

# Inhibition of glioblastoma growth by *Rhodiola rosea* L. and its active constituents: An *in vitro* and *in vivo* study

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## Introduction

Glioblastoma is the most common and malignant brain tumor in adults and also frequently occurs in children, with median overall survival of 14.6 months (Dong et al. 2019). Over the past 10+ years, hundreds of clinical trials have ended without any clinical benefit, due to the remarkable cellular heterogeneity and complexity of the disease, as well as some limitations due to technical issues and ethical restrictions (Dirks et al. 2020). Studies in humanized mice models can circumvent some of these limitations. Immunodeficient SCID mice are perfect recipients and reconstituted with human tumor cells developing symptoms specific for human diseases (Bleau et al. 2009).

Regarding this, many investigations have been focused on multi-target botanical agents such as *Rhodiola rosea* L., considering them as the nature's answer and simultaneous impact over multiple functional networks during disease treatment with fewer side effects over the human body (Marchev et al. 2016).

The aim of this study was to evaluate the anti-proliferative and apoptotic activity of 70% methanolic extract of *R. rosea* rhizomes, as well as its major compounds *in vitro*, towards human glioblastoma cell line U87 MG and *in vivo*, in humanized murine glioblastoma model.

## Materials and methods

### Cell culture

The human glioblastoma cell line U87 MG was maintained in Eagle's Minimum Essential Medium supplemented with 10% FBS and 1% streptomycin.

### Cell proliferation/viability assay

Colorimetric MTT assay was used for this test. The cells were seeded at a density of  $1 \times 10^5$  cells/well in 96-well plates and treated after 24 h with different concentrations of *R. rosea* extract and pure compounds. The absorption at 490 nm was measured after 48 h.

### Apoptosis detection

Cells were grown at a density of  $1 \times 10^5$  cells/well in 6-well plates. Following 24 h of incubation, cells were treated with different concentrations of *R. rosea* rhizome extract and pure compounds. After 72 h staining for annexin-V and propidium iodide was carried out using the Annexin V-FITC Apoptosis Detection Kit.

### Generation of humanized murine glioblastoma model

Cells at concentration of  $1 \times 10^5$  cells/ $\mu$ L were slowly

deposited in the SCID mice at a rate of 1  $\mu\text{L}/\text{min}$  in the right striatum at a depth of -3 mm from dura with a 10- $\mu\text{L}$  syringe.

## Results and discussion

### *In vitro studies*

*Rhodiola rosea* rhizome extract and its major compounds (the phenylethanoids salidroside and *p*-tyrosol and the phenylpropanoids rosarin, rosavin and rosin) were previously identified in the rhizomes and their respective amounts were 2.67, 0.02, 0.37, 1.92 and 0.04% (Marchev et al. 2020). The extract and the pure molecules were applied at concentrations in the range of 25 to 200  $\mu\text{g}/\text{mL}$ . The level of cell proliferation was evaluated on the last four hours after a three-day culture period. The most prominent activity was observed by the rhizomes extract at 100  $\mu\text{g}/\text{mL}$  with approximately 30% decrease of the cells proliferation. Among the identified metabolites the most active appeared to be rosarin and salidroside at 25  $\mu\text{g}/\text{mL}$  with 20.30% and 16.39% inhibition of the cell proliferation. Rosin, *p*-tyrosol and rosavin had an anti-proliferative activity in the same range, but at concentrations of 100 or 200  $\mu\text{g}/\text{mL}$ . The analysis of the cell apoptosis showed that incubation with the extract and the pure molecules influenced in a non-dose dependent manner this process. Further the cell apoptosis of the extract and the combined formulation of the identified molecules in a ratio corresponding to that in the rhizomes were evaluated on U87 MG glioblastoma cell line. At the doses investigated, the combined formulation of the pure substances decreased the cell proliferation with 17.3% at 300  $\mu\text{g}/\text{mL}$ . In comparison the extract inhibited the cell growth with 22.6% and 36.0% at 300 and 500  $\mu\text{g}/\text{mL}$ , respectively.

### *In vivo studies*

For mice treatment two therapies were developed: an intensive (simultaneous treatment with the extract and salidroside after injection of the glioblastoma cells in the mice) and normal (the treatment starts after tumor formation, which usually occurs after 2-3 weeks) therapy. The dose applied was 500 mg/day for human, calculated for mice with an average body weight of 25 g. The results showed

that the intensive treatment with the extract suppressed the tumor growth at the 10th week. The same treatment increased the life span of the mice with two weeks as well.

## Conclusion

Based on these results it can be concluded that the extract from *R. rosea* L. rhizomes its pure compounds can influence directly the cell proliferation and the cellular metabolism. The research in mice was conducted in immunodeficient animals and there was a direct increase effect on the life span of the treated groups, which reveals the positive effect of the extract, itself, even though no immune cells were involved.

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