CCAGCGTTGCCAGTTTTTAG-3'	

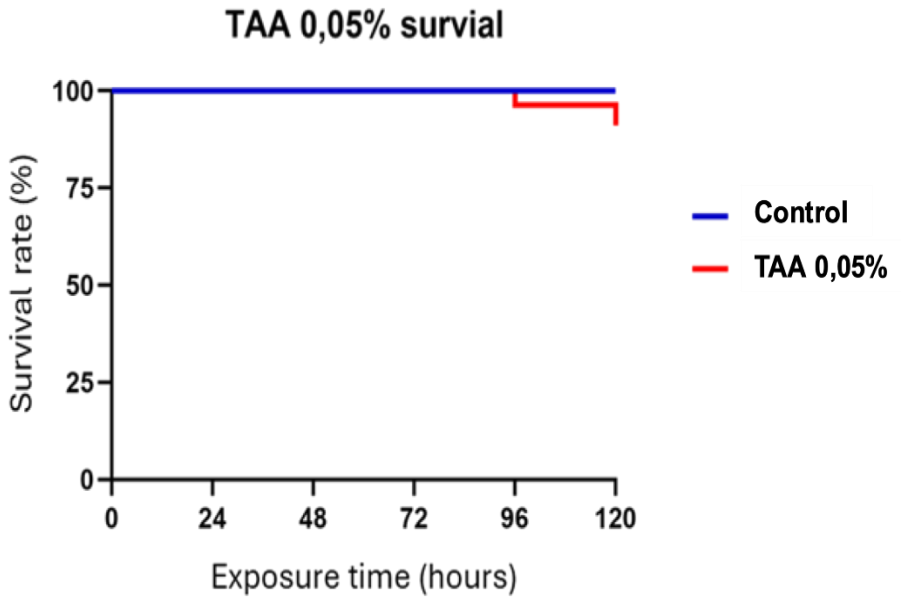

Fig. 1: Survival rate of zebrafish larvae following exposure to 0.05% TAA (n = 135).

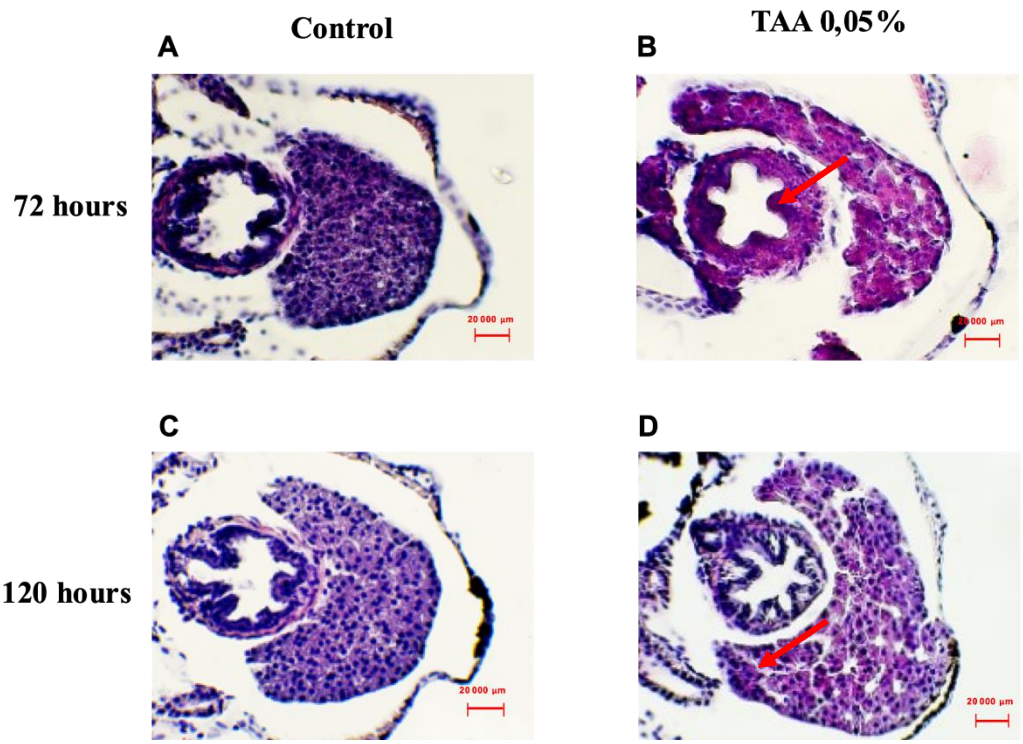

Fig. 2: Histological changes in zebrafish larval livers induced by TAA exposure. (A) Control liver at 7 dpf. (B) Livers of TAA-exposed (0.05%) larvae at 7 dpf (72 hours postexposure). (C) Control liver at 9 dpf. (D) Livers of TAA-exposed (0.05%) larvae at 9 dpf (120 hours postexposure). Scale bar: 20000 µm. (red arrow: damage to the liver tissue of zebrafish larvae)

