Comparative study of Rhodiola preparations on behavioral despair of rats

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Abstract

The antidepressant-like activity of an extract of the roots of \textit{Rhodiola rosea} (RR), its combination with piperine containing extract (RPE), pure substances isolated from \textit{Rhodiola}, such as rhodioloside, rosavin, rosin, rosarin, tyrosol, cinnamic alcohol, cinnamaldehyde and cinnamic acid has been assessed in laboratory animals through application of the Porsolt behavioural despair assay. RR increased the swimming time of rats in a dose dependent manner (ED\textsubscript{50} = 7 mg/kg) and, when administered at 20 mg/kg, exhibited a stronger anti-depressant type effect than either imipramine (at 30 mg/kg) or an extract of \textit{Hypericum perforatum} (at 20 mg/kg). Rhodioloside, and tyrosol were identified as active principles of the extract, whereas rosavin, rosarin, rosin, cinnamic alcohol, cinnamaldehyde, cinnamic acid were inactive. A fixed combination of rhodioloside, rosavin, rosarin and rosin was more active than any of the individual components alone, indicating a synergistic effect of the ingredients in RR extract. Piperine in combination with Rhodiola (RPE) distorts pharmacological effect of Rhodiola most probably due to changes of pharmacokinetic profile of rhodioloside and rosavin. RPE cannot provide predictable therapeutic effect due to herb–herb interaction. Moreover, concomitant treatment of RPE with other drugs should also be excluded due to drug–piperine interaction.

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\textbf{Keywords:} Rhodiola rosea; Rhodioloside; Rosavin; Piperine; Anti-depressant; Drug–herb interaction

Introduction

\textit{Rhodiola rosea} L. is a valuable medicinal plant known mainly as an adaptogen increasing resistance to the harmful effects of various stressors (Boon-Niermeijer et al., 2000; Darbinian et al., 2000; Panossian et al., 2007; Perfumi and Mattioli, 2007; Saratikov and Krasnov, 2004; Spasov et al., 2000; Shevtsov et al., 2003; Sokolov et al., 1985). In the course of our systematic research on chemistry and pharmacology of adaptogens (Boon-Niermeijer et al., 2000; Darbinian et al., 2000; Narimanian et al., 2005; Panossian and Wikman, 2005; Panossian and Wagner, 2005; Panossian et al., 1999a, b, 2007; Spasov et al., 2000; Shevtsov et al., 2003) we decided to evaluate a possible anti-depressant effect of \textit{Rhodiola rosea} extract. Clinical trials carried out in 1986 and 1987 by Brichenko and co-workers in Russia (Brichenko et al., 1986; Brichenko and Skorokhodova, 1987) demonstrated that when \textit{R. rosea} is administered together with tricyclic anti-depressants there is a marked reduction in the side effects of the drugs and an additional positive effect on psychopathological symptoms in patients with psychogenic depression. The results of a recent pilot clinical trial of RR demonstrated the anti-depressive potency of the drug in patients with mild to moderate depression (Darbinian et al., 2007).
One of the objectives of the present study was to identify active principle of RR extract and possible synergistic effect of active constituents, using an appropriate animal system. A subproject was to study the combination of RR and a piperine containing extract. As piperine itself has shown antidepressant effect (Lee et al., 2005; Li et al., 2007) and also is known to favor the uptake of active substances (Atal et al., 1981) the hypothesis was to have an efficient fixed combination of Rhodiola and piperine containing extract. Porsolt et al. (1977a, b, 1978) proposed a test involving the assessment of behavioural despair in laboratory animals as a model for determining anti-depressant activity. Rats and mice that are repeatedly forced to swim in a restricted space succumb to immobility as a characteristic behavioural outcome. This endpoint reflecting a state of despair is significantly changed by agents which have been shown to be therapeutically effective in human depression.

In the present study, we have been able to identify active compounds of Rhodiola which are active in Porsolt’s behavioral despair test and demonstrate their synergistic action in experiments involving laboratory animals in vivo. We found also that the combination of piperine with Rhodiola distorts pharmacological effect of Rhodiola most probably due to changes of the pharmacokinetic profile of active ingredients.

