Review paper

The importance of immunological studies on *Rhodiola rosea* in the new effective and safe herbal drug discovery

MALGORZATA HARTWICH

Abstract

Rhodiola rosea (roseroot, golden root) is the best known alpine-arctic medicinal plant species of the Family Crassulaceae, growing in Asia, Europe and North America. The rhizomes of this plant contain tonic, phytoadaptogen, antidepressant, anti-inflammatory, antitumor, anticancer, antiviral, anti-AD agents and are recommended to apply in many complementary therapies.

Rhodiola rosea is used in the traditional folk incl. Chinese medicine (known as Hongjingtian) and further studied in the Institute of Traditional Chinese Medicine at the China Academy of Traditional Chinese Medicine (Beijing, China). Many actually "excellent- advertised!?" commercial dietary supplements consisting of R. rosea pulverized rhizomes/extracts/constituents are to order and buy via Internet and also in many drugstories world-wide.

The genetic diversity of the roseroot (great number of clones) demonstrated directly in the unstable quantitative production of the biologically active compounds, and potentially therefore also with dose-dependent differences in the immunological responses between aqueous and dried hydroalcoholic extracts of R. rosea or in the comparison of R. rosea with other Rhodiola sp. rhizomes have been shown and discussed in this review.

The studies suggest to inform and warn the European Medicines Agency and the all self-medicated patients, especially with serious illnesses e.g. cancer, about the risks and danger for the health, and life by the use of any R. rosea products.

The standardized, effective, and safe, pesticides-/contaminates-/microorganisms-free EU-/EMA-controlled R. rosea drug/drugs should be elaborated and sale only in drugstories.

In my opinion, the sale of the R. rosea/potential all "pseudo-herbal" dietary supplements in Internet should be officially prohibited by EMA as soon as possible.

Key words: *Rhodiola sp., Rhodiola rosea, immunological studies, biological studies, Directive of EMA, European Medicines Agency, dietary supplements, Traditional Chinese Medicine, warning, self-medication, patients.*

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Rhodiola rosea L. (*Sedum roseum* Scop; roseroot, golden root) from the family *Crassulaceae* is the arctic-alpine plant species wild-grown especially in Russia, China, Norway, Sweden, Finland, Island, Switzerland, the North America and also cultivated. The characteristic of the plant and the history of the antitumor studies on *Rhodiola* rosea extracts/constituents were described by Skopinska-Rozewska *et al.* [1] and most of them are registered in Pubmed.

It is important to note, that the first antitumor and anti-metastatic effects of an official extract of *Rhodiola rosea* rhizomes were established by Dement´eva and Iaremenko in 80s years in experiments on inbred and non-inbred mice and rats with transplantable NK/Ly tumor, Ehrlich's adenocarcinoma, melanoma B16 and Lewis lung carcinoma. Application of the said preparation to sarcolysin-treated animals was followed by an increase in survival [2].

Correspondence: Malgorzata Hartwich PhD, Am Vollmersbusch 23, D-58706 Menden, Germany; e-mail: hartwich.m@t-online.de

The first immunological studies on *Rhodiola rosea* showing its antioxidative, immunomodulatory and angiogenic (using the wound-induced-angiogenesis model) activities were done by the polish research group of Prof. Ewa Skopinska-Rozewska and Prof. Miroslawa Furmanowa and documented by Furmanowa *et al*. [3-5] and Hartwich [6].

