

RESEARCH ARTICLE

Vanillic Acid Mitigates Alcohol-Induced Oxidative Stress by Modulating Lipid Peroxidation, Nitric Oxide Levels, and Antioxidant Enzyme Activity in Zebrafish Larvae

Amanda Nasrallah ^{a,*}, Mohamed Shaaban^b^a Department of General medicine, Alquds University, Palestine.^b Department of Biobanks & Complex Data Management, Cote d'Azur University, Av.Valrose, 06000 Nice, France

***Corresponding Author:** Amanda Nasrallah
Department of General medicine, Alquds University, Palestine
Email: amanda.nasrallah@students.alquds.edu

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Abstract

Alcohol consumption is a major contributor to oxidative stress-related diseases, including liver damage and neurotoxicity. Vanillic acid (VA), a natural phenolic compound, possesses strong antioxidant properties that may mitigate oxidative damage. This study evaluates the protective effects of VA against alcohol-induced oxidative stress in zebrafish larvae. Zebrafish were exposed to 1% ETOH for 24 hours to induce oxidative stress, followed by treatment with VA at concentrations of 50 µg/mL and 100 µg/mL for another 24 hours. The impact of VA on oxidative stress biomarkers was assessed by measuring lipid peroxidation, nitric oxide production, and antioxidant enzyme activities, including superoxide dismutase, catalase, glutathione, and glutathione S-transferase. Additionally, acridine orange staining was used to evaluate apoptosis, while reactive oxygen species levels were quantified using dichlorofluorescein diacetate and lipid peroxidation was analyzed using diphenyl-1-pyrenylphosphine fluorescence assays. Results demonstrated a significant increase in oxidative stress markers following 1% ETOH exposure, characterized by elevated ROS levels, MDA accumulation, and reduced antioxidant enzyme activities. However, VA treatment effectively counteracted these effects, with 100 µg/mL showing the highest efficacy in restoring antioxidant defenses, reducing lipid peroxidation, and inhibiting NO overproduction. Furthermore, fluorescence-based assays confirmed the reduction of apoptosis and oxidative damage in VA-treated zebrafish larvae. These findings suggest that VA mitigates alcohol-induced oxidative stress by modulating key biochemical pathways, thereby offering potential therapeutic benefits against alcohol-related toxicity. Future studies should focus on elucidating the molecular mechanisms underlying VA's protective effects.

1. Introduction

Excessive alcohol consumption is a major public health concern, contributing to a wide range of diseases, including liver damage, cardiovascular disorders, and neurotoxicity (Dai et al., 2021). One of the primary mechanisms underlying alcohol-induced toxicity is oxidative stress, which results from an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defense systems (Zhang et al., 2020). Ethanol (1% ETOH) metabolism generates ROS and free radicals, leading to lipid peroxidation, protein oxidation, DNA damage, and apoptosis. Chronic exposure to alcohol has been shown to impair the activity of key antioxidant enzymes, further exacerbating oxidative damage (Jeong et al., 2020; Kubiak-Tomaszewska et al., 2020).

Oxidative stress plays a crucial role in alcohol-related organ damage by triggering inflammatory responses and cellular apoptosis (Fan et al., 2022). The major antioxidant defense mechanisms include enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as non-enzymatic components like reduced glutathione (GSH) (Jomova et al., 2024; Torres-Ramos

et al., 2018). In alcohol-exposed organisms, these antioxidant systems become overwhelmed, leading to increased lipid peroxidation, nitric oxide (NO) overproduction, and apoptosis. Given the detrimental effects of alcohol-induced oxidative stress, it is essential to explore natural compounds with potent antioxidant properties that can counteract these effects and restore cellular homeostasis.

Vanillic acid (VA), a naturally occurring phenolic compound found in various plant-based foods such as vanilla beans, whole grains, and fruits, has garnered significant attention for its pharmacological properties (Tazon et al., 2024). VA exhibits strong antioxidant, anti-inflammatory, and neuroprotective effects (Bains et al., 2022), making it a promising candidate for mitigating alcohol-induced oxidative stress. Several studies have demonstrated that phenolic compounds, including VA, can effectively scavenge ROS, modulate antioxidant enzyme activities, and reduce lipid peroxidation (Elager et al., 2023). Furthermore, VA has been shown to exert protective effects against neurodegenerative diseases and liver toxicity by enhancing endogenous antioxidant defenses and suppressing pro-inflammatory

cytokines (Alamri et al., 2022; Ghaderi et al., 2024).

Zebrafish (*Danio rerio*) serve as an excellent model system for studying the effects of alcohol exposure and evaluating the protective potential of bioactive compounds. Due to their genetic similarity to humans, rapid development, and transparent embryos, zebrafish provide a reliable platform for assessing oxidative stress biomarkers, apoptotic responses, and antioxidant enzyme activities in a high-throughput manner (Katoch and Patial, 2021; Wang et al., 2022). Previous studies have successfully utilized zebrafish larvae to investigate the effects of alcohol exposure, demonstrating increased ROS accumulation, lipid peroxidation, and apoptotic cell death (Du et al., 2020). This model system offers a unique opportunity to evaluate the efficacy of VA in mitigating alcohol-induced toxicity at the biochemical and cellular levels.