Materials and methods

Details of the project were submitted to and approved by the Ethics Committee of the Armenian Drug and Medical Technology Agency of the Ministry of Health of the Republic of Armenia. The principles of laboratory animal care, as delineated in EEC Directive 75/318 (1994), were followed throughout the study.

Study drugs

Extracts of roots of Rhodiola rosea L. containing 2.7% rhodioloside, 6.0% rosavin and 0.8% tyrosol (DERgenuine 2.5–5.0:1. batches EX20404 and EX20465, HPLC fingerprint is on the Fig. 1) were supplied by the Swedish Herbal Institute (Gothenburg, Sweden). High purity reference standards of rosavin, rosarin, rosin, rhodioloside and tyrosol were kindly provided by G. Zapesochnaya (Vilar, Moscow, Russia). Cinnamic acid, cinnamaldehyde and cinnamic alcohol were from Sigma-Aldrich (St. Louis, MO, USA). The commercial product Jarsin 300® (batch 96021102) was purchased from Lichtwer Pharma (Berlin, Germany) and contained ca. 1% hyperforin, 0.1% hypericin and 0.1% pseudohypericin per dosage form. Apo-Amitriptyline® tablets were purchased from Apotex (Toronto, Canada) and contained 10 mg of amitriptyline per tablet (batch L D40Z0). Imipramine hydrochloride (Gedeon Richter, Budapest, Hungary: batch 63074098 containing 12.5 mg/ml) was diluted with water to a concentration of 6 mg/ml. Black Pepper (Piper nigrum) fruit was purchased from Alfred Galke GmbH (37534 Gittelde/Harz, Germany, total content of piperine analogs – 5%) and extracted with 50% ethanol. Fixed combination (RPE) of Rhodiola rosea (RR) and Piper nigrum extracts (PN) was prepared at Swedish Herbal Institute (the ratio
RR/PN in RPE was 10:1, calculated for herbal substance; piperine content in RPE ~0.1%.

Study animals

Male Wistar rats (170–200 g) were obtained from the Central Animal House of the Institute of Fine Organic Chemistry of the National Academy of Sciences, Yerevan, Armenia. All animals were clinically examined upon arrival and any that showed signs of abnormality or disease were excluded. Animals were kept in the animal house under a 12 h light:12 h dark cycle for 10–15 days prior to the commencement of the study and were offered standard rat chow ad lib. Animals considered unsuitable were replaced prior to the commencement of the study, but no animals were replaced after the study had begun.

During the study period, animals were kept separately in cages (55 × 35 × 25 cm) consisting of polystyrene cases with lattice-framed steel lids: wood-sawdust was used as bedding. The target ranges for the temperature and the relative humidity of the animal house were 22 ± 4 °C and 40 ± 5%, respectively, and the air was changed 1–2 times/h. Throughout the study, a standardised diet for rats (Combi/Yerevan Combi-Corm Plant; Zapadnyuk et al., 1983) was provided, but feeding was discontinued prior to the administration of a test substance. Only tap water was offered ad lib.

Determination of the anti-depressant effect of RR extract and its isolated components and metabolites

The procedure according to Porsolt et al. (1977a, b, 1978) was used with minor modifications as described by Chatterjee et al. (1998). Naive rats were forced individually to swim for 15 min inside a vertical glass cylinder (25 cm diameter, 50 cm high) containing a 20 cm column of water maintained at 25 °C. It has been found that when naive animals are re-exposed 2 days later to the forced swimming assay, their floating behaviour during the test period is reproducible within different sets of rats. Hence test samples (herbal medicines and the pure compounds 1–8; Fig. 2) were administered to test rats three times, once immediately after the initial

![Chemical formulas of compounds 1–8](image_url)

Fig. 2. Chemical formulas of compounds 1–8.
exposure of the animal to the forced swimming assay, and 24 and 1 h prior to re-exposure as described previously. Single doses only of the standard anti-depressants amitriptyline and imipramine hydrochloride were administered 30 min prior to re-exposure of the test animals. Treated rats were resubmitted to the forced swimming assay 48 h after their initial exposure, and the total immobility time (i.e. the time during which swimming ceased completely and the animal floated passively in a slightly hunched, but upright, position with its nose just above the surface of the water) was determined during the subsequent 5 min period. All behavioural studies were performed between 9.00 and 15.00 h and were recorded using a video camera. The tapes were later evaluated by an observer, who had no knowledge of the treatment that each animal had received, and dose–responses were finally evaluated.

