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Distinct metabolite profiles induced by alcohol and nicotine exposure in zebrafish: Implications for understanding shared dopaminergic pathways in addiction

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Abstract

Addiction to alcohol and nicotine, whether individually or in combination, represents a significant global health challenge. These substances are known to activate a shared final neuronal pathway within the dopaminergic system, which is crucial for mediating the pleasurable sensations associated with reward. To explore whether the brain metabolite profile of individuals with addiction could elucidate the mechanisms underlying this common neuronal pathway, we conducted a comprehensive analysis of the metabolite profiles from zebrafish brains exposed to alcohol, nicotine, and their combination under rigorously controlled experimental conditions. Our findings reveal that each addictive substance generates a distinct 1H NMR metabolite profile. Specifically, alcohol exposure was associated with a marked increase in taurine levels and a decrease in N-acetyl L-aspartate (NAA) levels, correlating with alcohol's antagonistic effects on excitatory neurotransmission and a decline in overall brain health. Conversely, nicotine exposure resulted in an increase in NAA and levels of common neurotransmitters, aligning with previous studies that highlight nicotine's role in enhancing excitatory neurotransmission, memory formation, and neuroprotection. Notably, our investigation did not identify a shared metabolite that could elucidate the mechanisms of the drug-induced common neuronal pathway. These results underscore the complexity of the metabolic alterations induced by alcohol and nicotine and suggest that distinct pathways may be activated by each substance, warranting further investigation into their individual and combined effects on brain metabolism and function.

Keywords: Alcohol; Nicotine; Addiction; Zebrafish; Metabolite Profile; 1H NMR

1. Introduction

Drug addiction is a pervasive global issue, with alcohol and nicotine being two of the most commonly abused substances. Both drugs, whether consumed independently or in combination, are known to activate a shared final neuronal pathway within the dopaminergic system, which is integral to the sensation of reward. However, substantial evidence indicates that nicotine and alcohol exert markedly different effects on the central nervous system (CNS). Nicotine has been associated with neuroprotective properties, primarily through the inhibition of proteolytic enzymes and the upregulation of the ubiquitination system, which enhances the clearance of cellular debris and misfolded proteins from lysosomes, thereby improving neuronal efficiency [1-2]. In contrast, alcohol consumption is linked to increased apoptotic neurodegeneration, primarily via the activation of the proteolytic enzyme caspase-3, which contributes to neuronal cell death [3-4].

The contrasting actions of these substances extend to their molecular effects on gene expression and cellular metabolism. Nicotine promotes the upregulation of genes associated with neuronal development, synaptic plasticity, and survival, while simultaneously increasing the rate of cell proliferation through enhanced protein turnover [5].

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Conversely, chronic alcohol exposure is known to downregulate numerous functional genes, decrease protein turnover, and lead to reductions in brain volume, which can have detrimental effects on cognitive function [6-7]. Furthermore, nicotine binds to nicotinic acetylcholine receptors (nAChRs), which are excitatory ligand-gated ion channels, and enhances synaptic strength by potentiating N-methyl-D-aspartate (NMDA) receptor function, thereby improving memory and cognitive performance [8-9]. In stark contrast, chronic alcohol consumption acts as a selective antagonist of NMDA receptors, leading to neurological deficits and cognitive impairments [4, 10].

The interplay between alcohol and nicotine becomes particularly complex in individuals who are co-addicted to both substances. While some studies suggest that nicotine and alcohol may mitigate each other's effects in co-abusers, the underlying mechanisms remain poorly understood [1,9]. Recent analyses of brain metabolite profiles indicate that both substances induce significant and distinct changes in brain metabolism, further complicating our understanding of their combined effects [4, 11]. Given the prevalence of co-addiction, it is crucial to investigate how the combined exposure to alcohol and nicotine influences brain metabolism and function.

In this study, we employed zebrafish as a model organism to analyze the brain metabolite profiles resulting from acute exposure to alcohol, nicotine, or a combination of both. Our findings reveal that co-exposure to alcohol and nicotine produces a metabolite profile that closely resembles that of the control group, suggesting a potential attenuation of the individual effects of each substance when administered together. This research contributes to the growing body of literature on the neurobiological effects of polysubstance use and underscores the need for integrated approaches to address addiction.

