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Original article

Effects of *Rhodiola rosea* supplementation on mental performance, physical capacity, and oxidative stress biomarkers in healthy men

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Abstract

Purpose: The objective of this study was to investigate the effects of chronic *Rhodiola rosea* (*R. rosea*) supplementation on mental and physical performance, as well as hormonal and oxidative stress biomarkers.
Methods: Twenty-six healthy male students received either *R. rosea* extract (600 mg/day; RR) or placebo (PL) in a randomized double-blind trial. Prior to supplementation (Term I) and following 4 weeks of supplementation (Term II), the students underwent psychomotor tests for simple and choice reaction time, included in the Vienna Test System. Also, the subjects performed VO_{2peak} test. Blood samples were obtained before and after the test to measure the hormonal profile (cortisol, testosterone, and growth hormone), as well as the biomarkers of oxidative stress (lipid hydroperoxides, total antioxidant capacity, and superoxide dismutase) and muscle damage (creatine kinase).
Results: *R. rosea* ingestion shortened reaction time and total response time. Moreover, a greater relative increase in the number of correct responses was observed in RR group as compared to the PL group. No changes in endurance exercise capacity and hormonal profile were observed after *R. rosea* ingestion. *R. rosea* ingestion raised plasma total antioxidant capacity. It did not, however, affect other measured parameters.
Conclusion: Chronic *R. rosea* ingestion does not affect physical performance, but can improve the results of some psychomotor tests (simple and choice reaction time) in young, healthy, and physically active men. The improvements in mental performance, however, at least in our study, seem not to be related to changes in cortisol release or antioxidant activity of *R. rosea* extract. Thus, the specific mechanisms responsible for these effects still need to be elucidated.

Keywords: Cortisol; Endurance capacity; Incremental exercise; Men; Oxidative stress; Simple and choice reaction time; Testosterone

1. Introduction

Rhodiola rosea (*R. rosea*) is the plant with roots containing biologically active substances including flavonoids and phenolic glycosides: salidrosides and rosavins.[1] These substances allow an organism to counteract adverse physical, chemical, or biological stressors by generating non-specific resistance. Due to these compounds *R. rosea* has been purported to possess anti-fatigue and ergogenic properties,[2] which may be reflected in an enhancement of work capacity.[3] Nonetheless, no improvements in exercise performance parameters were observed in humans after chronic *R. rosea* ingestion.[4–6]

Various studies involving young healthy human subjects have shown that chronic *R. rosea* supplementation can diminish mental fatigue as indicated by the improvement in the results of tests involving complex perceptive and cognitive cerebral functions,[7] as well as neuro-motoric function.[8] Conversely, another study[9] reported no changes in mental performance after *R. rosea* intake; however, the dose of *R. rosea* extract was similar to that previously described.[7,8]

It has been demonstrated that beneficial stress-protective activity of *Rhodiola* may be associated with the hypothalamic–pituitary–adrenal axis and regulation of key mediators of stress response including cortisol.[10] However, no studies

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investigated the effects of chronic *R. rosea* supplementation on cortisol release in healthy physically active people.

The mechanism by which *R. rosea* may exert its ergogenic effects is free radical mitigation.[2] It is well known that strenuous exercise increases free radical production in skeletal muscles which may contribute to fatigue by decreasing calcium sensitivity of the myofilaments and depressing force.[11] It has been also suggested that some antioxidants can inhibit oxidative stress and delay muscle fatigue.[11] Antioxidant potential of *R. rosea* has been shown during *in vitro* studies.[12,13] Four major bioactive substances (salidroside, rosin, rosavin, and rosarin) from *R. rosea* have been shown to scavenge the reactive oxygen species (ROS) in dose-dependent manner.[14] Moreover, alternative to free radical scavenging, the activation of enzymatic system by signal transduction pathway and protection against oxidative damage has been recently proposed as a major mechanism of action for plant antioxidants.[15] In a study of Huang et al.,[14] a 4-week treatment with *R. rosea* extract increased protein expression of antioxidant enzymes in rat liver. Moreover, rats treated with *R. rosea* had a significantly attenuated exercise-induced oxidative stress in blood, liver, and skeletal muscle, with concurrent enhanced swimming performance.[14] Unfortunately, these observations were not confirmed in trained athletes.[16] Similarly, 2 studies[6,17] reported an attenuation in exercise-induced increase in plasma creatine kinase (CK) activity as a result of *R. rosea* supplementation, whereas no effect of *R. rosea* on this parameter was observed in other studies.[16,18]