Four sets of experiments were conducted with different groups of rats. In sets A and D, appropriate amounts of Jarsin 300\(^{\text{b}}\), RR or RPE extract were suspended in 0.3% carboxymethylcellulose and administered (orally by oral gavage) in a volume equivalent to 5 ml/kg (p.o.) at the dose levels indicated in Table 1. The extracts (or water or 1% aqueous ethanol as negative control) were administered over 3 consecutive days as indicated above, and experiments were performed on the 3rd day, 1 h after the last application. The standard anti-depressants amitriptyline and imipramine were employed as positive controls and administered in single doses of 3 and 30 mg/kg (p.o.), respectively, with pre-treatment time of 30 min.

In sets B and C, isolated pure compounds were dissolved (and diluted, if necessary) in 1% ethanol and administered in a volume equivalent to 5 ml/kg (p.o.) at the dosage levels indicated in Table 1. The test samples (or the equivalent of 5 ml/kg of 1% aqueous ethanol as negative control) were administered over 3 consecutive days as indicated above, and experiments were performed on the 3rd day, 1 h after the last application.

**Pharmacokinetics of rhodioloside and rosavin**

**Chemicals**

HPLC-grade methanol (Rotisolv\(^{\text{b}}\); # 7342.1) and acetone (# 7328.2) were purchased from Carl Roth GmbH (Carlsruhe, Germany); HPCE-grade water (# 5062-85780), 50 mM borate buffer (pH 9.3; # 5062-8573) and 0.1 M sodium hydroxide (# 5062-8575) were from Hewlett-Packard (Palo Alto, CA, USA) and ethyl acetate

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**Table 1.** Effect of RR, pure compounds and potential metabolites derived there from them, Jarsin 300 and standard anti-depressant drugs on behavioural despair in rats as measured by the reduction in immobility time during the Porsolt assay

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of animals treated</th>
<th>Dose (equivalent)(^{a}) (mg/kg)</th>
<th>Depression (immobility time) compared with normalized control group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (water)</td>
<td>8</td>
<td>–</td>
<td>100 ± 18.1</td>
</tr>
<tr>
<td><em>Hypericum perforatum</em> extract Jarsin 300(^{\text{b}})</td>
<td>8</td>
<td>20</td>
<td>33.5 ± 12.0(^{*,b,c})</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>8</td>
<td>3</td>
<td>66.3 ± 18.7</td>
</tr>
<tr>
<td>Imipramine</td>
<td>6</td>
<td>30</td>
<td>42.8 ± 11.9(^{***})</td>
</tr>
<tr>
<td><em>Rhodiola rosea</em> extract(^{d})</td>
<td>8</td>
<td>10</td>
<td>30.4 ± 10.6(^{*})</td>
</tr>
<tr>
<td><em>Rhodiola rosea</em> extract</td>
<td>8</td>
<td>20</td>
<td>26.0 ± 10.9(^{*})</td>
</tr>
<tr>
<td><em>Rhodiola rosea</em> extract</td>
<td>7</td>
<td>50</td>
<td>12.7 ± 3.9(^{**})</td>
</tr>
<tr>
<td>Piperine containing <em>Rhodiola rosea</em> extract</td>
<td>8</td>
<td>5</td>
<td>23.4 ± 3.9(^{**})</td>
</tr>
<tr>
<td>Piperine containing <em>Rhodiola rosea</em> extract</td>
<td>8</td>
<td>10</td>
<td>14.9 ± 1.8(^{**})</td>
</tr>
<tr>
<td>Piperine containing <em>Rhodiola rosea</em> extract</td>
<td>8</td>
<td>20</td>
<td>20.1 ± 2.2(^{**})</td>
</tr>
<tr>
<td>Piperine containing <em>Rhodiola rosea</em> extract</td>
<td>7</td>
<td>50</td>
<td>21.9 ± 2.5(^{**})</td>
</tr>
<tr>
<td><em>Rhodioloside</em> (1)</td>
<td>8</td>
<td>0.26 [15]</td>
<td>23.18 ± 12.9(^{**})</td>
</tr>
<tr>
<td>Rosavin (3)</td>
<td>8</td>
<td>0.26 [10]</td>
<td>43.4 ± 6.8</td>
</tr>
<tr>
<td>Rosarin (4)</td>
<td>8</td>
<td>0.26 [20]</td>
<td>62.7 ± 4.3</td>
</tr>
<tr>
<td>Rosin (5)</td>
<td>7</td>
<td>0.26 [32]</td>
<td>69.3 ± 17.5</td>
</tr>
<tr>
<td>Mixture of 1–5 (each at a dose of 0.26 mg/kg)</td>
<td>7</td>
<td>1.04 [10–15]</td>
<td>18.9 ± 4.7(^{**})</td>
</tr>
<tr>
<td>Tyrosol (2)</td>
<td>8</td>
<td>0.25 [38]</td>
<td>45.66 ± 6.75(^{*})</td>
</tr>
<tr>
<td>Cinnamic alcohol (6)</td>
<td>8</td>
<td>0.12 [38]</td>
<td>92.6 ± 11.8</td>
</tr>
<tr>
<td>Cinnamic aldehyde (7)</td>
<td>8</td>
<td>0.12 [38]</td>
<td>−143.34 ± 4.54</td>
</tr>
<tr>
<td>Cinnamic acid (8)</td>
<td>8</td>
<td>0.12 [35]</td>
<td>82.9 ± 10.2</td>
</tr>
</tbody>
</table>