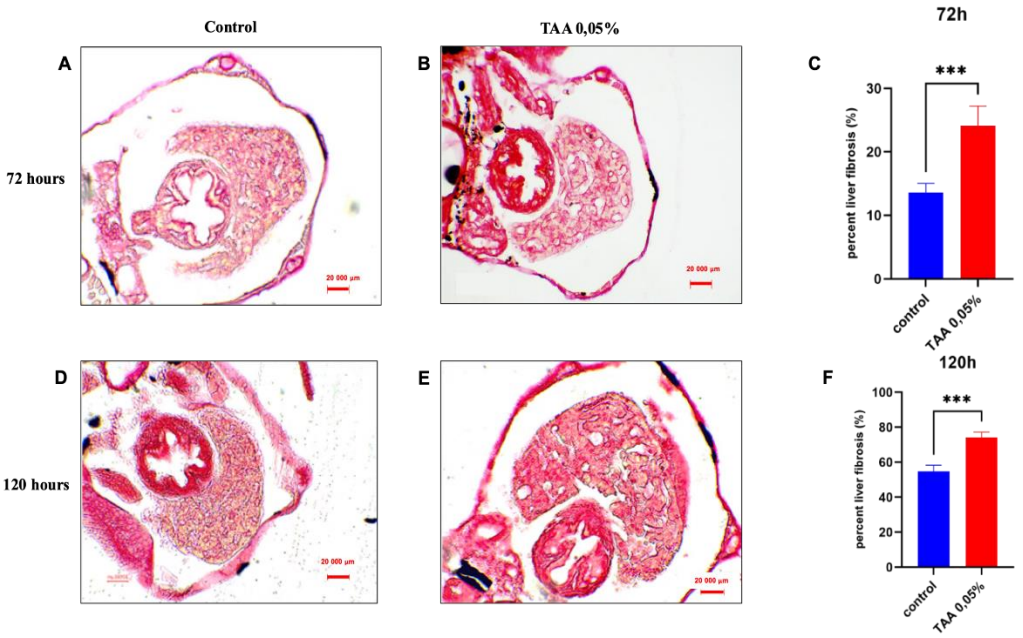

Fig. 3: Sirius Red staining of zebrafish larval liver sections. (A) Untreated control at 7 dpf. (B) TAA-treated plants (0.05%) at 7 dpf (72 hours after exposure). (C) Quantification of liver fibrosis at 72 hpf. (D) Untreated control at 9 dpf. (E) TAA treatment (0.05%) at 9 dpf (120 hours after exposure). (F) Quantification of liver fibrosis at 120 h after exposure. The data are presented as the means ± SEMs (n=8). ***$p \leq 0.001$.

Gene expression analysis: The expression of key genes involved in fibrosis, apoptosis and oxidative stress was analyzed via qPCR. The results revealed significant upregulation of *col1α1* (encoding type I collagen) at both 72 hpf (1.99-fold, $p < 0.001$) and 120 hpf (4.46-fold, $p < 0.001$) in the TAA-exposed group compared with the control group (Fig. 4A, B).