2. Materials and Methods

2.1. Materials

Adult zebrafish (*Danio rerio*), approximately 3 cm in length and weighing around 700 mg, were procured from a local aquarium store. The chemicals used in the study included ethanol (CAS number 64-17-5) and nicotine (CAS number 54-11-5) obtained from Sigma-Aldrich. Sodium phosphate dibasic (Na2HPO4, CAS number 7558-79-4) and sodium phosphate monobasic (NaH2PO4, CAS number 13472-35-0) were also sourced from Sigma-Aldrich. Chloroform (CDCl3, CAS number 865-49-6) and deuterium oxide (D2O, CAS number 7789-20-0) were supplied by ARMAR Chemicals. The NMR buffer, composed of Na2HPO4/NaH2PO4, was prepared in D2O at pH 6. All experiments were conducted using a 400 MHz Bruker NMR machine.

2.2. Methods

Zebrafish Maintenance and Selection** Approximately 100 healthy adult zebrafish of uniform size were maintained in a large aquarium (45×60×25 cm) under controlled conditions. The aquarium water had a conductivity of around 1,500 µS/cm, a pH of 7, and a temperature of 24 °C. Oxygen levels were sustained by continuous aeration using an air pump. Water quality was preserved through daily filtration for two hours, the addition of aquarium disinfectant, and a 30% water change each day using Millipore-filtered water with appropriate instant ocean salt. The zebrafish were fed twice daily (at 9 am and 5 pm) with bloodworms, ensuring that all food was consumed within five minutes. After a maintenance period of 10 days, only fish exhibiting normal swimming behavior, feeding activity, and morphological features (with no signs of disease) were selected for experimentation.

2.3. Brain Metabolite Profiling Procedure

- **Exposure of Zebrafish to Addictive Drugs:** To generate a model of addiction, healthy adult zebrafish of uniform size were treated with either 3% ethanol, 2 mg nicotine, or a combination of both (3% ethanol and 2 mg nicotine) in 1 L of filtered aquarium water for 1 hour, three times daily (8 am, 1 pm, and 6 pm) over a period of three weeks. Each treatment group consisted of approximately 20 healthy fish, with a parallel control group of 20 healthy fish maintained under identical conditions.
- **Procedure for Zebrafish Brain Removal:** Zebrafish were anesthetized by transferring them to chilled clean water for at least 30 minutes, following the protocol established by Tran and Gerlai Tran & Gerlai (2013) [12], which demonstrated that cold treatment provides comparable anesthetic properties to commercially available anesthetics. The head of each fish was excised using a razor blade, and soft tissues were meticulously removed from the ventral side of the skull using forceps. The optic chiasm was identified, and the eyes were carefully excised from the optic nerve with spring scissors. The skull was then broken, and the bone was removed from the ventral side of the brain. The cranial nerve was excised, and the skin was removed from the dorsal side of the brain without damaging the skull. The brain was subsequently extracted using a scalpel, washed in

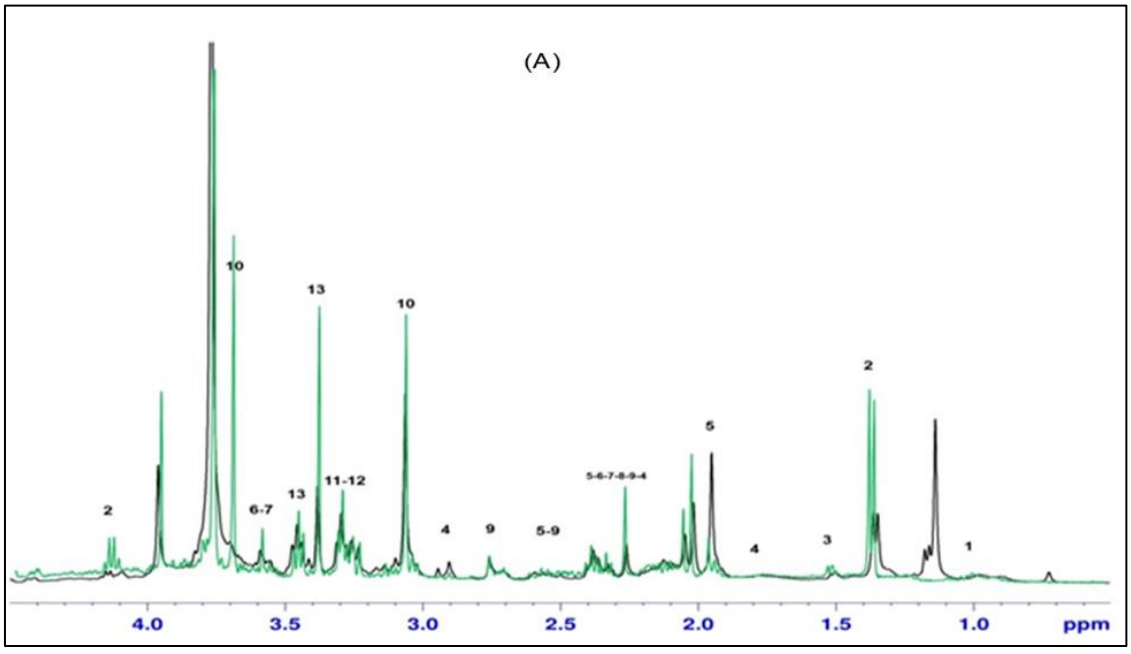
phosphate-buffered saline (PBS) to eliminate any blood traces, and excess PBS was carefully removed with tissue paper. The brain samples were immediately frozen in liquid nitrogen for future analysis.

