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Short communication

Modulation of adipogenesis by *Polygonum hydropiper* L. and *P. aviculare* L. extracts

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Introduction

Obesity is among the leading health problems of the human population during the last decades. Its complex etiology obscures obesity management (Savova et al., 2021).

Nature and its plant diversity provide thousands of bioactive compounds with (un)known medicinal properties. For centuries, people have used folk remedies as singe plant or plant combinations for different diseases including obesity.

Polygonum aviculare L. and *P. hydropiper* L. both from Polygonaceae are plants used in ethnopharmacological herbal combinations for obesity, hypertension and atherosclerosis treatment (Lee et al., 2011; Park et al., 2014).

The human Simpson-Golabi-Behmel syndrome (SGBS) preadipocyte cell strain is a relevant model for studying adipogenesis (Fischer-Posovszky et al., 2008).

The present study aimed to elucidate whether the extracts of *P. aviculare* and *P. hydropiper* areal parts affect human SGBS adipogenic differentiation and suggest their potential molecular mechanism of action.

Materials and methods

Plant material and extraction

Aerial parts of investigated plants were collected in September 2019 from the following locations: *P. hydropiper* - Zvanichevo village, Bulgaria, latitude 42.1908 N, longitude 24.2416 E; and *P. aviculare* - Sandanski, Bulgaria, latitude 41.5785 N; longitude 23.2869 E. Plant samples lyophilized and grounded were subjected to ultrasound-assisted extraction in 50% methanol, at room temperature for 20 minutes. The filtered extracts were concentrated on rotary evaporator, then lyophilized and stored at -20 °C prior to use.

Cell culture and treatment

The human SGBS cells were kindly provided by Prof. Wabitsch from the Ulm University (Ulm, Germany) and were cultured according to the optimal conditions described detailly by Tews et al. (2019). From first day of differentiation and on every fourth day with change of the media, extracts were added at concentrations of 5, 10 and 25 μg/mL. Extracts effect were compared to vehicle treatment of 0.02% DMSO.

Cell viability

The influence of plant extracts on cell viability was assessed by MTT assay on confluent SGBS preadipocytes.

Oil red O (ORO) adipogenesis assay

Differentiated SGBS adipocytes were fixed with 10% formalin for 10 min at room temperature, then washed twice with phosphate buffered saline and stained with the fresh filtered ORO dye solution for 15 min. Photomicrographs of the stained cells were captured using an Oxion Inverso OX.2053-PLPH inverted microscope

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and DC.10000-Pro CMEX camera (Euromex, The Netherlands). Estimation of the total lipid content was done by extraction of the ORO dye from the cells with isopropanol, followed by reading the absorbance at 495 nm.

Free glycerol assay

Concentration of the free glycerol, released in the culture media was measured with Adipolysis Assay Kit (MilliporeSigma) according to the manufacturer's instructions.

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

Total RNA was isolated and reverse transcribed to cDNA. Expression of the major adipogenic genes was detected with RT-qPCR as described by Savova et al. (2021).

Western blot analysis

Protein isolation and Western blotting were performed as described previously (Savova et al., 2021).

Results and discussion

Plant extracts from *P. aviculare* and *P. hydropiper* did not affect cell viability of SGBS cells.

Polygonum aviculare anti-atherosclerotic effect has been evaluated in vivo in ApoE knockout model on high-fat diet fed mice (Park et al., 2014). The anti-obesity effect of P. hydropiper has been assessed in vitro in murine 3T3-L1 adipocytes. It was suggested that the flavonoids - isoquercitrin and isorhamnetin - are responsible for activation of Wnt/β-catenin pathway and inhibition of adipocyte differentiation (Lee et al., 2011). Consistently, our data suggest that both plants from Polygonaceae family modulate adipogenesis.

Treatment with both *P. aviculare* and *P. hydropiper* extracts showed significant dose-dependent decrease in lipid accumulation in human SGBS adipocytes. Moreover, decrease in basal lipolysis as reduction of released glycerol in the culture media was observed. Analysis of relative mRNA expression revealed significant downregulation of *CEBPA*.

Decrease in protein abundance of PI3K was observed upon all treatments, while PPARγ protein expression was decreased by both extracts in the highest concentration.

Conclusion

Polygonum hydropiper and *P. aviculare* modulate adipogenesis and could be employed as natural sources of bioactive molecules or in combination with plant-derived compounds with anti-obesogenic activity.

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