\(^{a}\)The equivalent dose shown in brackets where appropriate corresponds to RR (batch EX20404).

\(^{b}\)Values are means ± SEM.

\(^{c}\)Significance of differences between means (versus the respective control: ANOVA, Dunnett’s multiple comparison test indicated by: \(*\ p<0.05, **\ p<0.01, ***\ p<0.001.\)

\(^{d}\)RR EX20404 (spissum).
Dosage and administration regimes

Animals were fasted for 16 and 2.5 h before breakfast (at 9.30 a.m.). RR and RPE were dissolved in sterile water (5 mg/ml) and administered at the dose of 50 mg/kg which is equivalent (in terms of rhodioloside) to the human mean daily dose of two tablets of Rosenroot. Two sets of experiments aimed at determining the pharmacokinetics of rhodioloside and rosavin in the blood of animals were performed.

In the first set of pharmacokinetic experiments, RR or RPE were administrated intravenously via the tail vein at a dose of 50 mg/10 ml/kg to 48 animals in each group 1 (mean weight – 124± 10 g; range – 120–140 g). Blood samples (4–6 ml each) were collected in heparinised centrifuge tubes immediately before administration of drug and at various time between 0.06 and 3 h after injection (Table 1). Following collection, blood samples were centrifuged at 300g for 15 min to obtain blood plasma which was stored at −20°C until required for analysis.

In the second set of pharmacokinetic experiments, RR or RPE were administrated orally by gavage at a dose of 50 mg/10 ml/kg to 48 animals in each group 1 (mean weight – 126±10 g; range – 120–140 g). Blood samples (4–6 ml each) were collected in heparinised centrifuge tubes immediately before administration of drug and at 0.5, 1.0, 1.5, 2, 3, 4, 6, and 8 h after drug administration. Following collection, blood samples were centrifuged at 300g for 15 min to obtain blood plasma which was stored at −20°C until required for analysis. Each animal received test sample solution in a volume proportional to the weight of an animal.

HPCE analyses

In order to analyse rhodioloside and rosavin in blood, 1.5 ml of acetone was added to 1.5 ml of the plasma sample, the mixture was vortex-mixed, a further 5 ml of acetone added, the whole vortex-mixed again and the proteins precipitated at 4°C by centrifugation at 2000g for 15 min. The supernatant was removed, evaporated to dryness at 40°C using a vacuum rotary evaporator and the residue dissolved in 100 µl of methanol, transferred into HPCE vials and subjected to analysis.