- **Extraction of Brain Metabolites:** Brain metabolite extraction was performed following the methodology developed by Cousin et al. (2014) [13]. In brief, the frozen brains were homogenized in a 4 ml methanol-water mixture (1:1). Following this, 4 ml of chloroform was added, and the mixture was sonicated for 15 minutes, followed by centrifugation at 5000 rpm at 4 °C. This process resulted in the formation of two distinct layers: an upper chloroform layer and a lower methanol-water layer (aqueous layer). The methanol-water layer was carefully separated, and any residual chloroform was removed by passing nitrogen gas at 4 °C. The aqueous phase was then dried using a cold trap dryer. The dried sample was reconstituted in 100 mM NMR buffer (pH adjusted with Na2HPO4/NaH2PO4 in D2O). The metabolite spectrum of the aqueous layer was analyzed using HNMR (400 MHz), and the resulting NMR data were processed with Chenomx NMR 7.1 software. The patterns of peaks and the metabolites associated with each peak were identified using databases provided by Cousin et al., 2014 [13] and Braida et al., 2020 [14].

3. Results and Discussion

The spectrum of zebrafish brain metabolites obtained from control samples using HNMR spectroscopy at 400 MHz exhibited a profile closely resembling that reported in previous studies, particularly the work by Kabli et al, 2009 [15] and Mahabir et al., 2014 [16]. Our control zebrafish metabolite profile also demonstrated significant similarity to earlier published data on rat brain metabolites [17]. These results indicate that our zebrafish housing conditions and the anesthetic protocol employed did not alter the brain metabolite profile. Furthermore, the methodology for metabolite extraction adapted from Mahabir et al.,2014 [16] proved to be highly optimized, allowing for accurate identification of metabolites.

When comparing the brain metabolites extracted from alcohol-addicted zebrafish to those from control samples, we observed near similarity in the spectral peak profiles, with notable exceptions at peak #3 (alanine), peak #5 (N-acetyl L-aspartate, NAA), and peak #13 (taurine). This finding aligns with previous reports indicating that chronic ethanol exposure alters metabolite profiles, particularly increasing alanine levels, which is known to enhance the activation of inhibitory neurons by competitively binding to GABA receptors [18]. Additionally, alcohol appears to selectively activate inhibitory neurons through increased taurine production, a known positive allosteric modulator of GABA and glycine receptors [19]. The observed decrease in NAA concentration in the ethanol-treated zebrafish suggests a shift in neurotransmission balance towards inhibition, as NAA is crucial for the rapid conversion to glutamate during excitatory processes and memory formation [20]. Given that NAA is considered an osmoregulator and an indicator of brain health, the reduction in its levels due to alcohol consumption indicates a compromise in brain health [21]. These findings corroborate earlier reports of alcohol-induced reductions in brain volume and impairments in excitability and memory processing [1, 22].