Altogether, above cited studies on *R. rosea*'s effects are ambiguous that may partially result from the dose of *R. rosea* extract. In fact, compared to animal studies, in the majority of human studies on chronic *R. rosea* supplementation the dose of the extract was relatively low, amounting to 100−200 mg,[6–9,16] while higher doses (≥600 mg) were administered for only 4−7 days, and followed by a lower dose.[4,5] Only in 2 studies[17,18] a higher dose for longer time of supplementation (600 mg for 30 days) was used; however, the only biochemical parameters measured were muscle damage and inflammatory markers. Moreover, no studies were performed thus far, analyzing simultaneously the effects of supplementation on mental performance, work capacity, hormonal profile, and oxidative stress biomarkers. Therefore, the aim of the present study was to investigate the effects of chronic *R. rosea* supplementation (600 mg daily for 4 weeks) on select parameters of mental performance, physical capacity, hormonal profile, exercise-induced oxidative stress, and muscle damage biomarkers in healthy physically active male students during the examination period.

2. Methods

2.1. Subjects and supplementation

Twenty-six male physical education students were enrolled in the study. All the students were healthy non-smokers without recent infections or joint or bone injuries; they were not engaged in high-performance sports and did not drink alcohol on a regular basis. The students did not ingest any supplements for at least 2 months prior to the study. Screening for the above mentioned criteria was accomplished via a special questionnaire filled out during subject recruitment.

The study was designed in agreement with the Declaration of Helsinki. All the students volunteered to the study and gave their informed consent. Potential risks and discomforts were explained to each student. The protocol of the study was approved by the Bioethical Committee at the Academy of Physical Education in Warsaw.

The recruited students ($n = 26$) were randomly assigned to 1 of 2 experimental groups. Using the double-blind approach, the treatment group ingested 600 mg of *R. rosea* extract per day (3 tablets per day in 3 divided doses, 1 tablet contained 200 mg of the extract) for a 4-week period (RR group; $n = 13$). At the same time, the control group ingested 3 placebo tablets per day (PL group, $n = 13$). All subjects and investigators (apart from 1 individual not directly involved in the data collection) were blind to treatment group allocation and remained blinded until data analysis. The compliance was measured by tablet counting. The participants who returned no more than 15% of their tablet dose were classified as "compliant".

Commercially available *R. rosea* extract was standardized to 3% rosavins (analyzed by high-performance liquid chromatography; Rhodiola, Naturell, Sweden). Total content of phenolic compounds in water and ethanol solution of *R. rosea* tablets was determined by the Folin−Ciocalteu method as previously described[19] and was expressed in milligram equivalents of gallic acid per tablet. The content of salidroside in *R. rosea* tablet was estimated spectrophotometrically according to the method described previously.[20] Placebo tablets, manufactured by Celon Pharma (Łomianki, Poland), contained maltodextrin, microcrystalline cellulose, magnesium stearate, and caramel (coloring) and appeared identical to the tablets of *R. rosea* extract.

2.2. Psychomotor tests

The study flowchart is displayed in Fig. 1. Prior to the experiment, and after 4-week supplementation with RR or PL, students performed tests assessing their reaction times: simple and choice reaction time, included in the Vienna Test System.[21] Tests were carried out in the morning the day before the incremental exercises were performed, following an overnight fast.

2.2.1. Simple reaction

An examined individual was seated in front of a monitor with the index finger of dominant hand placed on a sensor (so-called "stand-by key") located on the control panel. The student was instructed to maintain finger on the "stand-by key", and move it to the "reaction key" as soon as the stimulus (yellow light) appeared.