Analyses were carried out using a Hewlett Packard HPCE system comprising a model HP3DCE apparatus interfaced to a HP Kayak XA workstation and a HP Laser Jet 4000 printer, and equipped with a HP fused silica capillary (# G 1600-61232; total length 56 cm; effective length 50 cm; i.d. 50 µm; optical path length 150 µm) maintained at 10°C. The column was preconditioned by flushing for 1.0 min with 0.1 M sodium hydroxide, rinsing with water, and then flushing with mobile phase (50 mM borate buffer (pH 9.3):methanol in the ratio 85:15 (v/v) for rhodioloside and rosavin, and 60:40 (v/v) for tyrosol) for 2 min. Injection was at 50 mbar for 4 s, the running voltage was 25 kV, detection was at 201 nm (reference 300 nm), 251 nm (reference 450 nm) and 275 nm (reference 400 nm), and the run time was 15 min. The capillary was washed for 5 min after every fifth run and the mobile phase replaced. The mean concentrations of rhodioloside and rosavin in blood plasma (C_MEAN PLASMA) were calculated from the mean values measured by HPCE (C_MEAN HPCE) using the equation C_MEAN PLASMA = C_MEAN HPCE/(K1 × K2), where K1 is the concentration factor of the sample during pre-treatment ( = 15) and K2 is the appropriate coefficient of recovery (0.904 and 0.938 for rhodioloside and rosavin, respectively).

A capillary electropherogram of rhodioloside and rosavin shows migration times 11.86±0.06 and 12.39±0.06 min, respectively. Calibration curves for all analytes were linear in the range 2.50–125.00 mg/l with correlation coefficients of 0.9962 and 0.9984, respectively, for rhodioloside and rosavin. The limit of detection for all analytes was 0.05 mg/l (at a signal/noise ratio of 3) and the limit of quantification was ca. 0.25 mg/l. The accuracy was 98.59% for rhodioloside, and 97.20% for rosavin. The recoveries were 90.38 and 93.82%, respectively, for rhodioloside and rosavin from blood samples. The repeatability for all analytes was ca. 94%, and the relative standard deviation (RSD) value was established at <5% for all validation parameters.

Pharmacokinetic parameters

The pharmacokinetic parameters (Ritschel, 1999) were calculated using TOPFIT software (version 1.1; Godecke, Freiburg, Germany; Schering, Berlin, Germany; Thomae, Biberach-an-den-Riss, Germany). The measured parameters were: (i) C_MAX (ng/ml) – the maximum concentration taken directly from the concentration course; (ii) C0 (ng/ml) – initial drug concentration in blood plasma after the first dose; (iii) t1/2 (h) – the elimination half-life (0.693/k1); (iv) t1/2a (h) – the absorption half-life (0.693/k2); (v) AUC0→∞ (µg/ml) – area under the curve after extrapolation from time x to infinity, where x is the last time point with a concentration above the lower limit of quantification (= AUC0→t + Cx/K1).

Statistical analyses

Data were expressed as mean values ± standard error, standard deviation and coefficient of variation. Statistical analysis of the duration of the immobilisation time
of rats in the Porsolt forced swimming test was carried out using one-way repeated ANOVA with Dunnett’s multiple comparison post test with significance at the 95% confidence interval. Data management and calculations of mean values were performed using GraphPad PRISM statistical software (version 2.01, 1996).

Results and discussion

The anti-depressant-like activities of R. rosea root’s extract, of an extract of H. perforatum (Jarsin 300), and of the standard anti-depressants amitriptyline and imipramine were assessed in laboratory rats through application of the behavioural despair assay of Porsolt et al. (1977a, b, 1978). Table 1 shows that Radix rhodiola extract RR increased the swimming time of rats in a dose dependent manner, ED50 = 7 mg/kg. Moreover, the anti-depressant effects (as revealed by this model system) of RR administered in doses of 10, 20 and 50 mg/kg were greater than the effects of the standard anti-depressant drug imipramine and of the extract from H. perforatum.

The pure compounds rhodioloside (1), tyrosol (2), rosavin (3), rosarin (4) and rosin (5), isolated from the roots of R. rosea, were also submitted to the behaviour despair assay (Table 1). The highest anti-depressant effects were exhibited by tyrosol (2) and rhodioloside (1). Rosavin, rosarin (4) and rosin (5) were inactive in this test, as were the possible metabolites of the phenylpropanoid glycosides 3–5, namely, cinnamic alcohol (6), cinnamaldehyde (7) and cinnamic acid (8). Cinnamaldehyde, indeed, showed a significant depressive effect in the Porsolt assay.