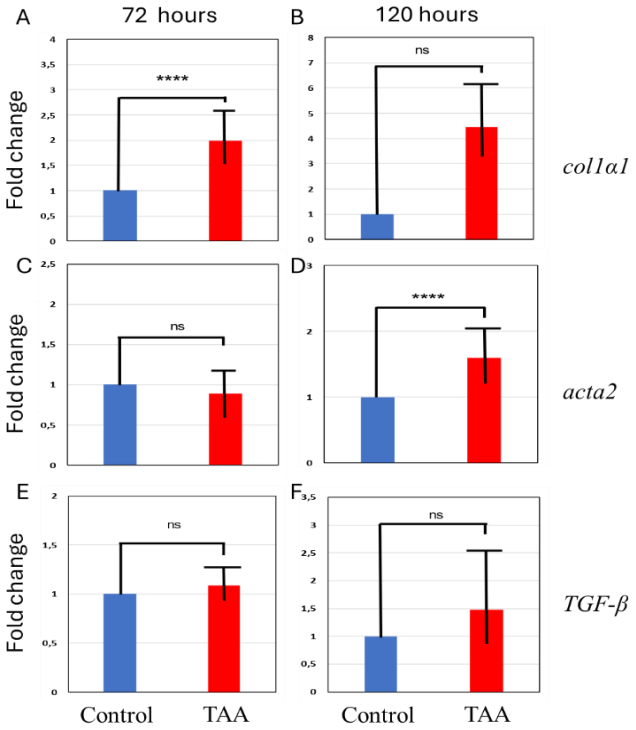

Fig. 4: Gene expression analysis of fibrosis-related markers in zebrafish larvae exposed to 0.05% TAA for 72 hours or 120 hours. (C) Relative mRNA expression levels of *col1α1*, (A) *acta2* and (B) *TGF-β* were determined via qPCR and normalized to those of the housekeeping gene ef1α. The data are presented as the mean fold change ± SEM compared with the control group (n=3). ns: not significant, ***p ≤ 0.001.

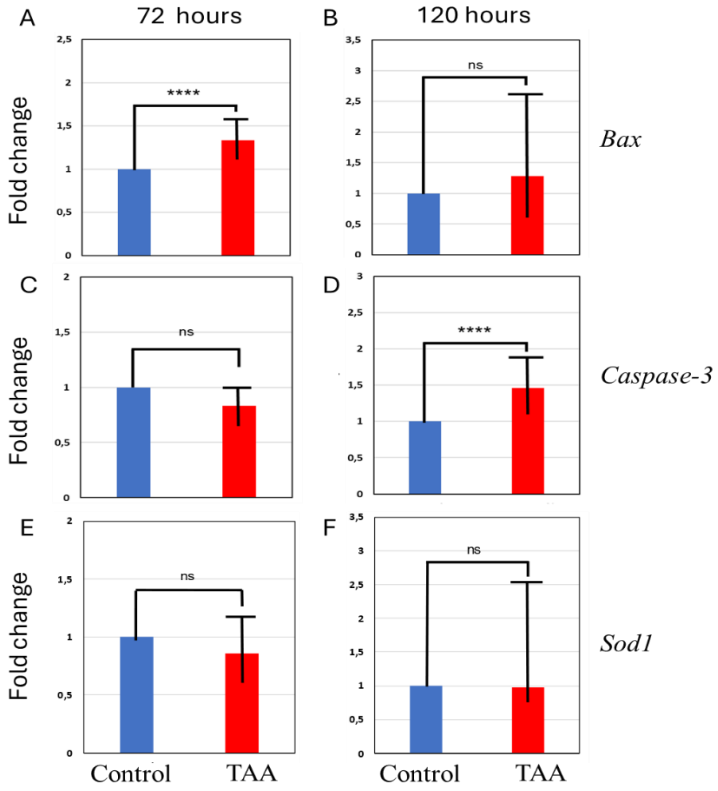

Fig. 5: Gene expression analysis of apoptosis- and oxidative stress-related markers in zebrafish larvae exposed to 0.05% TAA. (A, B) Relative mRNA expression levels of *Bax*, (C, D) *caspase-3* and (E, F) *Sod1* were determined by qPCR and normalized to those of the housekeeping gene ef1α at 72 hours (A, C, E) and 120 hours (B, D, F) post-TAA exposure. The data are presented as the mean fold change ± SEM compared with the control group (n=3). ns: not significant, ****p ≤ 0.0001.

Similarly, the expression of *acta2* (encoding α-SMA), a marker of activated hepatic stellate cells, was significantly increased at 120 hpf (1.59-fold, $p < 0.001$) but not at 72 hpf (Fig. 4C, D). These findings further support the development of liver fibrosis in TAA-exposed larvae. The expression of *TGF-β*, a profibrogenic cytokine, tended to be increased in the TAA-exposed group, although the difference was not statistically significant (Fig. 4E, F). These findings suggest a potential role for TGF-β signaling in TAA-induced liver fibrosis which warrants further investigation.