The present study aims to assess the protective effects of VA against alcohol-induced oxidative stress in zebrafish larvae by evaluating key oxidative stress markers, including lipid peroxidation, NO production, and antioxidant enzyme activities (SOD, CAT, GSH, and GST). Additionally, fluorescence-based assays such as acridine orange staining, diphenyl-1-pyrenylphosphine (DPPP) assay, and dichlorofluorescein diacetate (DCFDA) staining were employed to determine apoptosis, lipid peroxidation, and ROS accumulation, respectively. We hypothesize that VA will attenuate 1% ETOH-induced oxidative damage by modulating antioxidant defense mechanisms and inhibiting apoptosis. The findings from this study could provide valuable insights into the potential therapeutic applications of VA for alcohol-induced oxidative stress and related diseases.

2. Materials and Methods

2.1. Experimental Design and Zebrafish Maintenance

Zebrafish embryos were obtained from healthy adult zebrafish maintained in standard laboratory conditions at $28 \pm 1^\circ\text{C}$ with a 14:10-hour light-dark cycle. The embryos were collected and maintained in E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl_2 , and 0.33 mM MgSO_4). At 5 days post-fertilization (dpf), larvae were randomly divided into five experimental groups: (1) Control (untreated larvae), (2) 1% ETOH-exposed group (1% ETOH), (3) VA 50 $\mu\text{g}/\text{mL}$ -treated group, (4) VA 100 $\mu\text{g}/\text{mL}$ -treated group. 1% ETOH exposure was conducted by immersing zebrafish larvae in 1% ETOH (v/v) for 24 hours to induce oxidative stress. Following 1% ETOH exposure, larvae were treated with VA at the specified concentrations for another 24 hours. Larvae were monitored for mortality, morphological changes, and behavioral alterations under a stereomicroscope.

2.2. Determination of Antioxidant Enzyme Activity

Whole-body homogenates of zebrafish larvae were prepared in ice-cold phosphate-buffered saline (PBS, pH 7.4) using a sonicator. The homogenates were centrifuged at 12,000 rpm for 10 minutes at 4°C , and the supernatants were collected for biochemical assays (Jorge et al., 2023).

SOD Activity: SOD activity was determined using a colorimetric assay that measures the inhibition of nitroblue tetrazolium (NBT) reduction by superoxide radicals. The absorbance was recorded at 560 nm, and results were expressed as units per milligram of protein (U/mg protein). CAT activity was assessed by monitoring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm. The enzyme activity was

expressed as U/mg protein. GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The enzyme activity was expressed in U/mg protein. GSH levels were quantified using Ellman's reagent (DTNB), which reacts with free thiol groups to produce a yellow chromophore measurable at 412 nm. The levels were expressed as nmol/mg protein (Guo et al., 2022; Hanachi et al., 2021; Köktürk et al., 2020; Paduraru et al., 2021).

2.3. Determination of Lipid Peroxidation and NO Production

Malondialdehyde (MDA) levels were measured using the thiobarbituric acid reactive substances (TBARS) assay. Homogenates were incubated with thiobarbituric acid at 95°C for 30 minutes, and the absorbance of the resulting pink chromophore was recorded at 532 nm. The results were expressed as nmol MDA/mg protein. NO production was determined using the Griess reagent. Culture supernatants were mixed with the reagent, and the absorbance was measured at 540 nm to determine nitrite accumulation as an indicator of NO levels (Jiang et al., 2024; van den Boom et al., 2023).

2.4. Fluorescence-Based Assays

Acridine Assay for Apoptosis Detection: Zebrafish larvae were incubated with acridine orange (5 $\mu\text{g}/\text{mL}$) for 30 minutes in the dark, followed by PBS washing. Fluorescence images were captured under a fluorescence microscope using the FITC filter. The intensity of green fluorescence (indicative of apoptotic cells) was quantified using ImageJ software (Smirnova et al., 2021).

DPPP staining for lipid peroxidation detection. Larvae were incubated with DPPP (5 μM) for 30 minutes, followed by PBS washing. Fluorescence images were acquired, and lipid peroxidation levels were analyzed based on fluorescence intensity (Sudhakaran et al., 2024).

DCFDA Assay for ROS Measurement: Reactive oxygen species (ROS) levels were quantified using dichlorodihydrofluorescein diacetate (DCFDA). Zebrafish larvae were exposed to DCFDA (20 μM) for 30 minutes in the dark, washed with PBS, and analyzed under a fluorescence microscope. Fluorescence intensity was quantified using ImageJ. All assays were performed in triplicates to ensure reproducibility (Liu et al., 2022).

2.5. Statistical Analysis

Data were analyzed using GraphPad Prism 9 software and expressed as mean $\pm$ standard deviation (SD). One-way ANOVA followed by Tukey's post hoc test was used to determine statistical significance. Differences were considered significant at $p < 0.05$.

