The *in vivo* effect of *Rhodiola rosea* and *Rhodiola quadrifida* hydro-alcoholic extracts on chemokinetic activity of spleen lymphocytes in mice

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Abstract

Rhodiola rosea (RR) and Rhodiola quadrifida (RQ) roots and rhizomes are traditional natural drugs used in Asia as adaptogens and antidepressants. The aim of this work was to study the in vivo effect of 50% hydro-alcoholic extracts of RR and RQ roots and rhizomes on the ex vivo chemokinetic activity of splenic lymphocytes in mice. 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 (200 μg) only RQ extract was effective.

Key words: *Rhodiola rosea, Rhodiola quadrifida, mice, splenocytes, chemokinesis.*

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Introduction

Rhodiola rosea (RR) and *Rhodiola quadrifida* (RQ) belong to the big family of adaptogenic herbs, comprising medicinal plants from various species – *Panax ginseng*, *Schizandra chinensis*, *Eleutherococcus senticosus* and many others. *Rhodiola rosea* is considered to be one of the most active adaptogenic drugs. Adaptogenic plants are used in traditional medicine to decrease depression, to enhance work performance and resistance to high-altitude illness, and to treat consequences of physical and psychological stress [1-7].

Besides of this stimulatory action on the nervous system, adaptogenic plants also have immunotropic activity [8-16]. Our earlier experimental studies documented immunomodulatory effects of extracts from *Panax ginseng*, *Eleutherococcus senticosus*, *Schizandra chinensis* and three *Rhodiola species* (*R. rosea*, *R. quadrifida* and *R. kirilowii*) on various parameters of immunological response *in vivo* and *in vitro* [17-30]. In the present paper we evaluate the *in vivo* influence of *Rhodiola rosea* and *Rhodiola quadrifida* hydro-alcoholic extracts on chemokinetic activity of mice splenic lymphocytes *in vitro*.

Material and Methods

Rhodiola rosea (*Crassulaceae*) roots and rhizomes were cultivated, collected and identified in the Research Institute of Medicinal Plants (RIMP), Poznań, thanks to prof. Przemysław M. Mrozikiewicz and dr Waldemar Buchwald.

Rhizomes of *R. quadrifida* were collected in Altai mountain in Mongolia, thanks to dr H. Wiedenfeld. The Mongolian plant material was identified; voucher specimen was deposited at the herbarium of the Institute of Botany of Mongolian Academy of Science in Ulaanbatar. Samples for the study were obtained by Prof. Mirosława Furmanowa, head of the scientific project PBZ-KBN-092/PO5/2003. Sample extractions and their chemical analysis were performed by the scientists from RIMP (dr Alina Mścisz, dr Anna

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Krajewska-Patan, dr Sebastian Mielcarek), and from Warsaw Medical University (prof. Mirosława Furmanowa, dr Małgorzata Hartwich, dr Marek Malinowski) as was described before [24, 25, 30]. Briefly: air-dried finely powdered roots and rhizomes were extracted two times with 50% ethanol (hydro-alcoholic extracts, RRA and RQA), at 40-45°C, evaporated to dryness and lyophilized. For the study of chemical composition of extracts HPLC methods have been used [31, 32]. Extracts were dissolved in 10% ethyl alcohol before administration to the animals.

The study was performed on 8-10-weeks old female inbred Balb/c mice, 20-22 g of body mass, delivered from the Polish Academy of Sciences breeding colony.

For all experiments animals were handled according to the Polish law on the protection of animals and NIH (National Institutes of Health) standards. All experiments were accepted by the local Ethical Committee.

Rhodiola extracts were administered to Balb/c mice *per os* in 7 daily doses of 40 or 200 µg (each group consisted of 8 mice). These doses corresponded to 20 or 100 mg given to 70 kg person (applying the coefficient equal 7 for adjusting differences between mouse and human in relation of the surface to body mass). Mice received drugs by Eppendorff pipette, in 40 µl of 10 % ethyl alcohol, for 7 days. Control mice (16 animals) were fed 40 µl of 10% ethyl alcohol. On the day 8th mice were bled in anaesthesia from retro-orbital plexus and sacrificed with Morbital. Splenocytes were isolated from spleens under sterile conditions by straining through stainless sieve and cotton gauze and centrifugation on Lymphoprep in order to remove erythrocytes.

Spleen cells chemokinesis (spontaneous migration) assay *in vitro* was performed according to the Sandberg method [33] in own modification [19, 23]. Briefly, isolated splenocytes were resuspended in Parker culture medium with 5% inactivated FCS, at the final concentration of 30×10^6 cells/ml. Afterwards, siliconized capillary tubes were filled with cell suspension, sealed with plasticine, centrifuged (5 min 450 g) and fixed on the glass plates. Cells levels were marked. After 24 h incubation (37°C, 5% CO_2 humidified atmosphere) the distances of migration were measured in milimeters (mm) at a magnification of 6.5 × and presented as migration units (1 MU= 0.18 mm).

Stimulatory indices were calculated by dividing the results obtained for individual splenocytes cultures derived from *Rhodiola* fed animals by the mean of the results of accompanying control cultures.

Statistical evaluation of the results was done by 2-way analysis of variance (ANOVA) and Bonferroni post-test (GraphPad Prism software package).