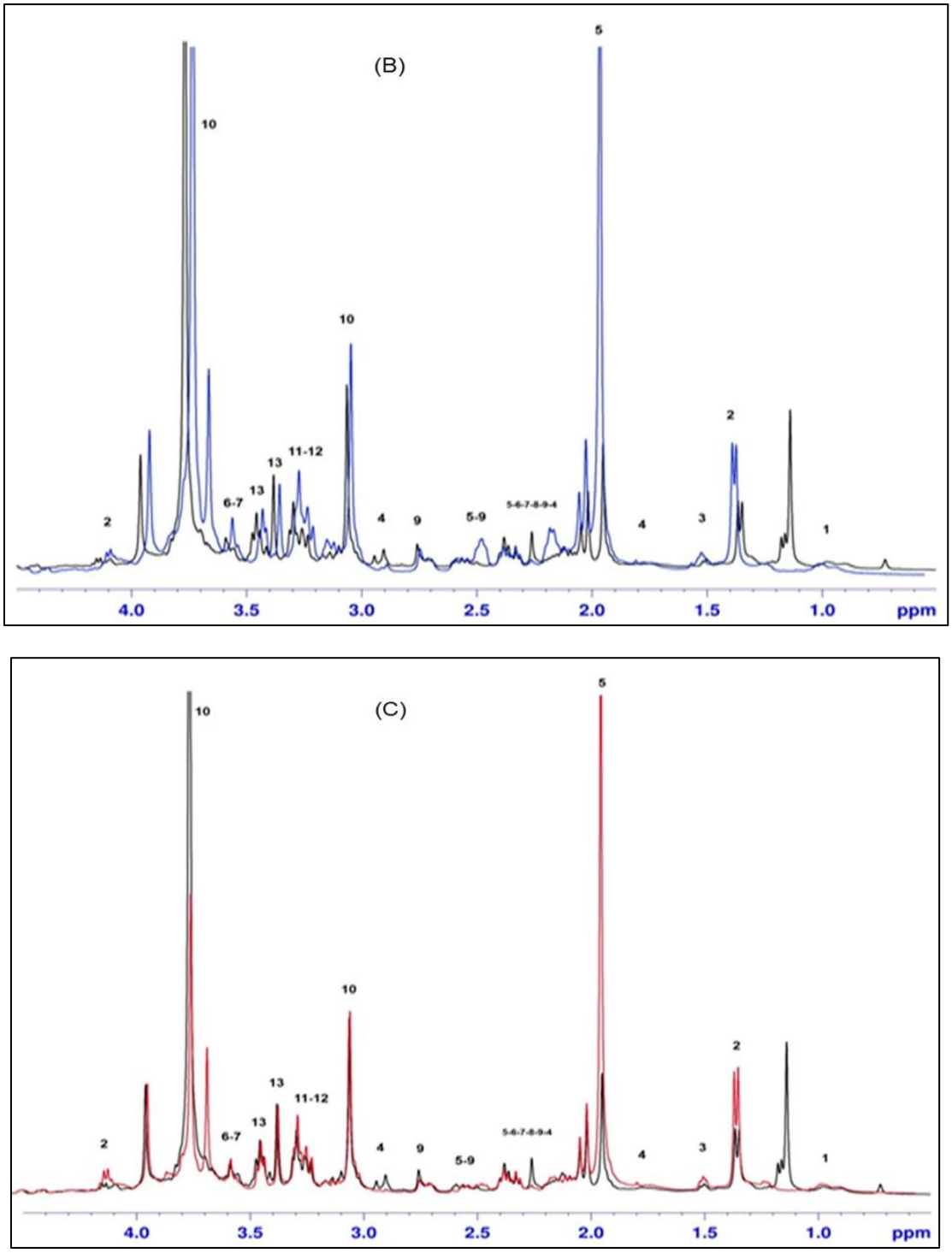


Figure 1 Comparison of zebrafish (Danio rerio) brain aqueous extract metabolite spectra between control and addictive drug-exposed brains, analyzed using high-resolution 400 MHz 1H NMR spectroscopy. (A) Comparison between control (black line) and ethanol-exposed (green line) samples; (B) Comparison between control (black line) and nicotine-exposed (blue line) samples; (C) Comparison between control (black line) and samples co-exposed to ethanol and nicotine (red line). Metabolites corresponding to each peak were identified based on the reference provided by Kabli et al., 2009 [15]. The assignments are as follows: Peak 1: valine/leucine/isoleucine; Peak 2: lactate; Peak 3: alanine; Peak 4: gamma-aminobutyric acid (GABA); Peak 5: N-acetyl L-aspartate (NAA); Peak 6: glutamate; Peak 7: glutamine; Peak 8: succinate; Peak 9: aspartate; Peak 10: creatine; Peak 11: choline; Peak 12: phosphocholine; Peak 13: taurine