3. Result

3.1. Protective Role of VA in 1% ETOH-Exposed Zebrafish Larvae

1% ETOH exposure significantly impacted the survival rate of zebrafish larvae (37%), leading to morphological abnormalities such as reduced body length and body curvature. The administration of VA markedly improved survival rates, with the 100 $\mu\text{g}/\text{mL}$ dose providing the greatest protective effect (92%). Larvae treated with VA exhibited a notable reduction in 1% ETOH-induced deformities, suggesting its

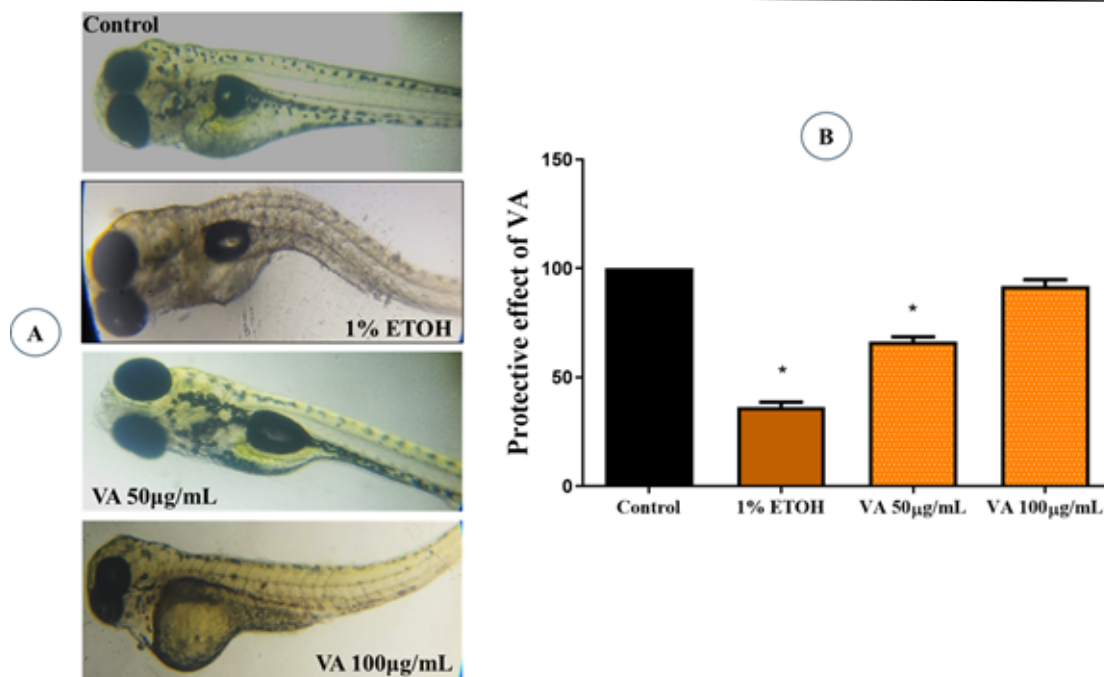


Figure 1: Representative images of zebrafish larvae under a stereomicroscope microscope after the treatment. The treatment and 1% ETOH group was compared with control ($n = 50$ larvae/group).

* represent significant difference at $p < 0.05$.

role in alleviating alcohol-induced toxicity (Figure 1).

3.2. Effect of VA on Antioxidant Enzyme Activities

A decline in SOD levels was observed in 1% ETOH-exposed larvae, indicating oxidative stress due to an accumulation of superoxide radicals (17 U/mg of Protein). Treatment with VA significantly increased SOD activity, particularly at 100 µg/mL (32 U/mg of Protein), restoring antioxidant balance and neutralizing superoxide radicals. 1% ETOH exposure impaired CAT enzyme function, leading to elevated hydrogen peroxide levels (8 µmol/mg of Protein). VA supplementation enhanced CAT activity, with the highest recovery noted at 100 µg/mL (22 µmol/mg of Protein), demonstrating its ability to counteract oxidative damage (Figure 2).

A marked reduction in GST activity was noted in 1% ETOH-treated zebrafish (15 nmol/mL), suggesting compromised detoxification pathways. VA treatment restored GST activity (28 nmol/mL), with the 100 µg/mL

concentration offering the most substantial improvement. 1% ETOH exposure significantly depleted intracellular GSH reserves (2.3 nmol/mg of Protein), reflecting oxidative imbalance. VA supplementation, particularly at 100 µg/mL (9 nmol/mg of Protein), effectively restored GSH levels, reinforcing its protective antioxidant properties.

3.3. Impact of VA on NO Levels and Lipid Peroxidation

1% ETOH exposure led to a surge in NO levels (71 µM), indicative of inflammation and oxidative stress. VA treatment significantly attenuated NO levels, with the most pronounced effect observed at 100 µg/mL (20 µM), highlighting its anti-inflammatory potential (Figure 3).

1% ETOH-induced toxicity caused excessive lipid peroxidation, as reflected by increased MDA levels (79%). VA-treated groups exhibited a significant reduction in MDA levels (20%), with 100 µg/mL demonstrating the most effective membrane-protective capability (Figure 4).