Tyrosol (2), an in vivo metabolite of 1, was the most active ingredient of RR reducing the immobility time significantly (by 52.98% compared with the control) at a dose of 0.006 mg/kg. Rhodioloside (1) showed the strongest anti-depressant effect with a reduction of 76.94% in immobility time (compared with the control) at a dose of 0.26 mg/kg. In studies involving humans, rhodioloside has been shown to exhibit an anti-fatigue effect and also to improve mental ability (Aksyonova, 1966; Panossian and Wagner, 2005). Recently we demonstrated that rhodioloside inhibits stress induced formation key mediators of stress response: cortisone, nitric oxide and phosphorylated JNK in blood of animals (Panossian et al., 2007). The SAPK/JNK pathway is known to be involved in the pathogenesis of glucocorticoid resistance (GR) found in subgroups of patients with major depression, and activation of SAPK/JNK has been reported to inhibit GR function (Wang et al., 2005). Moreover, physiologic activation of SAPK/JNK appears necessary for the induction of long term depression, and over-activation of these kinases by cytokines at pathophysiological concentrations is detrimental to long term potentiation. It has thus been suggested that SAPK/JNK pathways may represent a therapeutic target for the normalization of GR function in depression (Wang et al 2005). It can be hypothesized that the antidepressant effects of R. rosea and rhodioloside is associated with the inhibition of stress, induced by the over-activation of p-SAPK/p-JNK.

However, a strongest effect was observed with fixed combination of compounds 1–5, each at a dose of 0.26 mg/kg, reduced the immobility time compared with the control by 81.08%, strongly indicating a synergistic effect of the active ingredients in RR extract.

When combination of Rhodiola and piperine containing extract (RPE) was tested an abnormal dose dependant effect was observed – the activity of the preparation is decreasing when the dose is increasing in the range of doses 10–50 mg/kg RPE, Table 1, Fig. 3.

Combination of piperine with Rhodiola distorts pharmacological effect of Rhodiola most probably due to changes of pharmacokinetic profile of rhodioloside and rosavine (Table 2).

After the administration of RPE the absorption of Rosavin into the blood is much higher than it is after

![Fig. 3. Effect of RR and RPE on immobilization time of rats forced to swim. **Significant difference vs. control, p<0.01, ***p<0.001.](image-url)
administration of RR. The maximal concentration of Rosavin is increasing in 1.8 times at the 1.4 times dose increase. The pharmacokinetic of rhodioloside is completely differently changed, in particular a longer elimination time. It is also absorbed faster, but longer eliminated from the blood. However in both cases apparent volume of distribution is decreased, because of decreased bioavailability ($F$). It is known that piperine impairs cytochrome P4501A1 activity by direct interaction with the enzyme (Reen et al., 1996). It has a biphasic effect upon cytochrome P450 monoxygenase activity with an initial suppression followed by induction. Treatment of $D$. melanogaster with $P$. nigrum extract led to a greater than 2-fold upregulation of transcription of the cytochrome P450 phase I metabolism genes Cyp 6a8, Cyp 9b2, and Cyp 12d1 (Jensen et al., 2006). It can increase (Lala et al., 2004; Atal et al., 1985) or decrease (Hiwale et al., 2002) bioavailability of co-administered drugs, thereby adversely affecting their therapeutic efficacy. Two other bisalkaloids, dipiperamides D and E, were recently isolated as potent inhibitors of a drug metabolizing enzyme cytochrome P450 (CYP) 3A4 from $P$. nigrum (Tsukamoto et al., 2002). Thus, we conclude that combination of Rhodiola with piperine (RPE) cannot provide predictable therapeutic effect due to herb–herb interaction in the dose level of piperine higher of 0.08 mg/kg (in rat, that is theoretically equivalent to dose of 0.01 mg/kg in human). Moreover, concomitant treatment of this preparation (RPE) with other drugs should also be taken into consideration.

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References