The influence of the plant preparation AdMax (Nulab Inc., Clearwater, FL, USA) on immunity in ovarian cancer patients was studied by Kormosh *et al.* [7]. The preparation was a combination of dried ethanol/water extracts from roots of *Leuzea carthamoides*, *Rhodiola rosea*, *Eleutherococcus senticosus* and fruits of *Schizandra chinensis*. Twenty eight patients with stage III-IV epithelial ovarian cancer were treated once with cisplatin and cyclophosphamide. Peripheral blood was collected 4 weeks after the chemotherapy. Subclasses of T, B and NK lymphocytes were tested for in the blood samples: CD3, CD4, CD5, CD7, CD8, CD11B, CD16, CD20, CD25, CD38, CD45RA, CD50, CD71 and CD95. Immunoglobulin G, A and M concentrations were also determined. Changes were observed in the following T cell subclasses: CD3, CD4, CD5 and CD8. In patients who took AdMax (270 mg a day) for 4 weeks following the chemotherapy, the mean numbers of the four T cell subclasses were increased in comparison with the mean numbers of the T cell subclasses in patients who did not take AdMax. In patients who took AdMax, the mean amounts of IgG and IgM were also increased. It was suggested that the combination of extracts from adaptogenic plants may boost the suppressed immunity in ovarian cancer patients who are subjected to chemotherapy.

In vitro and *in vivo* effects of aqueous and 50% hydroalcoholic extracts of *Rhodiola rosea* on non-specific and specific cellular immunity in pigs, rats and mice were shown by Siwicki *et al.* [8]. Mice were fed 50, 100, 200, or 400 μg of *Rhodiola* extracts daily, for 7 days before cellular immunity study (local GVH reaction) Blood leukocytes collected from pigs and rats were cultivated *in vitro* with PHA or LPS in the presence of 1-50 μg/ml of *Rhodiola rosea* extracts for 72 hours The metabolic activity of blood phagocytes (mostly granulocytes) was determined based on the measurement of intracellular respiratory burst after stimulation by PMA (phorbol myristate acetate), and potential bactericidal activity was determined in isolated blood leukocytes stimulated with microorganisms. Additionally, some *in vitro* toxicological studies were performed. For these experiments GMK (monkey's kidney), EPC (fish epithelial cells), and KFC (Koy fins cells) cell lines, as well as lymphocytes and monocytes isolated from the blood of pigs and rats were used. Both extracts enhanced non-specific and specific cellular immunity to the various degrees; however, in higher doses or concentrations they presented inhibitory effects. In *in vitro* studies all extracts were non-toxic at concentrations 50, 100, 200, 400, 800, and 1000 μg/ml after 24, 48, and 72 hours of cells cultures.

Skopińska-Różewska *et al.* [1] have continued the studies. The aim of this next work was to study the effect of rosavin and aqueous and 50% hydroalcoholic extracts of *R. rosea* roots and rhizomes on neovascular reaction induced in the skin of Balb/c mice after grafting of L-1 sarcoma cells. Mice were treated *per os* with 50, 100, 200 and 400 μg of extracts, or were fed rosavin in daily doses 2, 4 and 8 μg. After 72 hours mice were sacrificed with lethal dose of Morbital. All newly formed blood vessels were identified and counted in dissection microscope. Both extracts in 100-400 μg daily doses and rosavin in the highest dose highly significantly decreased neovascular reaction.

In the further work [9] the effect of aqueous and 50% hydroalcoholic extracts of *Rhodiola rosea*, *Rhodiola kirilowii* and *Rhodiola quadrifida* roots and rhizomes on neovascular reaction induced in the skin of Balb/c mice after grafting of human kidney cancer tissue homogenate or, for comparison, L-1 sarcoma syngeneic cells was presented. Mice were fed 0.4 mg of extracts daily, for 3 days. After 72 hours mice were sacrificed with lethal dose of Morbital. All newly formed blood vessels were identified and counted in dissection microscope. Extracts of underground parts of *Rhodiola rosea* and *Rhodiola kirilowii* significantly decreased cutaneous neovascular reaction induced by human cancer homogenate, extracts prepared from *Rhodiola quadrifida* were ineffective in this model of angiogenesis. In syngeneic tumor cells model all extracts were effective, except aqueous extract of *R. kirilowii.*

The effect of feeding mice *R. rosea* extracts on *Pseudomonas aeruginosa* infection was studied by Bany *et al.* [10]. It was found that the infection intensity was highly significantly lower after treatment of mice for 7 days with daily dose 0.4 mg of aqueous extract than in the control group. The weaker effect of hydro-alcoholic extract was also statistically significant.

