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Short communication

Modulation of Nrf2 expression in human neutrophils by *Ballota nigra* extract

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Introduction

Transcription factor nuclear factor erythroid 2 p45-related factor 2 (Nrf2) is a main regulator of cellular antioxidant defense. The KEAP-Nrf2-ARE pathway plays important role on cellular homeostasis *via* modulation of cytoprotective enzymes, inflammatory cytokines and metabolic factors (Marchev et al., 2017).

Modulation of the Nrf2 activity is achieved through interactions with specific regions and is hence targeted by several types of modulators. The synthetic triterpenoid compound 2-cyano-3,12-dioxo-oleana-1,9(11)-dien-28-oic acid methyl ester (CDDO-Me) has been recently described as strong Nrf2 activator (Wong et al., 2020).

Neutrophils have a short live in circulation and accelerate greatly the local immune responses *via* increased granulopoiesis and migration at high numbers to infected or inflamed tissue (Dimitrova et al., 2019).

Plant metabolite profiles could be revealed with nuclear magnetic resonance (NMR) based metabolomics (Marchev et al., 2020). Different modes for NMR-based analysis, such as ^{1}H and 2D permit the identification of compounds in a complex mixture such as natural extracts (Salem et al., 2019).

The widespread perennial plant *Ballota nigra* L. has been extensively employed in traditional medicine for wound healing and as sedative for different nervous disorders (Pieroni, 2000). Among its major secondary metabolites are ballonigrine, ballotetroside, verbascoside and forsythoside (Vrchovska et al., 2007).

In the present study we have exploited NMR-based metabolomics to investigate the phytochemical profile of *B. nigra* extract. Furthermore, we have evaluated the potential modulation of the KEAP-Nrf2-ARE pathway in human neutrophils, treated with *B. nigra* extract, by performing NRF2 expression analysis.

Materials and methods

Plant material and extraction

Aerial parts from *B. nigra* were collected in 2018 from Plovdiv, Bulgaria. Following freeze-drying, the plant material was grounded and extracted with 50% aqueous methanol (1:30 w/v), in an ultrasonic bath at 35 kHz frequency for 20 min, at room temperature. The extract was filtrated and evaporated to dryness and stored at -20 °C further used for analysis.

NMR-based metabolite profiling

The NMR analysis followed the protocol, described by Amirova et al. (2021).

Human neutrophil culture

Human HL-60 cell line (kind gift from Dr. Giovanni Bernaridini, La Sapienza University Rome) was differentiated to human neutrophils incubated with 1% DMSO for 7 days. Differentiated neutrophils were then

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cultured in the presence of increasing concentrations of the extract and the pure substances. Cell viability was monitored using MTT reagent.

On the first hour after treatment, RNA isolation was performed to evaluate the expression of the gene encoding Nrf2, namely *NFE2L2*.

Quantitative real-time polymerase chain reaction (RT-qPCR)

Total RNA was isolated with RNAzol RT reagent and reverse transcribed using FirstStrand cDNA kit (Canvax). Gene expression was detected with RT-qPCR using Sso EvaGreen SuperMix (Bio-Rad) on CFX96 system (Bio-Rad). *ACTB* and *RLP13A* were used as reference genes.

Results and discussion

Metabolite profiling of B. nigra

According to the ^{1}H NMR spectral data the most abundant signals appeared to correspond to ballonigrine, verbascoside and forsythoside B.

Nrf2 expression in neutrophils

Plant extracts, as valuable source of bioactive molecules, could act as modulators of the function of neutrophils. The most abundant compound in *B. nigra* extract is verbascoside, which is reported to be activator of Nrf2 (Li et al., 2018a; Li et al., 2018b). In respect to immune cells, verbascoside decreases murine neutrophil respiratory burst, chemotaxis *in vitro* and migration to inflammatory sites (Li et al., 2018a; Li et al., 2018b). The strength and selectivity of verbascoside depended on the degree of activation and functional state of neutrophils, and could have a potential to affect neutrophil-related pathologies/conditions in heterogenic populations (Dimitrova et al., 2019).

The regulation of neutrophil function in the context of Nrf2-KEAP1 axis, *via* forsythoside B and ballonigrine, which are also present in crude extract, are described for the first time. We have performed a screening of compounds from *B. nigra* extract for potent modulation of Nrf2 expression in human neutrophils.

Conclusion

In the present study, we provide a broader insight of the biochemical status and Nrf2 expression in neutrophils, treated with extract of *B. nigra* and its secondary metabolites.

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