In addition to fibrosis-related genes, the expression of apoptosis-related genes (*bax* and *caspase-3*) and an oxidative stress-related gene (*sod1*) was also examined. The results revealed a significant increase in *bax* expression at 72 hpf (1.33-fold, $p < 0.05$) and a significant increase in *caspase-3* expression at 120 hpf (1.46-fold, $p < 0.0001$) in the TAA-exposed group (Fig. 5A-D). These findings suggest the involvement of apoptosis in TAA-induced liver injury and fibrosis. The expression of *sod1* showed a non-significant decreasing trend in the TAA-exposed group, indicating potential impairment of the antioxidant defense system (Fig. 5E, F).

Overall, the results of this study demonstrate that TAA exposure effectively induces liver fibrosis in zebrafish larvae. The observed histopathological changes, increased collagen deposition and altered gene expression patterns provide strong evidence for the development and progression of fibrosis in this model. These findings highlight the potential of zebrafish larvae as valuable tools for studying the molecular mechanisms of liver fibrosis and for screening potential therapeutic agents.

Discussion

The present study successfully established a zebrafish larval model of liver fibrosis induced by TAA exposure. The model recapitulated key pathological features observed in mammalian models and human liver fibrosis including hepatocyte necrosis, increased collagen deposition and activation of hepatic stellate cells. The zebrafish larval model offers several advantages for studying liver fibrosis, including its rapid development, optical transparency and genetic tractability[10,24], making it a valuable tool for investigating the molecular mechanisms underlying this complex disease and for screening potential therapeutic agents.

The observed upregulation of *col1a1* and *acta2* in TAA-exposed larvae is consistent with the findings of previous studies in zebrafish and other animal models of liver fibrosis[16,26,27]. The increased expression of these genes reflects the activation of hepatic stellate cells and the excessive production of extracellular matrix proteins, leading to the accumulation of scar tissue in the liver[27]. The trend toward the upregulation of *TGF-β* further suggests the involvement of this key profibrogenic cytokine in TAA-induced liver fibrosis[6,9]. These findings highlight the conserved molecular pathways involved in fibrosis across different species and support the translational relevance of the zebrafish model.

The induction of apoptosis, as evidenced by the increased expression of *bax* and *caspase-3*, is another important observation in this study. Apoptosis of hepatocytes is a well-recognized feature of liver injury and can contribute to the activation of hepatic stellate cells and the progression of fibrosis[2,25]. The temporal pattern of *bax* and *caspase-3* expression suggests a dynamic interplay between apoptosis and fibrosis during TAA-induced liver injury. The nonsignificant decrease in *sod1* expression suggests potential impairment of the antioxidant defense system[8] which could exacerbate liver damage and promote fibrosis development.

The findings of this study contribute to the growing body of evidence supporting the use of zebrafish larvae as a model for studying liver fibrosis. The ability of the model to recapitulate key pathological features and molecular changes associated with fibrosis, coupled with its numerous practical advantages, makes it a powerful tool for future research. Further studies using this model could focus on investigating the specific roles of different cell types and signaling pathways in fibrosis development and progression as well as in evaluating the efficacy of potential therapeutic interventions.

Conclusion

In conclusion, this study successfully established and characterized a zebrafish larval model of TAA-induced liver fibrosis. The model recapitulates key pathological features and molecular changes associated with fibrosis, providing a valuable platform for future research. The findings of this study contribute to a deeper understanding of the molecular mechanisms underlying liver fibrosis and highlight the potential of the zebrafish larval model for developing novel therapeutic strategies for this debilitating condition.

Acknowledgement

This research is supported by Laboratory of Regenerative Biomedicine, Faculty of Biology and Biotechnology, VNUHCM-University of Science, Ho Chi Minh City, Vietnam and Department of Aquacultural Biotechnology, Biotechnology Center of Ho Chi Minh City, Vietnam.

References

1. Bian L., Nguyen V.T., Tamaoki J., Endo Y., Dong G., Sato A. and Kobayashi M., Genetic hyperactivation of Nrf2 causes larval lethality in Keap1a and Keap1b-double-knockout zebrafish, *Redox Biology*, **62**, 102673, https://doi.org/10.1016/j.redox.2023.102673 **(2023)**

2. Canbay A., Friedman S. and Gores G.J., Apoptosis: The nexus of liver injury and fibrosis, *Hepatology*, **39(2)**, 273–278, https://doi.org/10.1002/hep.20051 **(2004)**

3. Chang C., Li H. and Zhang R., Zebrafish facilitate non-alcoholic

fatty liver disease research: Tools, models and applications, *Liver International*, **43(7)**, 1385–1398, https://doi.org/10.1111/liv.15601 **(2023)**

