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Rhodiola rosea AND FERULIC ACID ACTIVATE EXPRESSION OF GENES RELATED TO AUTOPHAGY AND RESISTANCE TO HEAT SHOCK IN MICE OF DIFFERENT AGE

O. I. DEMIANCHUK, M. V. IVANOCHKO, D. V. GOSPODARYOV, M. M. BAYLIAK

Department of Biochemistry and Biotechnology
of Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk, Ukraine

E-mail: oleh.demianchuk@pnu.edu.ua

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Rhodiola rosea is a medicinal plant, whose extracts extend lifespan of many animals. Ferulic acid, one of the active components of *R. rosea*, has recently been shown to prolong lifespan in the nematode *Caenorhabditis elegans* model [1]. Several studies have shown that active components of *R. rosea* activate transcription factor FoxO (forkhead box O) [2, 3]. In turn, FoxO regulates expression of genes encoding heat shock proteins, proteins involved in genome repair, autophagy, and antioxidant defence, etc. Also, many anti-aging preparations were shown to activate autophagy [4].

The aim of our study was testing whether *R. rosea* extract and ferulic acid activate expression of targets of FoxO, regulators of energy metabolism and autophagy in livers of young and old mice, and to what extent the effects of *R. rosea* extract and ferulic acid on the genes studied coincide.

Methods. C57BL/6J mice were reared at 22 ± 2 °C, 50–60% humidity, and 12/12 hour light/dark cycle. All groups were reared on a standard chow (4.8% fats, 21.8% protein, and 3.9% fibre). Experimental groups consumed water, supplemented with either sodium ferulate or *R. rosea* during 12 weeks prior sacrificing. The amounts of ferulate and *R. rosea* were adjusted to provide 4 mg of phenol-containing substances per 100 g weight, for a mouse, for 24 hours. We tested three-month-old ("young") and twelve-month-old males ("old").

The levels of messenger ribonucleic acid (mRNA) were assessed using AriaMx real-time polymerase chain reaction (RT-PCR) instrument (Agilent). Ribonucleic acid was purified using the Monarch Miniprep kit (New England BioLabs (NEB), T2010), complementary deoxyribonucleic acid synthesis was performed using the ProtoScript II kit (NEB, E6560), and quantitative RT-PCR (qRT-PCR) was performed using the Luna Universal kit (NEB, E3003). The expression of genes ATG5 (an autophagy marker), HSPB8 (a small heat shock protein, an FoxO target), UCP2 (uncoupling protein 2, a senescence marker), CDKN2 (cell cycle regulator, a senescence marker), PDK2 and PDK4 (pyruvate dehydrogenase kinases 2 and 4, regulators of oxidative metabolism), and TFEB (transcription factor EB, a transcriptional regulator of autophagy) was evaluated.

Results. Livers of young mice that consumed food supplemented with either sodium ferulate or *R. rosea* extract had 3.2-fold and 3.6-fold higher levels of mRNA of the small heat shock protein HspB8 than control mice, respectively (Fig. 1).

In old mice, the levels of mRNA for this protein were 3.3-fold higher in mice reared on the diet containing *R. rosea* extract as compared with the control (Fig. 2). However, there was no significant difference between control mice and those that consumed ferulate-supplemented food (Fig. 2).

In young mice, ferulate and *R. rosea* extract induced synthesis of mRNA of PDK4 by 4.3 and 6.6 times from the control level, respectively (Fig. 1). The difference in the levels of mRNA for PDK4 between control and experimental groups did not extinguish with age since old mice fed with ferulate and *R. rosea* supplemented food had 5.6- and 6.3-fold higher levels of mRNA for PDK4 as compared with the control (Fig. 2). Ferulate and *R. rosea* extract also affected the levels of mRNA of ATG5 and PDK2 in the livers of old mice. In particular, the levels of mRNA of ATG5 were 2.6- and 2.8-

