Investigating Natural Antioxidants as Protective Agents Against Alcohol and Nicotine-Induced Fetal Developmental Abnormalities

Introduction

- Maternal exposure to alcohol and nicotine during pregnancy remains a significant public health concern, leading to severe developmental abnormalities through oxidative stress mechanisms.
- While abstinence is the best prevention, there is a critical need to understand potential protective interventions for cases of exposure.
- Natural antioxidants present a promising avenue for investigation, but their protective effects against combined alcohol and nicotine exposure during fetal development remain poorly understood.

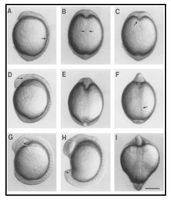
https://www.researchgate.net/figure/Oxidative-stress-induced-disease-in-humans-72_fig1_368254295/actionalreference

Review of Literature

Stages of Embryonic Development

Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B. and Schilling, T.F. (1995), Stages of embryonic development of the zebrafish. Dev. Dyn., 203: 253-310. https://doi.org/10.1002/aja.1002030302

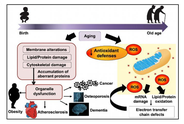
- Goal: To describe a series of stages for development of the embryo of the zebrafish, *Danio (Brachydanio) rerio*.
- Findings: The stages, and their names, are based on morphological features, generally readily identified by examination of the live embryo with the dissecting stereomicroscope.



Natural Antioxidants

Tan BL, Norhaizan ME, Liew WP, Sulaiman Rahman H. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. Front Pharmacol. 2018 Oct 16;9:1162. doi: 10.3389/fphar.2018.01162

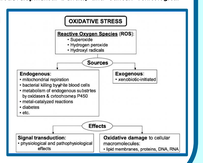
Goal: To evaluate natural antioxidants' role in preventing age-related diseases and promoting healthy aging by examining their effects on oxidative stress and inflammation.
Findings: Antioxidants demonstrated protective effects against multiple age-related diseases including cardiovascular disease, neurodegenerative disorders, diabetes, and cancer.



Oxidative Stress & Disease

Wells, P. G., McCallum, G. P., Chen, C. J., Henderson, J. T., Lee, C. J. J., Perstin, J., Preston, T. J., Wiley, M. J., & Wong, A. W. (2009). Oxidative Stress in Developmental Origins of Disease: Teratogenesis, Neurodevelopmental Deficits, and Cancer. Toxicological Sciences, 108(1), 4–18. https://doi.org/10.1093/toxsci/kfn263

Goal: To review how reactive oxygen species (ROS) and oxidative stress affect embryonic/fetal development and lead to birth defects, neurodevelopmental problems, and cancer.
Findings: Low levels of antioxidant enzymes in embryos make them vulnerable to oxidative damage.



Problem Statement

- This study aims to address this knowledge gap using zebrafish embryos as a model Organism, specifically examining the protective potential of spinach extract and vitamin C supplementation.

Goal

- To evaluate the protective effects of natural antioxidants (spinach extract and vitamin C) against alcohol and nicotine-induced developmental toxicity in zebrafish embryos, focusing on survival rates, cardiac function, morphological development, and the prevention of developmental abnormalities

Hypothesis

- Natural antioxidants supplementation (spinach extract and vitamin C) will provide significant protection against alcohol and nicotine-induced developmental toxicity in zebrafish embryos, as demonstrated by higher survival rates, normalized heart rates, improved body length development, and reduced morphological deformities (less than 15% combined mild and severe abnormalities).

Methodology

EMBRYO HARVEST
Follow embryo harvest protocol to collect *Danio rerio* embryos from two different populations.

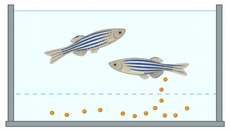


Image created by Finalist using BioRender, 2025

DEVELOPMENT
Allow embryos to develop 8 hours post-fertilization before solution exposure.

Image created by Finalist using BioRender, 2025

All methodology pictures taken by the teacher mentor and used by the Finalist with permission from P. Ramirez-Lumbreras (2025).

EXPOSURE
Divide embryos into the 9 groups. Expose each group to their solution for 1 hour. Then add antioxidants to the appropriate dishes.

Image created by Finalist using BioRender, 2025

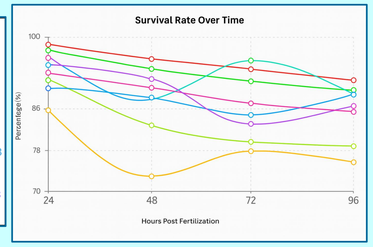
OBSERVATIONS
After embryos have been rinsed, return all groups to regular embryo solution water and observe

Image created by Finalist using BioRender, 2025

Results/ Analysis

Survival Rate

96
Control : 91.4
Nicotine : 75.2
Alcohol : 78.6
Spinach : 90.1
Vitamin C : 88.9
Nic+Spinach : 89.3
Nic+VitC : 82.8
Alc+Spinach : 87.3
Alc+VitC : 85.9



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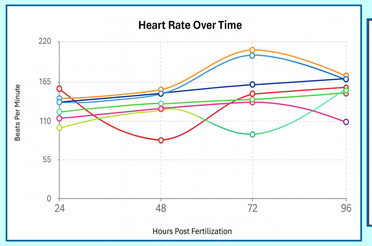
Survival Rate Patterns:
- Control group maintained the highest consistent survival (98.5%- 91.4%).
- Nicotine exposure showed more severe impact, with a sharp drop at 48 hpf (72.3%).
- Alcohol groups showed unexpectedly high initial survival (92.7%) but declined steadily.
- Both antioxidants spinach and vitamin C showed protective effects with survival rates close to control.
- Combined treatments of antioxidants and toxins showed intermediate survival rates between the control and toxic-only groups.

