The in vivo effect of Rhodiola kirilowii extracts on blood granulocytes metabolic activity in mice

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Abstract
Rhodiola kirilowii roots and rhizomes are traditionally used in China as a tonic, adaptogen, antimicrobial and anti-inflammatory drug. The plant is also used in the Chinese medicine for the enhancement of the ability of anti-anoxia, moreover it shows anticoagulative properties and decreases the level of blood sugar. R. kirilowii also protect people against cardiopulmonary function problems when moving to high altitude (4500 m).

The aim of this work was to study the in vivo effect of aqueous and 50% hydro-alcoholic extracts of R. kirilowii roots on metabolic activity of blood granulocytes in mice. Hydro-alcoholic extract of R. kirilowii exerted stimulatory effect in the lowest and in the highest dose applied. No difference from the control was observed in the group fed intermediate doses. In the case of aqueous extract intermediate doses presented inhibition of granulocytes chemiluminescence.

Key words: mice, Rhodiola kirilowii, granulocytes, chemiluminescence.

Introduction
Roots and rhizomes of plants belonging to various Rhodiola species are traditional natural drugs used in Asia as adaptogens and antidepressants. Rhodiola kirilowii is a plant used in the Chinese traditional medicine for the enhancement of the ability of anti-anoxia, and for its decreasing blood sugar and anticoagulative properties. Extracts of this plant has anti-inflammatory, bacterio- and fungo-static properties. Their in vitro activity against chronic hepatitis C virus and against Mycobacterium tuberculosis was also reported [1-3].

Information about immunotropic activity of Rhodiola species is scarce. We previously reported for the first time that extracts of R. rosea, R. quadrifida and R. kirilowii influenced some parameters of specific and non-specific cellular immunity and angiogenesis in mice, rats and pigs [4-12].

The aim of this work was to study the in vivo effect of aqueous (RKW) and 50% hydro-alcoholic (RKA) extracts of R. kirilowii roots on the metabolic activity of blood granulocytes in mice.

Material and methods
Plant
Rhodiola kirilowii (Reg.) Reg. (Crassulaceae) roots were collected in September and identified in the Research Institute of Medicinal Plants (RIMP), Libelta 27,
Table 2. Analysis of different extracts of *Rhodiola* samples (values in [%])

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Rosarin</th>
<th>Rosavin</th>
<th>Rosin</th>
<th>Salidroside</th>
<th>Tyrosol</th>
<th>Chlorogenic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. rosea</em></td>
<td>0.23*</td>
<td>0.003*</td>
<td>0.16*</td>
<td>0.46*</td>
<td>0.07*</td>
<td>0.07*</td>
</tr>
<tr>
<td></td>
<td>(0.17)</td>
<td>(0.277)</td>
<td>(0.14)</td>
<td>(0.73)</td>
<td>(0.12)</td>
<td>(0.11)</td>
</tr>
<tr>
<td><em>R. kirilowii</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>R. quadrifida</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.15*</td>
<td>1.00*</td>
<td>0.23*</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>–</td>
<td></td>
<td>(2.39)</td>
<td>(2.04)</td>
<td>(0.30)</td>
</tr>
</tbody>
</table>

* aqueous extract, ( ) hydro-alcoholic extract

Content of tannins and gallic acid in *R. kirilowii* extracts was compared to the content of these substances in extracts obtained previously from the roots of two other *Rhodiola* species – *R. rosea* and *R. quadrifida*. As presented in Table 1, content of tannins in hydro-alcoholic extract of *R. kirilowii* roots was substantially higher than in the aqueous extract. However, the situation with gallic acid was reverse: aqueous extracts contained three times more of this substance than alcoholic ones. It concerned extracts obtained from *R. kirilowii*, *R. rosea* and *R. quadrifida*. As presented in Table 2, other compounds identified in extracts from *R. rosea* and *R. quadrifida* were not found in *R. kirilowii* extracts.

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 Institute of Health) standards. All experiments were accepted by the local Ethical Committee (nr 1/N/WDP-1/19.01.2006).

*Rhodiola kirilowii* extracts were administered to the groups of 8 Balb/c mice each, *per os*, in daily doses of 0.05, 0.1, 0.2 or 0.4 mg. These doses corresponded to 25, 50, 100 or 200 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. Controls mice were fed 40 µl of 10% ethyl alcohol. On the day 8th the mice were bled in anaesthesia from retro-orbital plexus and sacrificed with Morbital.

Chemiluminescence test (CL)

Chemiluminescence test was measured using the method of Easmon and Cole with some modifications [14, 15] at room temperature, in scintillation counter (Rack-Beta 1218, LKB, Sweden). Briefly: samples of 0.05 ml heparinised blood were diluted 1 : 4 with PBS (Biomed Lublin, Poland) supplemented with 0.1% BSA (Sigma-Aldrich, USA) and 0.1% glucose (Polfa, Poland). Next, 0.05 ml of this diluted blood was mixed with 0.2 ml of luminol (Sigma-Aldrich, USA) solution (10-5 M) in PBS and placed in a scintillation counter in the “out of coincidence” mode for background chemiluminescence measurement. Then, the cells were activated by addition of 0.02 ml solution of opsonised zymosan (10 mg/ml) and chemiluminescence activity was measured for the next 15 min. Counting of leukocytes and blood smears examination were performed by routine methods and the results were shown as the maximum value of chemiluminescence (cpm) obtained for 10³ granulocytes.