The last up to date polish immunological studies on *Rhodiola rosea* were documented by Skopińska-Różewska *et al.* [11]. The *in vivo* effects of 50% hydro-alcoholic extracts of *R. rosea* and *R. quadrifida* roots and rhizomes on the *ex vivo* chemokinetic activity of splenic lymphocytes in mice have been shown. Mice were fed for 7 days *Rhodiola* extracts in daily doses 40 or 200 μg. The chemokinetic activity of splenocytes was determined in 24-hour cell cultures in capillary tubes. Both extracts stimulated splenocytes mobility in lower dose, in higher dose only *R. quadrifida* extract was effective.

The studies have resulted in significant differences in immunological activities between aqueous and alcoholic extracts of *Rhodiola rosea* and in the comparison between *Rhodiola rosea* and other *Rhodiola* sp., also dose-dependent.

Blomkvist *et al.* discussed in the "Planta Medica" the perspectives on Roseroot (*Rhodiola rosea*) studies. The *Rhodiola rosea* (roseroot) extract was up to date a commercially successful product, primarily used to reduce

the effect of fatigue on physical and mental performance. In this perspective the Authors presented own investigations of the most recent studies performed on human subjects. With a focus on the statistical methods they found considerable shortcomings in all but one of the studies that claim significant improvement from roseroot extract. Overall, the study designs have not been well explained. Experimental results have been confused and appear to be in some cases incorrect. Some of the conclusions are based on selected results and contradicting data have not been adequately taken into account. They point to other studies of higher quality performed on roseroot, several that found no significant effect and one that did. Blomkvist *et al.* conclude that the currently available evidence for the claimed effects is insufficient and that the effect of *Rhodiola rosea* is in need of further investigation before therapeutic claims can be made [12].

The presence in the extract of *Rhodiola rosea* rhizomes and roots, approximately 140 compounds - monoterpene alcohols and their glycosides, cyanogenic glycosides, aryl glycosides, phenylethanoids, phenylpropanoids and their glycosides, flavonoids, flavonlignans, proanthocyanidins and gallic acid derivatives was reported [13].

The rhizomes of 95 roseroot clones in the Norwegian germplasm collection were analysed and quantified by Elameen et al. for their content of the bioactive compounds rosavin, salidroside, rosin, cinnamyl alcohol and tyrosol using HPLC analysis. All five bioactive compounds were detected in all 95 roseroot clones but in highly variable quantities. The ranges observed for the different compounds were for rosavin 2.90-85.95 mg/g, salidroside 0.03-12.85 mg/g, rosin 0.08-4.75 mg/g, tyrosol 0.04-2.15 mg/g and cinnamyl alcohol 0.02-1.18 mg/g. The frequency distribution of the chemical content of each clone did not reflect a certain geographic region of origin or the gender of the plant. Significant correlations were found for the contents of several of these bioactive compounds in individual roseroot clones. A low, but not significant correlation was found between AFLP markers previously used to study the genetic diversity of the roseroot clones and their production of the chemical compounds. The maximum level of rosavin, rosin and salidroside observed were higher than for any roseroot plant previously reported in literature, and the roseroot clones characterized in this study might therefore prove to be of high pharmacological value [14]. Or can the "special great content" of the biologically active compounds in the extract initiate any health´s problems, when the dose will be too high!?