4. Chen W.Y., Thioacetamide-induced liver fibrosis is reduced in humanized immune system mice, *The Journal of Immunology*, **210(1_Supplement)**, https://doi.org/10.4049/jimmunol.210.Supp.229.26 **(2023)**

5. Cox A.G. and Goessling W., The lure of zebrafish in liver research: regulation of hepatic growth in development and regeneration, *Current Opinion in Genetics & Development*, **32**, 153–161, https://doi.org/10.1016/j.gde.2015.03.002 **(2015)**

6. Dewidar B. et al, TGF-β in Hepatic Stellate Cell Activation and Liver Fibrogenesis—Updated 2019, *Cells*, **8(11)**, 1419, https://doi.org/10.3390/cells8111419 **(2019)**

7. Ezhilarasan D., Molecular mechanisms in thioacetamide-induced acute and chronic liver injury models, *Environmental Toxicology and Pharmacology*, **99**, 104093, https://doi.org/10.1016/j.etap.2023.104093 **(2023)**

8. Gulesserian T., Engidawork E., Fountoulakis M. and Lubec G., Antioxidant proteins in fetal brain: superoxide dismutase-1 (SOD-1) protein is not overexpressed in fetal Down syndrome, In Protein Expression in Down Syndrome Brain, Springer Vienna, 71–84, https://doi.org/10.1007/978-3-7091-6262-0_6 **(2001)**

9. Hayashi H. and Sakai T., Biological Significance of Local TGF-β Activation in Liver Diseases, *Frontiers in Physiology*, https://doi.org/10.3389/fphys.2012.00012 **(2012)**

10. Katoch S. and Patial V., Zebrafish: An emerging model system to study liver diseases and related drug discovery, *Journal of Applied Toxicology*, **41(1)**, 33–51, https://doi.org/10.1002/jat.4031 **(2021)**

11. Khabib M.N.H., Sivasanku Y., Lee H.B., Kumar S. and Kue C.S., Alternative animal models in predictive toxicology, *Toxicology*, **465**, 153053 **(2022)**

12. Khomich O., Ivanov A.V. and Bartosch B., Metabolic Hallmarks of Hepatic Stellate Cells in Liver Fibrosis, *Cells*, **9(1)**, 24, https://doi.org/10.3390/cells9010024 **(2019)**

13. Liang Y., Wang J., Xu C., Han W., Wu S., Wu Y., Zhang J., Liu J., Zhang Z., Shi J. and Zhang K., Remodeling Collagen Microenvironment in Liver Using a Biomimetic Nano-Regulator for Reversal of Liver Fibrosis, *Advanced Science*, **10(18)**, https://doi.org/10.1002/advs.202300127 **(2023)**

14. Lowe K.O., Tanase C.E., Maghami S., Fisher L.E. and Ghaemmaghami A.M., Inflammatory Network of Liver Fibrosis and How It Can Be Targeted Therapeutically, *Immuno*, **3(4)**, 375–408, https://doi.org/10.3390/immuno3040023 **(2023)**

15. Matsuo M., Murata S., Hasegawa S., Hatada Y., Ohtsuka M. and Taniguchi H., Novel liver fibrosis model in Macaca fascicularis induced by thioacetamide, *Scientific Reports*, **10(1)**, 2450, https://doi.org/10.1038/s41598-020-58739-4 **(2020)**

16. Migdał M., Tralle E., Abu Nahia K., Bugajski Ł., Kędzierska K.Z., Garbicz F., Piwocka K., Winata C.L. and Pawlak M., Multi-omics analyses of early liver injury reveals cell-type-specific transcriptional and epigenomic shift, *BMC Genomics*, **22(1)**, 904, https://doi.org/10.1186/s12864-021-08173-1 **(2021)**

17. Morrison J.K., DeRossi C., Alter I.L., Nayar S., Giri M., Zhang C., Cho J.H. and Chu J., Single-cell transcriptomics reveals conserved cell identities and fibrogenic phenotypes in zebrafish and human liver, *Hepatology Communications*, **6(7)**, 1711–1724, https://doi.org/10.1002/hep4.1930 **(2022)**

18. Nguyen V.T., Nguyen T.T.T., Phuong Phi N., Mai Que D.N., Thu Hien L., Phuong Hanh L.L., Phuong Thao N.H., Nguyen X.T., Thanh Luu P., Thuy Vy N.H. and Thi Thuy D., Keap1/Nrf2-independent antioxidative activity of Phyllanthus amarus extract in zebrafish, *Vietnam Journal of Biotechnology*, **20(4)**, 653–661, https://doi.org/10.15625/1811-4989/17475 **(2022)**