Statistical analysis

The results were verified statistically by a one-way ANOVA analysis of variance (GraphPad Prism software package), and the significance of differences between the groups was verified with a Tukey-Kramer Multiple Comparisons Test.

Results

One-way ANOVA. Performed analysis of variance revealed, that variation among columns means is significantly greater than expected by chance. The p-value is <0.0001, considered extremely significant.

The results are presented graphically on Figure 1. ANOVA assumes that the data are sampled from populations with identical SDs. This assumption is tested using the method of Bartlett.

Tukey-Kramer Multiple Comparisons test:
RKA Control vs. RKA 0.05 p < 0.05,
"-“ RKA 0.4, p < 0.01,
0.05 vs. 0.1, p < 0.01,
0.1 vs. 0.2, p < 0.05,
0.1 vs. 0.4, p < 0.001.

Hydro-alcoholic extract of *R. kirilowii* exerted stimulatory effect in the lowest and in the highest dose applied. No difference from the control was observed in the group fed intermediate doses.

Water (aqueous) extract (RKW).

The p-value is 0.0006, considered extremely significant. Variation among column means is significantly greater than expected by chance:
RKW 0.05 vs. RKW 0.1, p < 0.05,
"-“ vs. RKW 0.2, p < 0.05,
RKW 0.1 vs. RKW 0.4, p < 0.05,
"-“ 0.2 vs. RKW 0.4, p < 0.01.

Aqueous extract (RKW) inhibited granulocyte metabolic activity in intermediate doses. In the lowest and the highest dose no statistically significant effect was observed.

Discussion

In this paper we present for the first time in vivo modulatory effect of *Rhodiola kirilowii* extracts on mice granulocytes luminol-dependent chemiluminescence, measured in scintillation counter. This test is widely accepted as a method of measuring granulocytes metabolic activity and their oxygen-dependent killing potential.

Previously, we have observed dose-dependent stimulation of chemiluminescent granulocytcs activity in mice fed for seven days extracts from another *Rhodiola* species – *Rhodiola quadrifida* [10]. Unexpectedly, in the present study we observed different pattern of response in the case of *Rhodiola kirilowii* extracts. The lowest and the highest dose was stimulatory (in the case of RKA) or without effect (in the case of RKW). Intermediate doses were inhibitory (in mice fed RKW) or without effect (in mice fed RKA). These differences may be connected with different chemical composition of extracts obtained from various *Rhodiola* species. The main group of chemical substances present in *Rhodiola* extracts are phenolic glycosides (rosavin characteristic for *R. rosea*, mongroside characteristic for *quadrifida* and salidroside characteristic for both species).
The following compounds were isolated by Wiedenfeld et al. [16] from root extracts of RK: rhiophyanoside A, arbutin, epigallocatechin gallate, fructopyryano-(1-4)-glucopyranose and lotaustralin. Lotaustralin, salidroside, daucosterol and tyrosol were already described by other authors.


Immunomodulatory activity of RK extracts may be partly connected with their epigallocatechin-gallate content. The effect of (−)-epigallocatechin 3-gallate (EGCG), a major polyphenol of green tea, on neutrophil migration has been studied by Takano et al. They reported, that EGCG inhibited rat neutrophil chemotaxis toward cytokine-induced neutrophil chemoattractant-1 (CINC-1) in a concentration-dependent manner. Oral administration of EGCG (1.0 mg or 1.5 mg/rat) at 1 h before the challenge with FITC-OVA suppressed neutrophil infiltration into the air pouch (inflammatory site) in the air-pouch type FITC-OVA-induced allergic inflammation in rats. Chemokine levels in the pouch fluids, however, were not influenced by EGCG administration. The results suggest that EGCG suppressed neutrophil infiltration by a direct action on neutrophils [17].

Other authors [18] investigate the ability of the major tea polyphenols: (−)-epigallocatechin gallate (EGCG), theaflavins (TF) and gallic acid (GA) to protect in vitro human neutrophils from oxidative damage induced by phorbol myristate acetate (PMA), and they estimated the level of reactive oxygen species (ROS) production in patients depending on the red tea Pu-Erh drinking. A decrease in ROS generation after red tea consumption was accompanied by the decrease of ROS in response to tested compounds in normal cells. EGCG and TF showed similar potency in antioxidative activities.

The potential health benefits ascribed to green tea and EGCG include antioxidative effects, cancer chemoprevention, improving cardiovascular health, enhancing weight loss, protecting the skin from the damage caused by ionizing radiation, and others were reported [19].

It was shown that (−)-epigallocatechin-3-gallate (EGCG), strongly inhibits neutrophil elastase, micromolar EGCG represses reactive oxygen species activity and inhibits apoptosis of activated neutrophils, and dramatically inhibits chemokine-induced neutrophil chemotaxis in vitro; moreover both oral EGCG and green tea extract block neutrophil-mediated angiogenesis in vivo in an inflammatory angiogenesis model [20].

Inhibitory and stimulatory effects observed by us in the present study may depend on the interplay between immunostimulatory and antioxidative factors present in RK extracts.

References