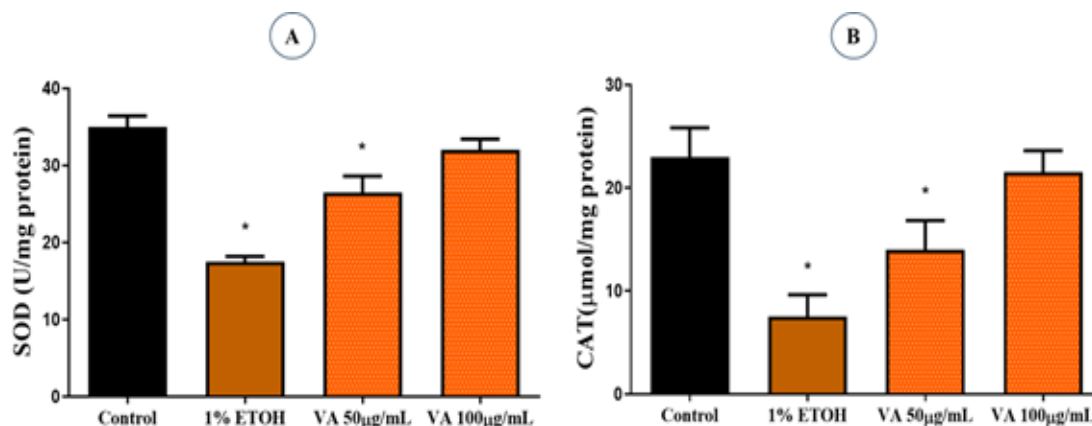


Figure 2: Reduction in SOD and CAT levels were significant in 1% ETOH larvae. The VA treatment in 1% ETOH larvae compared with control group ($n = 50$ larvae/group).

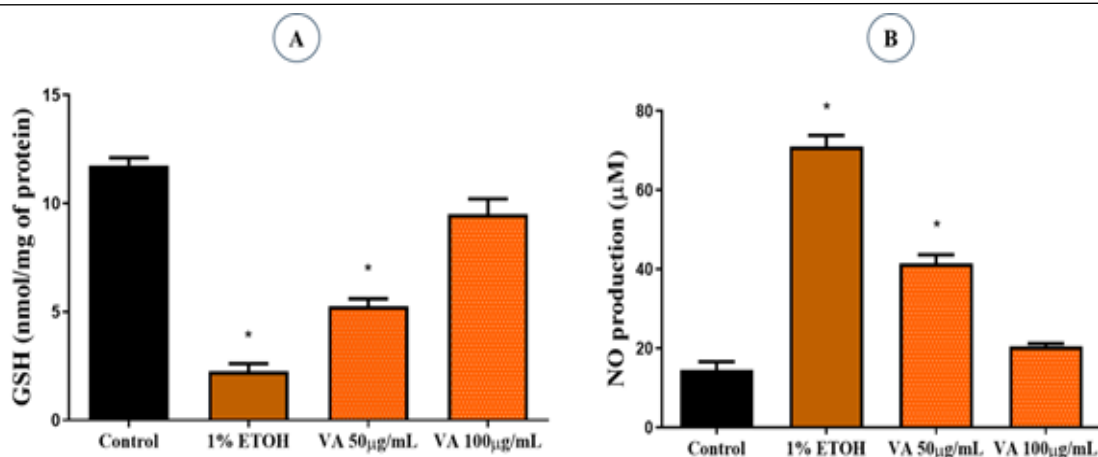


Figure 3: Reduction and increase in GSH and NO levels were significant in 1% ETOH larvae. The VA treatment in 1% ETOH larvae compared with control group (n = 50 larvae/group).

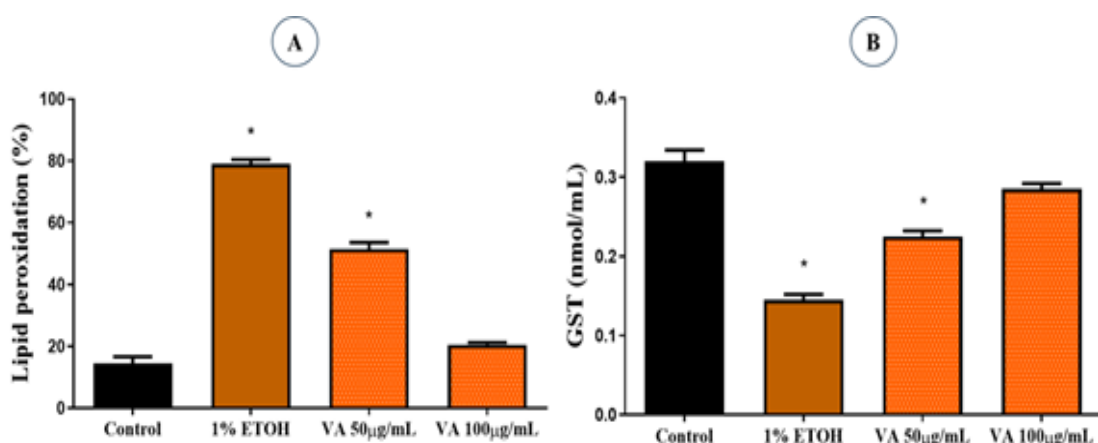


Figure 4: Reduction and increase in GST and lipid peroxidation levels were significant in 1% ETOH larvae. The VA treatment in 1% ETOH larvae compared with control group (n = 50 larvae/group).