In contrast, the analysis of brain metabolites from zebrafish subjected to long-term nicotine exposure revealed that most metabolite peaks remained similar to those in control samples, with a significant increase in NAA concentration. This increase is indicative of enhanced neuronal health and correlates with improved memory formation in nicotine-exposed individuals [23-24]. The slight increase in peaks representing neurotransmitters such as glutamine, succinate, and glutamate further supports the notion that nicotine contributes positively to overall brain health and cognitive function [25].

The comparison of brain metabolite profiles from zebrafish co-treated with alcohol and nicotine revealed a metabolite profile more similar to that of nicotine-treated zebrafish than to alcohol-treated or control samples. Specifically, the peaks for NAA and taurine were comparable to those observed in nicotine-treated brains. This observation is intriguing, as numerous studies suggest that co-exposure to nicotine and alcohol can mitigate the effects of each substance [26-27]. For instance, moderate alcohol consumption alongside nicotine has been shown not to impair nicotine associative learning, potentially enhancing cognitive performance [28]. Our treatment protocol, which involved moderate exposure to both substances, aligns with these findings, suggesting that the beneficial effects of nicotine may be preserved while the adverse effects of alcohol are masked.

However, the lack of significant changes in the peaks representing neurotransmitters such as glutamine, succinate, and glutamate in the alcohol-nicotine co-exposed zebrafish suggests that alcohol may inhibit some of the positive effects of nicotine. This hypothesis is further supported by the significant increase in lactate peak values in both alcohol and alcohol-nicotine treated samples, while only a moderate increase was observed in nicotine-treated samples. Lactate is essential for sustaining glutamatergic neurotransmission during long-term memory processing [7, 29]. Chronic nicotine infusion has been shown to enhance lactate production and the expression of monocarboxylate transport proteins, which facilitate lactate transport between astrocytes and neurons [30]. Therefore, the moderate increase in lactate in nicotine-treated zebrafish may reflect increased utilization of lactate during enhanced memory formation.

4. Conclusion

In summary, our findings indicate that while alcohol exposure leads to significant alterations in brain metabolite profiles, nicotine appears to exert neuroprotective effects that can be preserved even in the presence of alcohol. The interplay between these substances highlights the complexity of their combined effects on brain metabolism and function, warranting further investigation into the mechanisms underlying these interactions.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

Ethical approval was granted by the Mona Campus Research Ethics Committee (Ref: CREC-AN.005. 2022/2023).

Authors' Contribution

- **Mohammad Kutub Ali:** Research supervisor and coordinator, data analyser and manuscript drafting.
- **Derron Ricardo Taite:** Research scholar and data collection.

References

[1] McGinn, M., Paulsen, R., Itoga, C., Farooq, M., Reppel, J., Edwards, K., … & Edwards, S. (2016). Withdrawal from chronic nicotine exposure produces region-specific tolerance to alcohol-stimulated glua1 phosphorylation. Alcoholism Clinical and Experimental Research, 40(12), 2537-2547. https://doi.org/10.1111/acer.13258

[2] Chen, X., Owoseni, E., Salamat, J., Cederbaum, A., & Lu, Y. (2018). Nicotine enhances alcoholic fatty liver in mice: role of cyp2a5. Archives of Biochemistry and Biophysics, 657, 65-73. https://doi.org/10.1016/j.abb.2018.09.012

[3] Wen, W., Wang, Y., Li, H., Hu, D., Zhang, Z., Lin, H., … & Luo, J. (2023). Upregulation of mesencephalic astrocyte-derived neurotrophic factor (manf) expression offers protection against alcohol neurotoxicity. Journal of Neurochemistry, 166(6), 943-959. https://doi.org/10.1111/jnc.15921

