Preliminary pharmacological investigations of biotransformed roseroot (Rhodiola rosea L.) callus tissue

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Summary

Callus tissues from Rhodiola rosea L., a plant popular in traditional Russian and Asian medicine, treated with biosynthesis precursors of salidroside (p-tyrosol) and rosavins (cinnamic alcohol), were tested on the animals for the pharmacological activities of the extracts. Authors investigated the behavioural activity using locomotor activity and the motor coordination test. A single administration of extract from the callus tissue non treated with precursors resulted in stimulant effects on rats. The extract enriched in rosavins (after biotransformation) showed the inhibition of activity of tested animals. The more detailed studies concerning the exact nature of the effects especially after chronic administration are needed.

Key words: Rhodiola rosea L., biotransformation, callus cultures, Compact Callus Aggregate (CCA), salidroside, rosavins, cinnamyl alcohol, precursors, pharmacological studies
INTRODUCTION

Roserooot (*Rhodiola rosea* L., Crassulaceae), a plant popular in traditional Russian and Asian medicine, grows in the Arctic and in the mountain regions of Asia, North America and Europe. *R. rosea* is used for enhancing the physical and mental abilities of human body [1].

The roots of *R. rosea* contain biologically active compounds: phenylpropanoids (rosavins) – [2, 3], phenolic compounds (salidroside, tyrosol) [3, 4], flavonoids [5], phenolic acids [1, 6], monoterpenes [1], β-sitosterol, daukosterol [7], tannins [1], fatty acids [8], cyanogenic glucoside – lotaustralin [9] and essential oils [10].

Extracts from *R. rosea* roots are attributed to physiological and pharmacological activities influencing central nervous system (CNS): stimulating CNS activity [15-17], enhancing physical and mental work performance [18-20], eliminating fatigue and adaptogenic activity [21-23]. Only some of these activities have been proved in pharmacological and clinical studies [19, 20]. Two groups of active compounds – phenolic compounds (salidroside, tyrosol and phenylopropanoids (rosavins) are suggested to be responsible for the CNS-stimulating and adaptogenic properties. This kind of psychostimulating activity seems to be the most interesting aspect of *R. rosea* activities.

Biotransformation *in vitro* on *R. rosea* tissues have been carried out in Poland (by Furmanowa et al. [24-27] and by Krajewska-Patan et al. [28-30]) as well as in Finland (by György et al. [13, 14, 31, 32]). Our previous obtained results [29] confirmed that the callus tissue can produce the characteristic active substances even on a solid medium. The concentration of some of them, mainly salidroside, rosin and rosavin, can be significantly improved by addition of the precursors to the medium.

The main goal of this research, as a continuation of previously published investigations on supplementation tissue cultures with p-tyrosol [28, 29] and cinnamyl alcohol [30] was to search the influence of exogenous addition of salidroside precursor – p-tyrosole and cinnamyl alcohol - a rosavin precursor, on the changing of psychostimulating activity of extracts tested in some behavioral tests in rats.

MATERIALS AND METHODS

Animals

The experiments were performed on male and female Swiss mice (body weight 21-37 g) and male Wistar rats (body weight 180-200 g) kept on 12/12 h day/night cycle (light 7.00 - 19:00) under constant ambient conditions (20±2° C, relative humidity - 65%). The rats were fed a standard laboratory diet (pellets-Labofeed B (LSM) - Feeds and Concentrates Production Plant, Poland, ISO 9001:1996) and had tap water freely available in their home cages.
Extracts

The detailed procedure of callus tissue and extracts obtaining was described elsewhere [29]. The studies were performed using bellow listed extracts (50%EtOH) from rose-root (*Rhodiola rosea* L.):

- extract from *R. rosea* root (RIMP, Plewiska near Poznań)
- extract from callus H line + 2.5 mM p-tyrosol (solid medium)
- extract from callus H line – control for p-tyrosol (solid medium)
- extract from callus ZP/S line + 2.5 mM cinnamyl alcohol (solid medium)
- extract from callus ZP/S line – control for cinnamyl alcohol (solid medium)
- extract from callus ZP/S line + 2.5 mM cinnamyl alcohol (bioreactor)
- extract from callus H line + 2.5 mM p-tyrosol (bioreactor)
- control group – distilled water

Group of rats were treated with a single dose of the extracts (500 mg/kg b.w., p.o.) suspended in distilled water. The control group received appropriate volumes of distilled water.

