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Short communication

BSLA based cytotoxic profiling of *Verbena officinalis*

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Introduction

Verbena officinalis L. (Verbenaceae), commonly known as common vervain in some commercial preparations, is a perennial herb that has been traditionally used in European, Mediterranean and Asian traditional medicine for the treatment of nervous disorders, digestive complaints, inflammatory conditions and as a mild sedative (Calvo, 2006). The aerial parts of the plant are still widely consumed in herbal teas and incorporated into a variety of dietary supplements and phytotherapeutic products available on the global market. From a phytochemical point of view, the plant is rich in iridoids, phenylpropanoid glycosides and flavonoids; among these, verbascoside is considered the most relevant marker compound due to their well-documented antioxidant, anti-inflammatory, neuroprotective and vasoprotective activities (Alipieva et al., 2014). Despite widespread traditional use, toxicological data on *V. officinalis* preparations remain limited. Therefore, the aim of this study was to evaluate the cytotoxic potential of a hydroethanolic extract of *V. officinalis*, with quantified contents of verbascoside, using the Brine shrimp lethality assay (BSLA), in order to provide preliminary safety information for its potential therapeutic use.

Material and methods

Plant material and preparation of the extract

Dried aerial parts of *Verbena officinalis* L. (Alkaloid, Skopje) were extracted using 0.5 g plant material and 50 mL of 50% (v/v) ethanol. The extract was filtered and adjusted to 50 mL, yielding a 10 mg/mL stock solution used for all analyses.

Brine Shrimp Lethality Assay (BSLA)

The cytotoxic activity of the *V. officinalis* hydroethanolic extract was evaluated using the Brine Shrimp Lethality Assay (BSLA) by recording mortality of *Artemia salina* nauplii after 1, 2, 4, 6, and 24 h exposure (Meyer et al., 1982). Test solutions (0.01–5 mg/mL) were prepared from a 10 mg/mL stock using sodium chloride solution. Each assay contained 10 nauplii in 5 mL solution, with potassium dichromate ($K_2Cr_2O_7$) and sodium chloride used as positive and negative controls, respectively. LC_{50} values were calculated by probit analysis and toxicity was classified according to Meyer's and Clarkson's scales.

Results and discussion

The cytotoxic activity of the V. officinalis hydroethanolic extract was evaluated using the Brine Shrimp Lethality Assay (BSLA) by monitoring the

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mortality of Artemia salina nauplii over a 24-hour exposure period across a concentration range of 0.01–5 mg/mL. The LC_{50} values demonstrated a pronounced and consistent time-dependent decrease, indicating a progressive enhancement of cytotoxic potency with prolonged exposure. Specifically, LC_{50} values declined from 824.03 µg/mL (1 h) to 645.98 µg/mL (2 h), 546.91 µg/mL (4 h), and 513.84 µg/mL (6 h), culminating in a markedly low value of 53.78 µg/mL after 24 h. According to Meyer's toxicity criterion, the extract remained biologically active (LC_{50} < 1000 µg/mL) throughout all exposure intervals. In contrast, Clarkson's hierarchical classification revealed a distinct temporal shift in toxicity profile, transitioning from low toxicity (500–1000 µg/mL) during the initial 6 hours to high toxicity (LC_{50} < 100 µg/mL) at 24 hours (Meyer et al., 1982; Clarkson et al., 2004). This abrupt reduction in LC_{50} at the terminal time point suggests a non-linear toxicodynamic response, potentially reflecting delayed intracellular accumulation, progressive membrane disruption, or time-dependent metabolic activation of phytochemical constituents. The observed bioactivity is consistent with the phytochemical profile of *V. officinalis*, particularly the abundance of phenylpropanoid glycosides such as verbascoside. This compound has been reported to exhibit concentration- and context-dependent pro-oxidant behavior, in addition to modulating apoptotic pathways, mitochondrial dysfunction, and membrane integrity. Such multifaceted mechanisms may act synergistically, thereby amplifying cytotoxic outcomes under prolonged exposure conditions (Alipieva et al., 2014). The steep decline in LC_{50} at 24 hours further suggests that cumulative intracellular exposure and possible metabolic biotransformation of active constituents significantly contribute to the observed toxicity. The exceptionally low LC_{50} observed at 24 hours (53.78 µg/mL) positions the extract within a highly bioactive range, comparable to potent plant-derived cytotoxins reported in the literature. This underscores its potential pharmacological relevance, particularly as a source of lead compounds for further anticancer-oriented investigations.

Conclusion

Overall, the hydroethanolic extract of *V. officinalis* exhibits a robust, time-dependent cytotoxic profile characterized by a sharp intensification of activity at prolonged exposure durations. These findings not only highlight the importance of exposure time in toxicological evaluation but also support the hypothesis that verbascoside-rich extracts may exert significant bioactivity through cumulative and multi-target mechanisms. Further studies involving fractionation, compound isolation, and mechanistic validation in cell-based systems are warranted to elucidate its therapeutic potential and safety profile.

References

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