3.4. Reduction of ROS, Apoptosis, and Lipid Peroxidation by VA

1% ETOH-induced oxidative stress resulted in heightened ROS accumulation in zebrafish larvae. VA treatment led to a significant reduction in ROS levels, with the highest protective effect at 100 µg/mL (Figure 5). 1% ETOH exposure triggered extensive apoptosis, as evident from increased fluorescence intensity in acridine orange-stained larvae. VA treatment notably decreased apoptotic cell numbers, reinforcing its protective role in cellular integrity (Figure 6). 1% ETOH toxicity elevated lipid peroxidation, reflected by intense DPPP fluorescence signals. VA significantly diminished lipid peroxidation, with 100 µg/mL providing maximum protection against membrane damage (Figure 7).

4. Discussion

This study highlights the significant protective effects of VA in counteracting 1% ETOH-induced oxidative damage in zebrafish larvae. The observed benefits were demonstrated through increased survival rates, restoration of antioxidant defenses, and inhibition of lipid peroxidation and apoptosis. These findings reinforce the efficacy of plant-derived antioxidants in mitigating alcohol-related oxidative stress. 1% ETOH metabolism generates ROS, which induce cellular damage and impair physiological functions (Contreras-Zentella et al., 2022). The marked

increase in ROS observed in 1% ETOH-treated larvae was significantly reversed following VA supplementation. These findings align with prior research demonstrating the efficacy of VA antioxidants in scavenging free radicals in invitro cells (Taqvi et al., 2021). The enhanced activities of SOD and CAT further support the hypothesis that VA strengthens endogenous antioxidant defense mechanisms, providing resistance against 1% ETOH-induced toxicity.

Elevated NO levels in 1% ETOH-exposed larvae suggest an activated inflammatory response. VA treatment significantly lowered NO levels, corroborating its anti-inflammatory potential. Similar studies have shown that phenolic acids, including VA, possess strong anti-inflammatory properties, reducing NO-mediated damage in oxidative stress conditions (Chen et al., 2020). Additionally, the substantial decrease in MDA levels following VA supplementation confirms its role in preventing lipid peroxidation and maintaining membrane integrity. Apoptosis is a crucial factor in 1% ETOH-induced toxicity, leading to extensive cellular damage. The acridine orange assay revealed heightened apoptotic activity in 1% ETOH-treated larvae, which was significantly reduced upon VA treatment. This protective effect aligns with reports highlighting the anti-apoptotic properties of natural phenolic compounds. Furthermore, the reduction

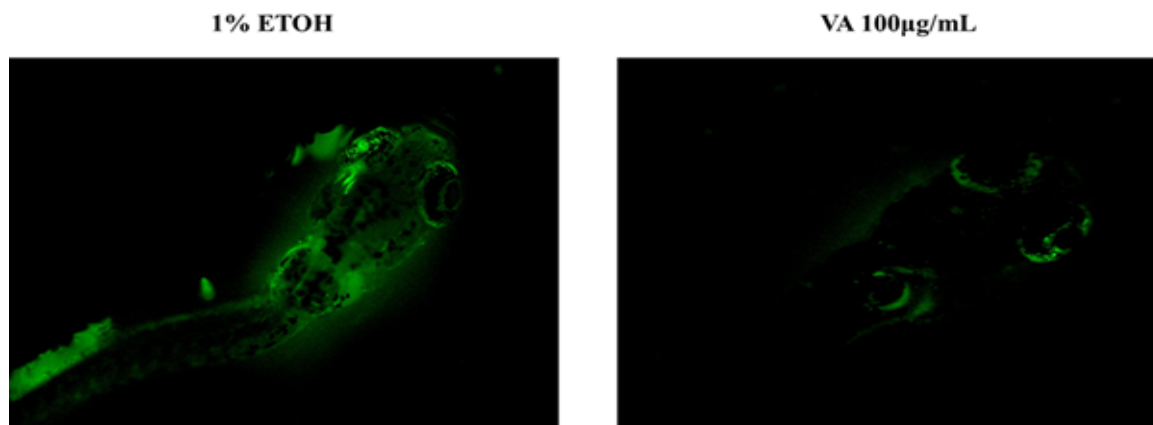


Figure.5 : Effect of VA on ROS Levels in 1% ETOH Zebrafish Larvae. (A) Representative DCFDA green fluorescence images illustrating ROS levels in 1% ETOH group (B) VA (100µg/mL) treatment group.