Acute toxicity assessment

Acute toxicity assessment after intragastric administration according to OECD guidelines on chemical substances investigations (“Wytyczne OECD do badań substancji chemicznych”, Dz. Ust. Nr 251 of 31.12.2005, poz. 2119) was performed on mice. The animals were divided into 2 groups (10 females and 10 males in each group) and after administration of the extracts in dose of 2000 mg/kg b.w. during the period of 14 days the following observations were done:

- behavioral changes (e.g. respiration rate, seizures, tremors, locomotor activity changes, changes in skeletal muscle tension in the hind legs)
- body mass change
- outer appearance change (eyes, hair, mucosal membranes)

In the case of significant changes the treated animals were isolated.

Locomotor activity

Locomotor activity was evaluated using a “PAN - licensed activity meter, Poland” by placing animals in the center of the apparatus and recording their activity with electromechanical counters [33]. The obtained data were expressed as signals corresponding with spontaneous movements for 5 minutes. The activity of animals was measured 60 min. after a single dose of the extract or water.
Motor coordination

Motor coordination was evaluated using 'chimney' test described originally for mice [34]. 60 min after an intragastrical administration of the extract or water, rat was allowed to enter a glass laboratory cylinder 50 cm long and 8 cm in diameter laid on its side. Immediately after the animal reached its bottom the position of the cylinder was rapidly changed from horizontal to vertical and the timer was started. The animal immediately begun to move backwards. The timer was stopped after the rat left the cylinder and assumed a sitting posture on the top of the vessel. The time of exit from the cylinder was accepted as a measure of motor coordination [34-36].

Any distracting factors were reduced to the minimum (noise, presence of people, presence of other rats) during all experiments.

Statistical analysis

The data were expressed as mean ± SEM. Statistical comparison of the results was carried out using a nonparametric analysis of variance (Kruskal-Wallis test) followed by the Dunn post-hoc test.

All the experiments were conducted in compliance with relevant Polish standards related to experiments on animals (Dz. Ust. Nr 251 of 31.12.2005, poz. 2119). The study has been approved by Local Ethic Committee of the Use Laboratory Animals in Poznań.

RESULTS AND DISCUSSION

Results of acute toxicity assessment after intragastric administration after of the extracts in dose of 2000 mg/kg b.w. did not show any toxic events or mortality in investigated animals. Therefore, it can be concluded that all investigated extracts are safe and probably belong to the 5th category of Globally Harmonized Classification System for acute oral toxicity [37].

The results of influence of single treatment of the extracts on locomotor activity and motor coordination are shown in table 1. The dose of extracts was chosen according to our previous studies [38-39]. We found that, generally, these substances produced statistically significant differences of locomotor activity (Kruskal-Wallis $H(7.60)=26.0; p<0.01$). However, using a detailed analysis, we observed that a single administration of the extract from callus ZP/S line + 2.5 mM cinnamyl alcohol (solid medium) lowered locomotor activity when compared with control rats ($p<0.05$), whereas the extract from callus H line – control for p-tyrosol (solid medium) showed a tendency to increase this paradigm ($p<0.1$). The values for the other groups did not reach statistically significant differences.
as compared with those obtained for control animals (p>0.05). In the experiment testing the acute extracts effect on motor coordination the overall analysis of the results expressed as time exit from “chimney” showed significant differences among the groups (Kruskal-Wallis [H(7,49)=18,1; p<0.05]). Using detailed analysis, we found that the major part of extracts shorten the exit time of rats (tab. 1). The differences between values for extract from root, extract from callus H line – control for p-tyrosol (solid medium), extract from callus ZP/S line + 2.5 mM cinnamyl alcohol (solid medium), extract from callus ZP/S line + 2.5 mM cinnamyl alcohol (bioreactor), extract from callus H line + 2.5 mM p-tyrosol (bioreactor) when compared with control group were statistically significant (p<0.05). Moreover, the extract from callus ZP/S line - control for cinnamyl alcohol (solid medium) also decreased the time of exit, but the effects did not reach significance (p<0.1). On the contrary, the extract enriched in 2.5 mM p-tyrosol (solid medium) produced opposite effect, since after its oral application the increase of test values was found when compared with control group (p<0.05).

### Table 1.

Acute effect of extracts from roseroot (*Rhodiola rosea* L.) (500 mg/kg, p.o.) on some parameters of their pharmacological profile in rats