Heart Rate

96
Control : 160
Nicotine : 175
Alcohol : 150
Spinach : 155
Vitamin C : 157
Nic+Spinach : 170
Nic+VitC : 172
Alc+Spinach : 106.3
Alc+VitC : 147



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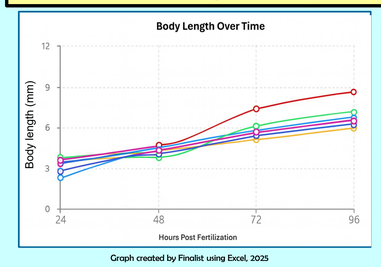
Heart Rate Trends:
- Control shows a steady increase in heart rate (156.5- 160 bpm)
- Nicotine exposure caused a significant elevation and heart rate, peaking at 72 hpf (208.9 bpm)
- Alcohol exposure decreased heart rate initially (95.6 bpm) with gradual recovery
- Antioxidant treatments moderated the heart rate changes in toxin-exposed groups.
- Most extreme variations occurred at 72 hpf across all groups.

Body Length



96
Control : 8.27
Nicotine : 5.1
Alcohol : 5.4
Spinach : 6.6
Vitamin C : 6.48
Nic+Spinach : 5.61
Nic+VitC : 5.51
Alc+Spinach : 5.94
Alc+VitC : 5.83

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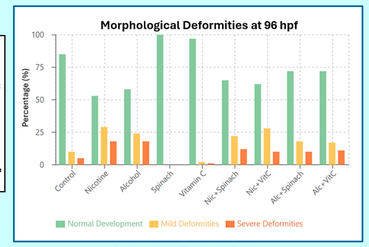
Body Length Trends:
- Control group showed steady growth, reaching 8.27mm by 96 hpf.
- Both toxins inhibited growth, with nicotine showing stronger growth suppression.
- Spinach treatment resulted in better growth outcomes compared to vitamin C.
- Early development to stages (24 ~48 hpf) showed minimal differences between groups.
- Greatest treatment effects observed after 72 hpf.

Morphological Deformities

Mild Deformities:
- Small variations in spine curvature
- Minor changes in body length
- Slight alterations in heart chamber development
- Moderate yolk sac edema
- Minor fin malformations

Severe Deformities:
- Major spinal deformations/severe curvature
- Significant reduction in body length
- Severe cardiac malformations/pericardial edema
- Multiple combined deformities



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Deformities Trends:
- Control Short baseline deformities (10% mild 5% severe).
- Nicotine exposure caused the highest rate of deformities 29% mild 18% seven-year.
- Alcohol exposure showed a similar severe deformity rate as nicotine 18%.
- Spanish treatment was most effective to preventing deformities.
- Antioxidant treatments reduce severe deformities more effectively than mild ones.

Applications

Medical/Clinical Applications

Development of preventive supplements for at-risk pregnancies where complete abstinence from alcohol/nicotine is challenging

Creation of guidelines for timing and dosage of antioxidant supplementation during pregnancy

Food Science/Nutritional Applications

Development of specialized prenatal supplements incorporating natural antioxidant complexes

Formulation of pregnancy-safe functional foods rich in protective antioxidants

Research Model Applications

Establishment of zebrafish as a cost-effective model for testing protective compounds against developmental toxicity

Development of standardized protocols for screening natural antioxidants for protective effects during embryonic development

Conclusion

Goal

- Determine natural antioxidants' effectiveness as protective agents against alcohol and nicotine-induced developmental toxicity

Methods

- Exposed zebrafish embryos to toxins and treated them with antioxidants (spinach extract and vitamin C). Analyzed survival rates, heart rate, body length, and morphological deformities
- Monitored development over 96 hours post-fertilization

Findings

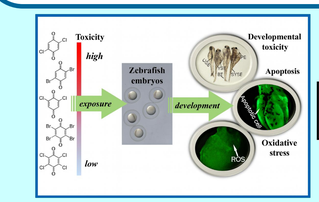
- Antioxidant treatment reduced developmental abnormalities, shown by improved survival and reduced deformity rates
- Treatment with vitamin C showed better protection than spinach extract.
- Decrease in developmental defects confirms antioxidants' protective role against toxin-induced damage

Importance

- Addresses the underlying cause of developmental toxicity (oxidative stress), preventing further damage
- Shows potential for developing preventive strategies for at-risk pregnancies
- Could improve developmental outcomes in cases of toxin exposure

Future Research

- Investigation of additional natural antioxidant sources (such as green tea extract or berries) could expand our understanding of which plant-based compounds offer optimal protection against developmental toxicity.
- Long-term studies tracking zebrafish development beyond 96 HPF would provide valuable insights into the lasting effects of antioxidant protection on organ systems and behavioral outcomes.



Wang, C., Vang, X., Zheng, Q., Moe, B., & Li, X.-F. (2018). Halobenzoquinone-Induced Developmental Toxicity, Oxidative Stress, and Apoptosis in Zebrafish Embryos. *Environmental Science & Technology*, 52(18), 10590–10598. https://doi.org/10.1021/acs.est.8b02831