The immunological experiments of the Skopińska-Różewska´ s research group [8] showed immunomodulatory effects exerted by *Rhodiola rosea* extracts (obtained from rhizomes grown in the Garden of the Medicinal Plants at the Research Institute of Medicinal Plants, Poznan, Poland) *in vitro* and *in vivo*. Both types of extracts stimulated non-specific and specific cellular immunity in lower doses, and were inhibitory in the highest dose applied. However, cytotoxic effects of this highest dose was not observed, what may suggest stimulation of non-specific suppressor cells in cultures containing 50 μg/ml of the extract. In experiments in mice, feeding cell donors 400 μg daily doses of extracts resulted in suppression of splenic lymphocytes proliferative and angiogenic activity, what may suggest the presence of non-specific suppressor cells in cultured or grafted cell suspension. This dose corresponds to 200 mg human dose, unfortunately being recommended by producers of such dietary supplements as FORMA-REGENERIX-ANTYSTRES (Biofarm, Poznan) and LENTAYA (Hermes Arzneimittel GmbH). So, in the opinion of the polish immunologists, use of these dietary supplements in recommended doses may be dangerous for human immune system, leading to the suppression of some specific and, possibly, some non-specific cellular immune responses. However, in daily doses of 25 and 50 mg *R. rosea* extracts may mildly stimulate cellular immunity. Since 1975, the *R. rosea* extract (40% EtOH) was produced in a large-scale and was registered in the Pharmacopoeia of Soviet Union (1989) with the name "*Rhodiola* Extract liquid" as a medicine and a tonic. The Extract is standardized up-to-date on salidroside and rosavin. In Sweden, *R. rosea* was recognized as an Herbal Medicinal Product "Arctic Root" in 1985 and has been described as an antifatigue agent and saled by Swedish Herbal Institute. In Germany, a dietary supplement of *R. rosea* "Lentaya" (Hermes) was earlier (but now no more) in the sale in drugstories and/or via Internet (among many other international *R. rosea* products) and in Poland – a product Antistress-Forma. Many, if not most, cancer patients will try such remedies in the course of their disease, often without the knowledge of their surgeon or oncologist.

Rhodiola rosea rhizomes have a low level of toxicity. However, should be taken early in the day because it can interfere with sleep or cause vivid dreams. *Rhodiola rosea* rhizomes/extracts have an antidepressant effect, it should not be used in individuals with bipolar disorder who are vulnerable to becoming manic when given anti-depressants or stimulants.

The risks of the complementary use of *Rhodiola rosea* preparations with antiestrogens tamoxifen and faslodex in breast cancer *in vitro* studies were also noted.

Rhodiola rosea may be effective and potent growth stimulator of the estrogen receptor-positive cell lines. Consumption of *Rhodiola* could, therefore, pose a significant hazard to the patients with breast cancer who have ER-positive tumors and who are being treated with antiestrogens [15]. This problem should be further, as soon as possible explained.

Conclusion

The immunological [1, 3-11] and/or phytochemical [13, 14] studies on *Rhodiola rosea* shown that the new e.g. anticancer/antidepressive/others supplements/potential traditional medicinal drugs in the future based on the native extract of *Rhodiola rosea rhizomes*/or combination of many herbal extracts, can be not enough effective! Additionally, it exists too great risk and danger for the human/animals health and life during the complementary use of the product/products.

Warning: *Rhodiola rosea* plants/extracts/products can contain also too high residues of pesticides/other chemicals/microorganisms/aflatoxins/. Side-effects after the use of the *R. rosea* products, dependent on daily nutrition/drinks and/or other used drugs, can be observed.

What do You think about this directive now?

"…the European Commission is prepared to consider extending the simplified registration procedure **to products other than herbal substances with a long tradition of safe use.** … The proposed extension would enable certain medicinal products from specific European or non-European medicine systems (such as – in alphabetical order – anthroposophic, Ayurvedic, Chinese, Kampo Korean, Mongolian, Thai, Tibetan Unani, or Vietnamese medicine) as well as traditional products with a longstanding tradition in the European Union (such as honey, royal jelly, propolis, fish oils, minerals, microorganisms and other substances) to be eligible for the simplified registration procedure with a view to placing them on the market as traditional medicinal products" [16].

The sale of *Rhodiola rosea* herbal products used often up to date in Traditional Chinese [17], Tibet o Russian Medicine should be prohibited in the EU-Drugstories and in Internet.

How might be treated responsibly i.e. effectively and safely so complicated illnesses as many cancer forms; viral diseases e.g. AIDS, Hepatitis C, flu; Alzheimer´s disease, depression and others?

My suggestion is to elaborate standardized mono-drugs of the herbal origin e.g. of the *Rhodiola rosea* registered as the EMA-Monograph, and often controlled during the sale-license-time!

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