<table>
<thead>
<tr>
<th>group</th>
<th>locomotor activity</th>
<th>motor coordination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[number of impulses /5 min.]</td>
<td>exit time(^b) [s]</td>
</tr>
<tr>
<td>control</td>
<td>37.4±4.4</td>
<td>22.8±4.0</td>
</tr>
<tr>
<td>extract from root (Plewska)</td>
<td>30.9±2.6</td>
<td>13.1±1.6*</td>
</tr>
<tr>
<td>extract from callus H line + 2.5 mM p-tyrosol (solid medium)</td>
<td>43.7±1.7</td>
<td>25.2±4.2</td>
</tr>
<tr>
<td>extract from callus H line – control for p-tyrosol (solid medium)</td>
<td>46.0±1.5*</td>
<td>2.0±3.2*</td>
</tr>
<tr>
<td>extract from callus ZP/S line + 2.5 mM cinnamyl alcohol (solid medium)</td>
<td>20.4±1.7*</td>
<td>9.8±0.9*</td>
</tr>
<tr>
<td>extract from callus ZP/S line - control for cinnamyl alcohol (solid medium)</td>
<td>34.3±4.9</td>
<td>17.4±1.5</td>
</tr>
<tr>
<td>extract from callus ZP/S line + 2.5 mM cinnamyl alcohol (bioreactor)</td>
<td>35.0±4.3</td>
<td>13.4±1.8*</td>
</tr>
<tr>
<td>extract from callus H line + 2.5 mM p-tyrosol (bioreactor)</td>
<td>29.4±4.9</td>
<td>12.8±1.9*</td>
</tr>
</tbody>
</table>

n – number of animals =12  
values expressed as means±SEM  
b – exit time from „chimney”  
\* , \*\* – statistically significant difference vs control, p<0.05 and p<0.1, respectively

It seems that our results showed rather positive effects, because we observed generally positive aspects of activity of the extracts in „chimney” tests what is in line with the results of the others [40]. They found that rats treated with *Rhodiola rosea* extract (50 mg/kg per day) significantly prolonged (by 24.6%) their swim time to exhaustion as compared with control rats. It means that the extracts rather produce rather simulative effects on motor coordination in rats. However, the nature
of these events is difficult to explain directly on the basis of our data. It is known that salidroside, one of the main components of *Rhodiola rosea* roots, can produce significant dose-dependent sedative-hypnotic effect in rats [41]. They found that this phenolic compound decreased the sleep latency and prolonged the sleeping time of mice produced by pentobarbital sodium (55 mg/kg, i.p.). Therefore, we expected that after supplementation with tissue cultures with p-tyrosol addition as a precursor of salidroside the weakness of motor coordination or lowering of locomotor activity should be observed. However, we did not find such statistically significant effects.

In general, *Rhodiola rosea* extract has been shown to have a positive influence on the central nervous system, increasing attention span, memory, strength and mobility of the human body, as well as weight management. It is believed that *Rhodiola rosea* can act as a COMT inhibitor where higher brain levels of serotonin and dopamine have been observed [42]. Therefore we also expected that the extracts from *Rhodiola rosea*, especially enriched in rosavines obtained after supplementation with the cinnamyl alcohol as a rosavin precursor, should affect locomotor activity in simulative manner. However, we observed rather inhibition than stimulation in rats treated both with the extract from callus ZP/S line + 2.5 mM cinnamyl alcohol (solid medium) and the extract from callus ZP/S line + 2.5 mM cinnamyl alcohol (bioreactor). We hope that these discrepancies may be explained by the fitochemical analysis.

**CONCLUSION**

Concluding, it seems that there are differences in the pharmacological profile of the extracts, but the more detailed studies concerning exact nature of the effects especially after chronic administration are needed.

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**REFERENCES**

WSTĘPNE BADANIA FARMAKOLOGICZNE BITRANSFORMOWANYCH TKANEK KALUSOWYCH RÓŻEŃCA GÓRSKIEGO (RHODIOLA ROSEA L.)

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S t r e s z c z e n i e

Badano tkanki kalusowe uzyskane in vitro z różeńca górskiego (Rhodiola rosea L.), rośliny tradycyjnie stosowanej w medycynie dalekowschodniej. Materiał roślinny poddawany był procesowi biotransformacji poprzez dodatek do pożywek wzrostowych prekursorów biosyntezy: p-tyrozolu (prekursora salidrozydu) i alkoholu cynamonowego (prekursora rozawin). Badano aktywność behawioralną ekstraktów otrzymanych z kalusa poddanego biotransformacji, stosując testy na szczurach na aktywność motoryczną i koordynację ruchową. Ekstrakt z kalusa nie poddanego biotransformacji po jednorazowym podaniu działał stymulująco w zastosowanych testach, natomiast wyciąg z kalusa o zawartości rozawin zwiększonej po biotransformacji działał hamująco na aktywność badanych zwierząt. Konieczne są dalsze badania wyjaśniające działanie wyciągów z tkanek kalusowych R. rosea, szczególnie po długotrwałym podawaniu.

Słowa kluczowe: Rhodiola rosea L., biotransformacja, kultury kalusowe, hodowle dużych agregatów, salidrozyd, rozawiny, alkohol cynamonowy, prekursory, badania farmakologiczne