

PPAR γ inhibition mediates *Alchemilla monticola* Opiz anti-adipogenic effect in human adipocytes

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Introduction

Obesity is a persistent and expanding social health concern. Excessive fat mass is associated with increased risk for chronic diseases including diabetes, atherosclerosis, non-alcoholic steatohepatitis, reproductive dysfunctions and certain types of cancer (Vasileva et al., 2020).

Medicinal plants have centuries of exploitation in management of weight control, mainly based on empirical ethnopharmacological knowledge. Deciphering the mechanism of action of such plants with the contemporary “-omics” approaches such as metabolomics, transcriptomics and proteomics could lead to the discovery of new bioactive leads with anti-obesity potential (Vasileva et al., 2020).

Alchemilla monticola Opiz (Rosaceae) is a perennial plant traditionally used to treat inflammatory conditions, wounds and burns. Additionally, when administered as infusion from the aerial parts weight loss effect is described. Phytochemically *Alchemilla* species are known as sources of flavonoids, flavonoid glycosides, phenolic acids and tannins (Mandrone et al., 2018). Our preliminary findings suggested that *A. monticola* inhibits adipogenesis *in vitro* in human Simson-Golabi-Behmel syndrome (SGBS) preadipocyte cell strain.

In the search of the mechanism for a potential anti-adipogenic effect of *A. monticola* extract, we have employed the human SGBS preadipocytes as obesity model system and performed gene and protein expression analysis. Furthermore, nuclear magnetic resonance (NMR)

based metabolomics was used to obtain data for the actual phytochemical profile of the extract.

Materials and methods

Plant material and extraction

Aerial parts from *A. monticola* were collected in 2018 from Rhodope Mountain, Bulgaria. Following freeze-drying and grounding, the plant material was extracted in 50% aqueous methanol (1:30 w/v) with ultrasound at 35 kHz frequency for 20 min, at room temperature. The extract was filtrated, evaporated to dryness under vacuum at 40 °C and stored at -20 °C prior to analysis.

NMR-based metabolomics

The NMR analysis was performed according to the protocol described by Mladenova et al. (2021).

Cell culture and treatment

The human SGBS cells were kindly provided by Prof. Wabitsch from the University of Ulm, Germany and were maintained and differentiation under the described optimal conditions (Tews et al., 2019). From first day of differentiation and on every fourth day with media replacement the cells were exposed to either *A. monticola* extract at final concentrations of 5, 10 and 25 $\mu\text{g/mL}$ or 0.02% DMSO as vehicle.

Oil red O (ORO) staining and free glycerol measurement

Lipid staining was performed as described by Mladenova et al. (2021). Briefly, fixed differentiated SGBS adipocytes were stained with ORO dye solution for 15 min. Microphotographs of the stained cells were captured. Total accumulated lipids were quantified by measuring the absorbance of the extracted ORO staining at 495 nm.

Released in the culture media free glycerol as a product from hydrolysis of triglycerides was determined using Adipolysis Assay Kit (MilliporeSigma) following the manufacturer's instructions.

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

Total RNA extraction, cDNA synthesis and real-time qPCR were performed as described (Mladenova et al., 2021). *RPL13A* and *TUBB* were used as reference genes.

Western blotting

Total protein lysates were prepared and analyzed through Western blotting as previously described (Mladenova et al., 2021).

Results and discussion

Phytochemical profiling of A. monticola

The metabolite characterization of *A. monticola* extract was performed by ¹H NMR and HSQC profiling. According to the ¹H NMR spectral data the most abundant signals corresponded to quercetin, isoquercetin, quercetin-3-*O*-β-glucuronide, rutin, catechin, vitexin and vanillic acid.

Antiadipogenic and lipolytic effect of A. monticola

Adipose tissue expansion is characterized by both increase in adipocytes size (hypertrophy) and cell numbers (hyperplasia or adipogenesis, Arner et al., 2019). Further, the tandem of the transcription factors CAAT/enhancer-binding protein alpha (C/EBPα) and peroxisome proliferator-activated receptor gamma (PPARγ) orchestrates adipogenesis (Vasileva et al., 2018).

Lipid staining revealed anti-adipogenic activity of the *A. monticola* extract and increased glycerol concentration suggests its lipolytic potential.

Expression of *CEBPA* and *PPARG* was dose-dependently decreased upon *A. monticola* treatment in human SGBS adipocytes. The similar effect was observed

with the protein abundance of C/EBPα and PPARγ. These results suggest that the studied plant extract worth further exploration as source of bioactive leads with anti-obesity effect.

Conclusion

In the present study, we demonstrate antiadipogenic effect of *A. monticola* on human SGBS adipocytes. Furthermore, PPARγ is identified as potential target for its mechanism of action. Additionally, we provide a broader insight of the phytochemical composition of *A. monticola*. Hence, the identified metabolites worth further exploration as potentially active in obesity management.

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