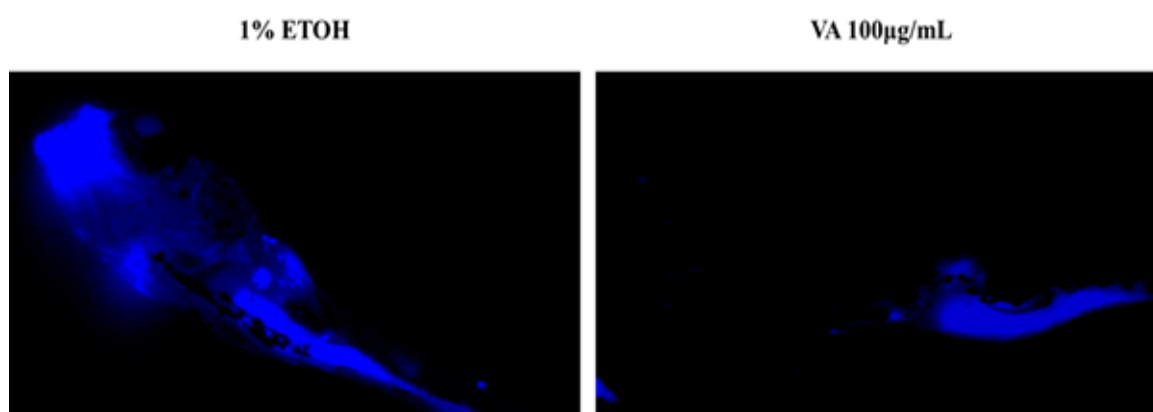


Figure 6: Effect of VA on lipid peroxidation levels in 1% ETOH Zebrafish Larvae. (A) Representative DPPP blue fluorescence images illustrating ROS levels in 1% ETOH group (B) VA (100µg/mL) treatment group

in lipid peroxidation, as observed in the DPPP assay, further substantiates VA's role in safeguarding cellular membranes from oxidative degradation.

Overall, these findings underscore the therapeutic potential of VA in managing 1% ETOH-induced oxidative stress. Its efficacy in restoring antioxidant balance, reducing apoptosis, and inhibiting lipid peroxidation establishes VA as a promising candidate for mitigating alcohol-related toxicity. Further investigations into its molecular mechanisms and potential synergistic interactions with other natural compounds could enhance its clinical applicability.

5. Conclusion

VA exhibited profound protective effects against 1% ETOH-induced oxidative stress in zebrafish larvae by enhancing antioxidant enzyme activity, reducing ROS accumulation, inhibiting lipid peroxidation, and mitigating apoptosis. These findings indicate that VA may serve as a potential therapeutic agent for alcohol-related oxidative damage. Future studies should explore its clinical applications and molecular pathways in greater depth.

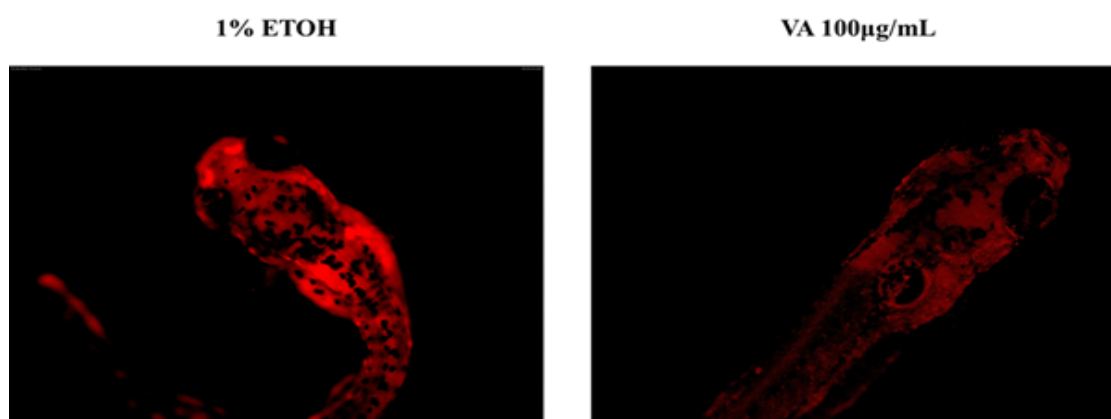


Figure 7: Effect of VA on apoptosis Levels in 1% ETOH Zebrafish Larvae. (A) Representative acridine orange red fluorescence images illustrating apoptosis levels in 1% ETOH group (B) VA (100µg/mL) treatment group.

Declarations**Ethics approval statement**

No ethical approval was required for the current study as it did not deal with any human or animal samples.

Consent to participate

Not applicable

Consent to publish

Not applicable

Data Availability Statement

The data are available from the corresponding author upon reasonable request

Competing Interests

The authors declare that they have no conflict of interest

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Not Applicable

Author contribution

Conceptualization, Data curation, Investigation: A.N. Formal analysis: M.S Writing A.N & M.S review and editing: All authors have read and agreed to the published version of the manuscript

