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Short communication

Concentration-dependent embryotoxicity of *Nepeta grandiflora* and *Nepeta laevigata* methanol extracts

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Introduction

The pharmacological activities of *Nepeta* species are usually ascribed to iridoid monoterpenoids and polyphenolics. Iridoids can be found in the form of aglycones (IAs) and glycosides (IGs), although some representatives of the genus *Nepeta* produce only the glycosylated iridoids. Previous phytochemical studies assigned *Nepeta grandiflora* M.Bieb. and *N. laevigata* (D.Don) Hand.-Mazz. to a chemotype producing substantial amounts of IGs, predominantly 1,5,9-*epi*-deoxyloganic acid (1,5,9-*e*DLA), but containing no nepetalactone-type iridoids (Banjanac et al., 2026). *N. grandiflora* is an ornamental herbaceous perennial native to Caucassus and Crimea, recognized for its repellent activities (Birkett et al., 2010). On the other hand, *N. laevigata* is a perennial spreading from Afghanistan to S. Central China, displaying prominent antimicrobial and antioxidant potential (Almas et al., 2024). To the best of our knowledge, these two *Nepeta* species have not previously been described for their zebrafish embryotoxicity, which was the objective of this study.

Materials and methods

Plant material

Nepeta grandiflora M.Bieb. and *N. laevigata* (D.Don) Hand.-Mazz. (Lamiaceae) were cultivated in the greenhouse of the Institute for biological research „Siniša Stanković"- National Institute of the Republic of Serbia, University of Belgrade. Above ground parts with flowering tops were harvested in 2022, dried at the room temperature until constant mass, mechanically grinded into a fine powder, and used for the preparation of methanol extracts.

Extraction procedure and metabolic profiling

Plant material (10 g) was dissolved in 96% methanol (*w*:*v* = 1:10), vortexed for 1 min, and extracted in an ultrasonic bath for 1 h at 4 °C, which was followed by overnight extraction at 4 °C. Following centrifugation at 10000*g* for 10 min, supernatant was filtered, and evaporated in a vacuum concentrator (Rotavapor RII, Buchi, Switzerland). Samples were dissolved in 96% methanol (*w*:*v* = 1:10), filtered, and transferred into HPLC vials. Subsequently, samples were injected onto HPLC/Orbitrap MS instrument for untargeted metabolomics of methanol-soluble compounds, as well as onto GC/MS instrument towards profiling of volatile organic compounds (VOCs).

Zebrafish maintenance

Adult zebrafish (*Danio rerio*, AB wild-type strain) were housed in a custom-built recirculating aquaculture system under standardized environmental conditions (water conductivity of

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approximately 600 µS, pH 7.0–7.5, and temperature of 26 °C, with a photoperiod of 14 h light and 10 h dark). Fish were fed three times daily, consisting of two feedings with commercial dry diet and one feeding with live *Artemia salina* nauplii. Embryos were obtained using 1.7 L breeding tanks (Techniplast, Italy), with each breeding group composed of two males and four females. Experimental assays were carried out in triplicate.

Embryotoxicity

Treatment was performed using OECD protocol (OECD 2013) modified as described in Aleksić et al. (2018). Fertilized eggs were divided in 24 well plate (10 embryos per well, each containing 2 mL of E3 medium). Embryos were exposed to extracts of *N. grandiflora* and *N. laevigata* in concentrations: 100, 250, 500, and 1,000 µg/mL. Experiments also included negative (E3 medium) and positive control (3,5-dichloroaniline). Exposure started at 4 h post fertilization (hpf) and lasted until 96 hpf in dark. The embryos were observed under the stereomicroscope (63× magnification) and imaged. Coagulation, non-detached tail, and lack of somites were assessed as lethal endpoints at 24 and 48 hpf, while at 48 hpf lack of heartbeat was considered lethality. Embryo hatching rates were assessed at 48, 72 and 96 hpf. Following developmental malformations were regularly assessed: head malformation, eye and body pigmentation, tail malformation, scoliosis, yolk morphology, heart malformation, pericardial edema and growth retardation.

Results and discussion

N. grandiflora and *N. laevigata* displayed similar IG profiles, predominated by 1,5,9-*e*DLA, which is not surprising, as they are phylogenetically closely related (Banjanac et al., 2026). On the other hand, rosmarinic and caffeic acids were the most abundant polyphenolics. Among VOCs, 1,8-cineole and germacrene D were recorded as the major constituents of methanol extracts.

N. grandiflora extracts exhibited concentration-dependent effects on embryonic development. Exposure to the highest tested concentration (1,000 µg/mL) caused evident embryotoxicity beginning at 24 hpf, resulting in complete embryo mortality by 96 hpf. No adverse effects on embryo survival, hatching rate, or sublethal developmental endpoints were observed at the lowest tested concentration (100 µg/mL). Similarly, *N. laevigata* induced concentration-dependent embryotoxic responses. The highest tested concentration resulted in pronounced embryotoxicity accompanied by developmental delay. In contrast, embryos exposed to the lowest concentration did not show evident effects on survival, hatching success, or the occurrence of sublethal developmental alterations. In both cases impact of intermediate concentration (250 and 500 µg/mL) was manifested by delayed embryonic development, depigmentation and morphological abnormalities, predominantly pericardial edema.

Conclusion

Embryotoxic effects of *N. grandiflora* and *N. laevigata* rely on high iridoid and phenolic content, and are concentration-dependent. Concentrations of extracts up to 100 µg/mL do not interfere with early development, organ formation, or survival in zebrafish embryos, which candidates them for therapeutic use in mammalians and humans.

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