Neurotoxicity and pharmacokinetic evaluation of the designer drug N-ethyl pentedrone: a toxicometabolomic approach

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New Psychoactive Substances (NPS) are compounds with central nervous system activity not regulated by international drug conventions. Among various NPS classes, synthetic cathinones (SC) are notably numerous. N-ethyl pentedrone (NEP) is a SC linked to numerous cases of intoxication worldwide. However, comprehensive studies on its toxicity are still lacking. Elucidating the metabolism of xenobiotics is pivotal for predicting their effects, toxicities, and potential treatments. Zebrafish models are reliable due to their genetic and physiological similarities to mammals, alongside their cost-effectiveness. The Zebrafish Water Tank (ZWT) model is valuable for studying drug metabolism, offering reliable insights into metabolite identification. Furthermore, toxicometabolomics allows qualitative and quantitative analysis of small molecules associated with cellular toxic events. This study aimed to investigate the metabolic profile and neurotoxic effects of NEP in zebrafish.

Six-month-old male zebrafish (N = 40) were equally distributed into five tanks: two negative controls (without drug exposure) and three exposure tanks ([NEP]=0.5 ?g/mL). After an 8-hour exposure, animals were euthanized, and had their brain collected. Brain samples were pooled in a scheme of five per sample. Metabolites were extracted using a homogenization protocol with methanol added with internal standard (methamphetamine-d5, 10 ?g/mL). Blank samples were prepared following the same procedure, but without tissues. Quality control (QC) were prepared by pooling all samples. Homogenates were injected into a LCMS9030 QToF (Shimadzu, Japan). MS-Dial was used for peak picking, chromatogram deconvolution, alignment, and integration, referencing public metabolomics databases. MS-FINDER and HMBD database were employed to enhance metabolite annotation. The resulting matrices were manually curated and analyzed using MetaboAnalyst. All experiments were approved by the Ethical Committee for Animal Research (protocol 6253-1/2023).

Seven NEP metabolites were identified in zebrafish brain tissues. Metabolite 1 (M1), characterized by N-dealkylation, was the most abundant metabolite, while M2 resulted from ?-ketone reduction. The second most abundant NEP metabolite was M4, produced by M1 ?-ketone reduction. Hydroxylation reactions were observed in the aromatic ring (M3) and the aliphatic chain (M5). Two phase II metabolites were detected: M6, produced by aromatic O-glucuronidation, and M7, formed by M6 N-dealkylation. The analysis performance was evaluated before and throughout data acquisition using QC samples, demonstrating substantial robustness. Differences in the metabolome between the two groups were highlighted using OPLS-DA models and univariate analysis. In the

exposed group, 28 metabolites were found upregulated and 28 downregulated. Associated pathways involving mainly the energetic metabolism, including propionylcarnitine, and the lipid metabolism, including tricosanoic acid (23:0) and 6,7-diketolithocholic acid.

Seven NEP metabolites were identified in zebrafish brain and pathways associated to its neurotoxicity were purposed. Phase I and phase II metabolites were detected. Multivariate analyses revealed significant alterations between exposed and non-exposed groups, indicating alterations in lipid and energy metabolism. These findings enhance our understanding of NEP toxicity mechanisms and provide valuable insights for toxicological assessments.

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