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Reference

1. Alamri, E.S., El Rabey, H.A., Alzahrani, O.R., Almutairi, F.M., Attia, E.S., Bayomy, H.M., Albalwi, R.A., Rezk, S.M., 2022. Enhancement of the Protective Activity of Vanillic Acid against Tetrachloro-Carbon (CCl₄) Hepatotoxicity in Male Rats by the Synthesis of Silver Nanoparticles (AgNPs). *Molecules* 27, 8308. <https://doi.org/10.3390/molecules27238308>
2. Bains, M., Kaur, J., Akhtar, A., Kuhad, A., Sah, S.P., 2022. Anti-inflammatory effects of ellagic acid and vanillic acid against quinolinic acid-induced rat model of Huntington's disease by targeting IKK-NF-κB pathway. *Eur. J. Pharmacol.* 934, 175316. <https://doi.org/10.1016/j.ejphar.2022.175316>
3. Chen, S., Lin, R., Lu, H., Wang, Q., Yang, J., Liu, J., Yan, C., 2020. Effects of phenolic acids on free radical scavenging and heavy metal bioavailability in kandelia obovata under cadmium and zinc stress. *Chemosphere* 249, 126341. <https://doi.org/10.1016/j.chemosphere.2020.126341>
4. Contreras-Zentella, M.L., Villalobos-García, D., Hernández-Muñoz, R., 2022. Ethanol Metabolism in the Liver, the Induction of Oxidant Stress, and the Antioxidant Defense System. *Antioxidants* 11, 1258. <https://doi.org/10.3390/antiox11071258>
5. Dai, W., Chen, C., Feng, H., Li, G., Peng, W., Liu, X., Yang, J., Hu, X., 2021. Protection of Ficus pandurata Hance against acute alcohol-induced liver damage in mice via suppressing oxidative stress, inflammation, and apoptosis. *J. Ethnopharmacol.* 275, 114140. <https://doi.org/10.1016/j.jep.2021.114140>
6. Du, W., Chen, X., Shi, M., Bian, F., Zhao, Z., 2020. Ethanol affects behavior and HPA axis activity during development in zebrafish larvae. *Sci. Rep.* 10, 21402. <https://doi.org/10.1038/s41598-020-78573-y>
7. Eelager, M.P., Masti, S.P., Chougale, R.B., Hiremani, V.D., Narasgoudar, S.S., Dalbanjan, N.P., S.K., P.K., 2023. Evaluation of mechanical, antimicrobial, and antioxidant properties of vanillic acid induced chitosan/poly (vinyl alcohol) active films to prolong the shelf life of green chilli. *Int. J. Biol. Macromol.* 232, 123499. <https://doi.org/10.1016/j.ijbiomac.2023.123499>
8. Fan, H., Tu, T., Zhang, X., Yang, Q., Liu, G., Zhang, T., Bao, Y., Lu, Y., Dong, Z., Dong, J., Zhao, P., 2022. Sinomenine attenuates alcohol-induced acute liver injury via inhibiting oxidative stress, inflammation and apoptosis in mice. *Food Chem. Toxicol.* 159, 112759. <https://doi.org/10.1016/j.fct.2021.112759>
9. Ghaderi, S., Gholipour, P., Safari, S., Sadati, S.M., Brooshghalan, S.E., Sohrabi, R., Rashidi, K., Komaki, A., Salehi, I., Sarihi, A., Zarei, M., Shahidi, S., Rashno, M., 2024. Uncovering the protective potential of vanillic acid against traumatic brain injury-induced cognitive decline in male rats: Insights into underlying mechanisms. *Biomed. Pharmacother.* 179, 117405. <https://doi.org/10.1016/j.biopha.2024.117405>
10. Guo, D., He, R., Luo, L., Zhang, W., Fan, J., 2022. Enantioselective acute toxicity, oxidative stress effects, neurotoxicity, and thyroid disruption of uniconazole in zebrafish (Danio rerio). *Environ. Sci. Pollut. Res.* 29, 40157–40168. <https://doi.org/10.1007/s11356-022-18997-3>
11. Hanachi, P., Kazemi, S., Zivary, S., Karbalaee, S., Abolghasem Ghadami, S., 2021. The effect of polyethylene terephthalate and abamectin on oxidative damages and expression of vtg and cyp1a genes in juvenile zebrafish. *Environ. Nanotechnology, Monit. Manag.* 16, 100565. <https://doi.org/10.1016/j.enmm.2021.100565>
12. Jeong, M.S., Park, S., Han, E.J., Park, S.Y., Kim, M.J., Jung, K., Cho, S.-H., Kim, S.-Y., Yoon, W.-J., Ahn, G., Kim, K.-N., 2020. Pinus thunbergii PARL leaf protects against alcohol-induced liver disease by enhancing antioxidant defense mechanism in BALB/c mice. *J. Funct. Foods* 73, 104116. <https://doi.org/10.1016/j.jff.2020.104116>
13. Jiang, Y., Cao, Y., Li, Y., Bi, L., Wang, L., Chen, Q., Lin, Y., Jin, H., Xu, X., Peng, R., Chen, Z., 2024. SNP alleviates mitochondrial homeostasis dysregulation-mediated developmental toxicity in diabetic zebrafish larvae. *Biomed. Pharmacother.* 177, 117117. <https://doi.org/10.1016/j.biopha.2024.117117>
14. Jomova, K., Alomar, S.Y., Alwasel, S.H., Nepovimova, E., Kuca, K., Valko, M., 2024. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Arch. Toxicol.* 98, 1323–1367. <https://doi.org/10.1007/s00204-024-03696-4>
15. Jorge, S., Félix, L., Costas, B., Valentim, A.M., 2023. Housing Conditions Affect Adult Zebrafish (Danio rerio) Behavior but Not Their Physiological Status. *Animals* 13, 1120. <https://doi.org/10.3390/ani13061120>
16. Katoch, S., Patial, V., 2021. Zebrafish: An emerging model system to study liver diseases and related drug discovery. *J. Appl. Toxicol.* 41, 33–51. <https://doi.org/10.1002/jat.4031>
17. Köktürk, M., Alak, G., Atamanalp, M., 2020. The effects of n-butanol on oxidative stress and apoptosis in zebra fish (Danio rerio) larvae. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* 227, 108636. <https://doi.org/10.1016/j.cbpc.2019.108636>