Results

Performed analysis of variance revealed, that variation among column means is highly significantly greater than expected by chance (Table 1). Bonferroni Multiple Comparison Test indicated differences between control group and groups of mice fed 0.04 mg daily dose of both extracts. Higher 0.2 mg dose was ineffective in the case of *Rhodiola rosea* (RRA) extract (Table 2 and Fig. 1).

Discussion

The aim of this study was to evaluate, for the first time, the *in vivo* effect of extracts obtained from the roots and rhizomes of *R. rosea* and *R. quadrifida* on chemokinetic activity of mice splenocytes in 24 h tissue cultures *in vitro*. The results obtained show higher in vitro migratory activity of splenocytes collected from mice fed lower dose of *Rhodiola* extracts. It may reflect the higher incidence in these cell suspensions of T lymphocytes, having better migratory properties than B cells.

Earlier experiments performed by us with some other plant adaptogens (extracts from *Eleutherococcus senticosus*, *Centella asiatica*, *Lithospermum canescens*) revealed stimulatory effect of lower doses and no effect or inhibitory influence of higher doses of these substances on mobility of splenocytes of treated mice [17, 19]. In the present study we observed similar effects. Moreover, our earlier studies on various other parameters of cellular specific and nonspecific immune response in mice, rats and pigs, revealed that *Rhodiola* extracts *in vivo* and *in vitro* enhanced these responses in lower doses and were ineffective or suppressed them in higher ones [24, 25, 27]. In experiments in mice, feeding cell donors 400 µg daily doses of *R. rosea* extracts

Table 1. Two-way ANOVA

Source of Variation	**% of total variation**	**p value**		
Interaction	5.90	p<0.0001		
Dose	53.36	p<0.0001		
Drug	8.61	p<0.0001		
Source of Variation	**p value summary**	**Significant?**		
Interaction	***	Yes		
Dose	***	Yes		
Drug	***	Yes		
Source of Variation	**Difference**	**Sum-of-squares**	**Mean square**	**F**
Interaction	2	0.3253	0.1627	15.60
Dose	2	2.943	1.471	141.2
Drug	1	0.4748	0.4748	45.54
Residual	170	1.772	0.01042	

Table 2. Bonferroni posttest

Control vs. 0.04				
Drug	**Difference**	**t**	**p value**	**Summary**
RRA	0.2300	7.748	p<0.001	***
RQA	0.4800	17.71	p<0.001	***
Control vs. 0.2 mg				
Drug	**Difference**	**t**	**p value**	**Summary**
RRA	0.01000	0.3369	p>0.05	ns
RQA	0.1300	4.379	p<0.001	***
0.04 vs. 0.2 mg				
Drug	**Difference**	**t**	**p value**	**Summary**
RRA	-0.2200	5.901	p<0.001	***
RQA	-0.3500	9.925	p<0.001	***

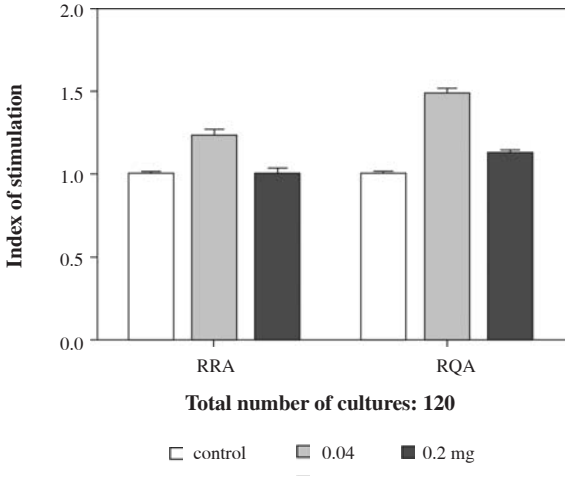

Fig. 1. The effect of feeding mice for 7 days *Rhodiola* hydro-alcoholic extracts on chemokinetic activity of splenic lymphocytes in 24-hours tissue culture (mean stimulation index ± SEM)

resulted in suppression of splenic lymphocytes angiogenic activity, what may suggest the presence of suppressor cells or production of suppressory cytokines. This dose corresponds to the doses recommended by producers of some dietary supplements which contain *Rhodiola rosea* (Anty-stress, Lentaya) [24]. In the case of *R. quadrifida* hydro-alcoholic extract, the best stimulation of lymphocytes angiogenic activity was obtained feeding mice 200 µg daily dose (what corresponds to 100 mg of human dose). Dose of 400 µg was not inhibitory, as in the case of *R. rosea*, but gave lower stimulatory effect [25]. It corresponds to the effects obtained by us for these two *Rhodiolas* in the present work.

Conclusion

In human, daily doses 20 mg, 50 mg (and in the case of *R. quadrifida* also 100 mg) of *Rhodiola* extracts would effectively stimulate lymphocyte-dependent immunity. Use of dietary supplements containing *R. rosea* in daily doses higher than 50 mg, might be dangerous because of the possibility of suppression of some lymphocyte activities. However, anti-bacterial activity of peritoneal macrophages increased in mice fed 400 µg of *Rhodiola rosea* extracts [in press], and metabolic activity of mice blood granulocytes fed *R. quadrifida* presented dose-dependent increase up to the 400 µg daily dose [28].

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