[4] Kamal, H., Tan, G., Ibrahim, S., Shaikh, M., Mohamed, I., Mohamed, R., … & Kumar, J. (2020). Alcohol use disorder, neurodegeneration, alzheimer's and parkinson's disease: interplay between oxidative stress, neuroimmune response and excitotoxicity. Frontiers in Cellular Neuroscience, 14. https://doi.org/10.3389/fncel.2020.00282

[5] Majdi, A., Kamari, F., Sadigh-Eteghad, S., & Gjedde, A. (2019). Molecular insights into memory-enhancing metabolites of nicotine in brain: a systematic review. Frontiers in Neuroscience, 12. https://doi.org/10.3389/fnins.2018.01002

[6] Qin, L., Zou, J., Barnett, A., Vetreno, R., Crews, F., & Coleman, L. (2021). Trail mediates neuronal death in aud: a link between neuroinflammation and neurodegeneration. International Journal of Molecular Sciences, 22(5), 2547. https://doi.org/10.3390/ijms22052547

[7] Bullock, K., Cservenka, A., & Ray, L. (2016). Severity of alcohol dependence is negatively related to hypothalamic and prefrontal cortical gray matter density in heavy drinking smokers. The American Journal of Drug and Alcohol Abuse, 43(3), 281-290. https://doi.org/10.1080/00952990.2016.1257632

[8] Gubner, N., Kozar-Konieczna, A., Szołtysek-Bołdys, I., Słodczyk-Mańkowska, E., Goniewicz, J., Sobczak, A., … & Goniewicz, M. (2016). Cessation of alcohol consumption decreases rate of nicotine metabolism in male alcohol-dependent smokers. Drug and Alcohol Dependence, 163, 157-164. https://doi.org/10.1016/j.drugalcdep.2016.04.006

[9] Crummy, E., O'Neal, T., Baskin, B., & Ferguson, S. (2020). One is not enough: understanding and modeling polysubstance use. Frontiers in Neuroscience, 14. https://doi.org/10.3389/fnins.2020.00569

[10] Moorman, D. (2018). The role of the orbitofrontal cortex in alcohol use, abuse, and dependence. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 87, 85-107. https://doi.org/10.1016/j.pnpbp.2018.01.010

[11] Soliman, N., Ghafar, M., AbuoHashish, N., Ibrahim, M., Eid, A., El-Gohary, R., … & Elshamy, A. (2022). The possible role of naringenin in the prevention of alcohol-induced neurochemical and neurobehavioral deficits. Neurochemical Research, 48(2), 537-550. https://doi.org/10.1007/s11064-022-03775-x

[12] Tran, S. and Gerlai, R. (2013). Time-course of behavioural changes induced by ethanol in zebrafish (danio rerio). Behavioural Brain Research, 252, 204-213. https://doi.org/10.1016/j.bbr.2013.05.065

[13] Cousin, M., Ebbert, J., Wiinamaki, A., Urban, M., Argue, D., Ekker, S., … & Klee, E. (2014). Larval zebrafish model for fda-approved drug repositioning for tobacco dependence treatment. Plos One, 9(3), e90467. https://doi.org/10.1371/journal.pone.0090467

[14] Braida, D., Ponzoni, L., Moretti, M., Viani, P., Pallavicini, M., Bolchi, C., … & Sala, M. (2020). Behavioural and pharmacological profiles of zebrafish administrated pyrrolidinyl benzodioxanes and prolinol aryl ethers with high affinity for heteromeric nicotinic acetylcholine receptors. Psychopharmacology, 237(8), 2317-2326. https://doi.org/10.1007/s00213-020-05536-6

[15] Kabli, N., Spaink, H. P., De Groot, H., & Alia, A. (2009). "Metabolomic analysis of zebrafish embryos: A new tool for studying the effects of environmental pollutants." *Environmental Science & Technology*, 43(12), 4547-4552. doi:10.1021/es803136b.