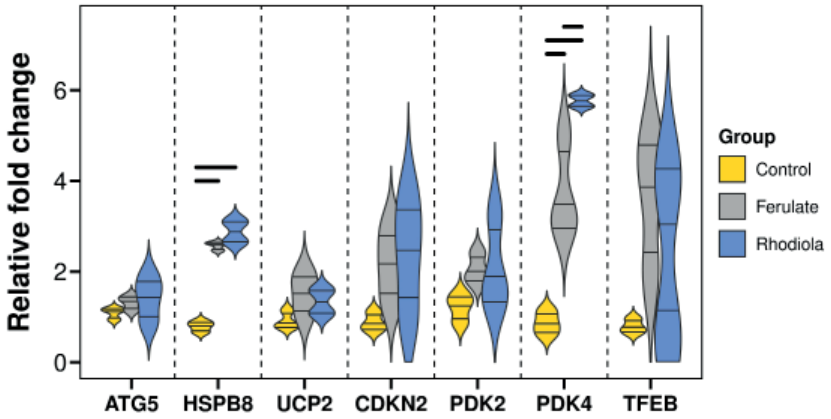

Fig. 1. **Relative levels of mRNA of the genes studied in the livers of three-month-old mice consuming food with or without sodium ferulate or *R. rosea* extract:**
horizontal bars denote statistically significant differences (Duncan's test, $P < 0.05$, $n = 3$)

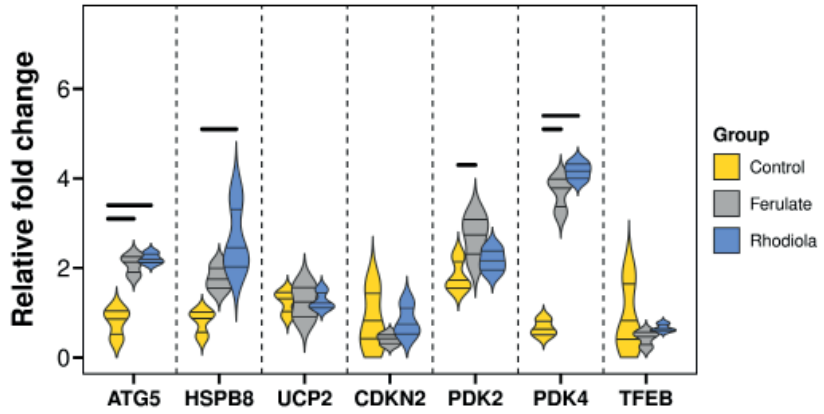

Fig. 2. **Relative levels of mRNA of the genes studied in the livers of twelve-month-old mice consuming food with or without sodium ferulate or *R. rosea* extract:**
horizontal bars denote statistically significant differences (Duncan's test, $P < 0.05$, $n = 3$)

fold higher in old mice consumed ferulate- and *R. rosea* supplemented food, respectively, as compared with the control. The levels of PDK2 were 1.5-fold higher in the livers of mice that consumed ferulate-supplemented food than in control mice.

Discussion. *R. rosea* preparations were shown to increase expression of small heat shock proteins [3]. This is confirmed by our current data. In addition, we have shown that *R. rosea* and ferulic acid may suppress generation of adenosine triphosphate (ATP) in the oxidative phosphorylation by increasing expression of PDK2 and PDK4. In turn, PDK2 and PDK4 inhibit pyruvate dehydrogenase complex (PDC). The inhibition of PDC blocks entry of pyruvate into the tricarboxylic acid cycle (TCA). Consequently, it suppresses production of reducing equivalents required for the operation of oxidative phosphorylation. Decrease in production of ATP leads to concomitant increase in the levels of adenosine monophosphate, a well-known inducer of autophagy [4]. Activation of autophagy markers, such as ATG5, we observed in the livers of old mice reared on the food with ferulate and *R. rosea*.

Our data identify a set of molecular targets that may account for the adaptogenic and anti-aging properties of ferulic acid and *R. rosea*. In addition, we have shown that *R. rosea* and ferulic acid treatments do not affect such well-known markers of aging as CDKN2, and do not influence autophagy via TFEB.

Conclusions. Both, *R. rosea* extract and one of its active components — ferulic acid — promote increasing in the levels of mRNA for genes HSPB8 and PDK4, coding for small heat shock protein and pyruvate dehydrogenase kinase 4, respectively. In old mice, *R. rosea* promote expression of HSPB8, ATG5, PDK2, and PDK4. Thus, ferulic acid and *R. rosea* exert

similar effects on gene expression by supposed activation of heat shock response and autophagy, and concomitant inhibition of mitochondrial metabolism via boosting expression of PDK2 and PDK4.

Key words: Rhodiola rosea; ferulic acid; mice; PDK4; autophagy; mRNA.

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Authors' contribution. OID composed the diets and assisted in qRT-PCR, MVI carried about the animals and assisted in qRT-PCR, DVG conducted qRT-PCR and wrote the abstract, MMB conceived and designed the study, and provided resources for the work.

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