18. Kubiak-Tomaszewska, G., Tomaszewski, P., Pachecka, J., Struga, M., Olejarz, W., Mielczarek-Put, M., Nowicka, G., 2020. Molecular mechanisms of ethanol biotransformation: enzymes of oxidative and nonoxidative metabolic pathways in human. *Xenobiotica* 50, 1180–1201. <https://doi.org/10.1080/00498254.2020.1761571>
19. Liu, F., Zhang, Y., Wang, F., 2022. Environmental relevant concentrations of triclosan affected developmental toxicity, oxidative stress, and apoptosis in zebrafish embryos. *Environ. Toxicol.* 37, 848–857. <https://doi.org/10.1002/tox.23448>
20. Paduraru, E., Flocea, E.-I., Lazado, C.C., Simionov, I.-A., Nicoara, M., Ciobica, A., Faggio, C., Jijie, R., 2021. Vitamin C Mitigates Oxidative Stress and Behavioral Impairments Induced by Deltamethrin and Lead Toxicity in Zebrafish. *Int. J. Mol. Sci.* 22, 12714. <https://doi.org/10.3390/ijms222312714>
21. Smirnova, A., Mentor, A., Ranefall, P., Bornehag, C.-G., Brunström, B., Mattsson, A., Jönsson, M., 2021. Increased apoptosis, reduced Wnt/ β -catenin signaling, and altered tail development in zebrafish embryos exposed to a human-relevant chemical mixture. *Chemosphere* 264, 128467. <https://doi.org/10.1016/j.chemosphere.2020.128467>
22. Sudhakaran, G., Ramamurthy, K., Dhaareshwar, V.N., Rajagopal, R., Alfarhan, A., Arockiaraj, J., 2024. Neurotoxic and developmental effects of scented incense stick smoke: Network toxicology and zebrafish model study. *Toxicol. Lett.* 402, 15–26. <https://doi.org/10.1016/j.toxlet.2024.10.008>
23. Taqvi, S., Ahmed Bhat, E., Sajjad, N., Sabir, J.S.M., Qureshi, A., Rather, I.A., Rehman, S., 2021. Protective effect of vanillic acid in hydrogen peroxide-induced oxidative stress in D.Mel-2 cell line. *Saudi J. Biol. Sci.* 28, 1795–1800. <https://doi.org/10.1016/j.sjbs.2020.12.023>
24. Tazon, A.W., Awwad, F., Meddeb-Mouelhi, F., Desgagné-Penix, I., 2024. Biotechnological Advances in Vanillin Production: From Natural Vanilla to Metabolic Engineering Platforms. *BioChem* 4, 323–349. <https://doi.org/10.3390/biochem4040017>
25. Torres-Ramos, Y., Montoya-Estrada, A., Cisneros, B., Tercero-Pérez, K., León-Reyes, G., Leyva-García, N., Hernández-Hernández, O., Magaña, J.J., 2018. Oxidative Stress in Spinocerebellar Ataxia Type 7 Is Associated with Disease Severity. *Cerebellum* 17, 601–609. <https://doi.org/10.1007/s12311-018-0947-0>
26. van den Boom, R., Vergauwen, L., Koedijk, N., da Silva, K.M., Covaci, A., Knapen, D., 2023. Combined western diet and bisphenol A exposure induces an oxidative stress-based paraoxonase 1 response in larval zebrafish. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* 274, 109758. <https://doi.org/10.1016/j.cbpc.2023.109758>
27. Wang, C., Yuan, Z., Li, J., Liu, Y., Li, R., Li, S., 2022. Acute effects of antimony exposure on adult zebrafish (*Danio rerio*): From an oxidative stress and intestinal microbiota perspective. *Fish Shellfish Immunol.* 123, 1–9. <https://doi.org/10.1016/j.fsi.2022.02.050>
28. Zhang, L., Meng, B., Li, L., Wang, Y., Zhang, Y., Fang, X., Wang, D., 2020. *Boletus aereus* protects against acute alcohol-induced liver damage in the C57BL/6 mouse via regulating the oxidative stress-mediated NF- κ B pathway. *Pharm. Biol.* 58, 905–914. <https://doi.org/10.1080/13880209.2020.1812672>