[16] Mahabir, S., Chatterjee, D., & Gerlai, R. (2014). Strain dependent neurochemical changes induced by embryonic alcohol exposure in zebrafish. Neurotoxicology and Teratology, 41, 1-7. https://doi.org/10.1016/j.ntt.2013.11.001

[17] Mao, K., Li, X., Chen, Z., Dong, X., Zhangsun, D., Zhu, X., … & Luo, S. (2022). A-conotoxin txib improved behavioral abnormality and changed gene expression in zebrafish (danio rerio) induced by alcohol withdrawal. Frontiers in Pharmacology, 13. https://doi.org/10.3389/fphar.2022.802917

[18] Fang, T., Liu, R., Fan, C., Sun, Y., Huang, X., Nie, Z., … & Pu, X. (2020). Effects of thymoquinone on small-molecule metabolites in a rat model of cerebral ischemia reperfusion injury assessed using maldi-msi. Metabolites, 10(1), 27. https://doi.org/10.3390/metabo10010027

[19] Mahabir, S., Chatterjee, D., Buske, C., & Gerlai, R. (2013). Maturation of shoaling in two zebrafish strains: a behavioral and neurochemical analysis. Behavioural Brain Research, 247, 1-8. https://doi.org/10.1016/j.bbr.2013.03.013

[20] Tran, S. and Chatterjee, D. (2015). An integrative analysis of ethanol tolerance and withdrawal in zebrafish (danio rerio). Behavioural Brain Research, 276, 161-170. https://doi.org/10.1016/j.bbr.2014.02.034

[21] Nowicki, M., Tran, S., & Chatterjee, D. (2015). Inhibition of phosphorylated tyrosine hydroxylase attenuates ethanol-induced hyperactivity in adult zebrafish (danio rerio). Pharmacology Biochemistry and Behavior, 138, 32-39. https://doi.org/10.1016/j.pbb.2015.09.008

[22] Viscarra, F., González-Gutiérrez, J., Esparza, E., Figueroa, C., Paillali, P., Hödar-Salazar, M., … & Iturriaga-Vásquez, P. (2020). Nicotinic antagonist ufr2709 inhibits nicotine reward and decreases anxiety in zebrafish. Molecules, 25(13), 2998. https://doi.org/10.3390/molecules25132998

[23] Ponzoni, L., Teh, M., Torres-Pérez, J., Brennan, C., Braida, D., & Sala, M. (2020). Increased response to 3,4-methylenedioxymethamphetamine (mdma) reward and altered gene expression in zebrafish during short- and long-term nicotine withdrawal. Molecular Neurobiology, 58(4), 1650-1663. https://doi.org/10.1007/s12035-020-02225-5

[24] Cuevas, E., Trickler, W., Guo, X., Ali, S., Paule, M., & Kanungo, J. (2013). Acetyl l-carnitine protects motor neurons and rohon-beard sensory neurons against ketamine-induced neurotoxicity in zebrafish embryos. Neurotoxicology and Teratology, 39, 69-76. https://doi.org/10.1016/j.ntt.2013.07.005

[25] Sharma, R., Sahota, P., & Thakkar, M. (2014). Nicotine administration in the cholinergic basal forebrain increases alcohol consumption in c57bl/6j mice. Alcoholism Clinical and Experimental Research, 38(5), 1315-1320. https://doi.org/10.1111/acer.12353

[26] Boiangiu, R., Mihăşan, M., Gorgan, D., Stache, B., & Hriţcu, L. (2021). Anxiolytic, promnesic, anti-acetylcholinesterase and antioxidant effects of cotinine and 6-hydroxy-l-nicotine in scopolamine-induced zebrafish (danio rerio) model of alzheimer's disease. Antioxidants, 10(2), 212. https://doi.org/10.3390/antiox10020212

[27] Liu, M. (2023). Comparative analysis of the metabolites and biotransformation pathways of fentanyl in the liver and brain of zebrafish. Frontiers in Pharmacology, 14. https://doi.org/10.3389/fphar.2023.1325932

[28] Mathur, P., Berberoglu, M., & Guo, S. (2011). Preference for ethanol in zebrafish following a single exposure. Behavioural Brain Research, 217(1), 128-133. https://doi.org/10.1016/j.bbr.2010.10.015

[29] Majdi, A., Kamari, F., Sadigh-Eteghad, S., & Gjedde, A. (2019). Molecular insights into memory-enhancing metabolites of nicotine in brain: a systematic review. Frontiers in Neuroscience, 12. https://doi.org/10.3389/fnins.2018.01002

[30] Kedikian, X., Faillace, M., & Bernabeu, R. (2013). Behavioral and molecular analysis of nicotine-conditioned place preference in zebrafish. Plos One, 8(7), e69453. https://doi.org/10.1371/journal.pone.0069453