19. Nguyen V.T., Thao V.T.M., Hanh L.L.P., Rol T.H., Thao N.H.P., Nguyen T.X., Luu P.T. and Thuy D.T., Exploring the Phytochemical Diversity and Antioxidant Potential of the Vietnamese Smilax glabra Roxb: Insights from UPLC-QTOF-MS/MS and Zebrafish Model Studies, *Applied Biochemistry and Biotechnology*, https://doi.org/10.1007/s12010-024-04930-6 **(2024)**

20. Pei Q., Yi Q. and Tang L., Liver Fibrosis Resolution: From Molecular Mechanisms to Therapeutic Opportunities, *International Journal of Molecular Sciences*, **24(11)**, 9671, https://doi.org/10.3390/ijms24119671 **(2023)**

21. Robinson N.B., Krieger K., Khan F.M., Huffman W., Chang M., Naik A., Yongle R., Hameed I., Krieger K., Girardi L.N. and Gaudino M., The current state of animal models in research: A review, *International Journal of Surgery*, **72**, 9–13, https://doi.org/10.1016/j.ijsu.2019.10.015 **(2019)**

22. Roehlen N., Crouchet E. and Baumert T.F., Liver Fibrosis: Mechanistic Concepts and Therapeutic Perspectives, *Cells*, **9(4)**, 875, https://doi.org/10.3390/cells9040875 **(2020)**

23. Sepehrinezhad A., Shahbazi A., Sahab Negah S., Joghataei M.T. and Larsen F.S., Drug-induced-acute liver failure: A critical appraisal of the thioacetamide model for the study of hepatic encephalopathy, *Toxicology Reports*, **8**, 962–970, https://doi.org/10.1016/j.toxrep.2021.04.011 **(2021)**

24. Sujatha G., Mathunika Devi A., Lesy Josephine J., Punitha P. and Ramanathan P., Efficient biological activity of acridine synthesised by using efficient nano catalyst, *Res. J. Chem. Environ.*, **28(3)**, 56-60 https://doi.org/10.25303/283rjce056060 **(2023)**

25. Tu T., Calabro S.R., Lee A., Maczurek A.E., Budzinska M.A., Warner F.J., McLennan S.V. and Shackel N.A., Hepatocytes in liver injury: Victim, bystander, or accomplice in progressive fibrosis?, *Journal of Gastroenterology and Hepatology*, **30(12)**, 1696–1704, https://doi.org/10.1111/jgh.13065 **(2015)**

26. van der Helm D., Groenewoud A., de Jonge-Muller E.S.M., Barnhoorn M.C., Schoonderwoerd M.J.A., Coenraad M.J., Hawinkels L.J.A.C., Snaar-Jagalska B.E., van Hoek B. and Verspaget H.W., Mesenchymal stromal cells prevent progression of liver fibrosis in a novel zebrafish embryo model, *Scientific Reports*, **8(1)**, 16005, https://doi.org/10.1038/s41598-018-34351-5

(2018)

27. Van Wettere A.J., Law J. Mac, Hinton D.E. and Kullman S.W., Anchoring Hepatic Gene Expression with Development of Fibrosis and Neoplasia in a Toxicant-induced Fish Model of Liver Injury, *Toxicologic Pathology*, **41(5)**, 744–760, https://doi.org/10.1177/0192623312464308 **(2013)**

28. Wallace M., Hamesch K., Lunova M., Kim Y., Weiskirchen R., Strnad P. and Friedman S., Standard Operating Procedures in Experimental Liver Research: Thioacetamide model in mice and rats, *Laboratory Animals*, **49(1_suppl)**, 21–29, https://doi.org/10.1177/0023677215573040 **(2015)**

29. Zhang Y., Liu K., Hassan H.M., Guo H., Ding P., Han L., He Q., Chen W., Hsiao C.D., Zhang L. and Jiang Z., Liver Fatty Acid Binding Protein Deficiency Provokes Oxidative Stress, Inflammation and Apoptosis-Mediated Hepatotoxicity Induced by Pyrazinamide in Zebrafish Larvae, *Antimicrobial Agents and Chemotherapy*, **60(12)**, 7347–7356, https://doi.org/10.1128/AAC.01693-16 **(2016)**.

(Received 17th September 2024, accepted 18th October 2024)