The tested parameters are: A1—median of reaction time (interval between the beginning of a given stimulus and the release of the "stand-by key", in ms); A2—median of movement time (interval between the release of the "stand-by key" and pressing the "reaction key", in ms); A3—median of total response time (interval between the beginning of a given stimulus

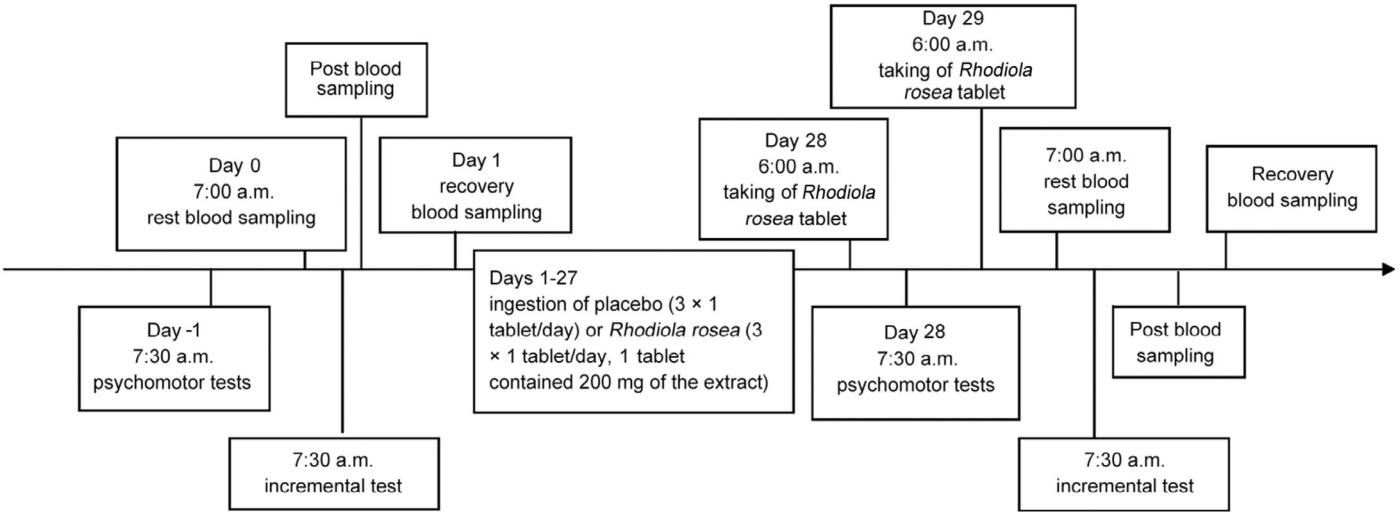

Fig. 1. Study flowchart. Rest: pre-exercise blood sampling; post: 3 min post-exercise blood sampling; recovery: 24-h recovery blood sampling.

and pressing the "reaction key", in ms). Reliability coefficient was $r = 0.90$ and $r = 0.95$ for A1 and A2, respectively.

2.2.2. Choice reaction

An examined individual was instructed to respond appropriately and as soon as possible to the stimuli appearing on the screen using upper and lower limbs. Five colored (white, yellow, blue, green, and red), circle-shaped optical stimuli appeared on the screen. Each circle appearing on the monitor was assigned its own "reaction key" on the control panel that corresponded to the color of the stimulus. The examined individual was asked to respond to the stimulus by pressing the matching "reaction key" with right or left hand. Additionally, the subject was instructed to use the foot to press the right or left pedal whenever a white rectangular light appeared on the black background of the screen. Finally, the test included reaction to acoustic stimuli. The participant was instructed to press either a gray or a black rectangular button as soon as possible after hearing high or low sound, respectively.

The tested parameters are: B1—number of correct responses (n); B2—number of incorrect and missed responses (n); B3—median of response time (s). Reliability coefficient was $r = 0.99$ for all 3 above mentioned variables.

2.3. Exercise protocol

Each student performed 2 incremental cycle ergometer tests to volitional fatigue on 2 separate occasions: before (Term I) and after 4 weeks of supplementation (Term II). The students were asked not to perform any strenuous exercise 3 days before testing. The tests were conducted on cycle ergometer (Ergomedic 839E; Monark, Vansbro, Sweden). The tests were performed in the morning following 12 h overnight fast, at air temperature between 19°C and 21°C and with 40%−60% relative humidity. Each subject was tested at the same time of day at the baseline (Term I) and the endpoint (Term II) to control the influence of circadian and diurnal rhythms. Throughout the test, pedal frequency was fixed at 60 revolutions per minute (rpm). Initial workload was set at 1 W/kg, after which workload was increased by 0.75 W/kg every 3 min until volitional exhaustion, with 1 min rest periods between 3 min exercises. Capillary blood samples were drawn before the test, during each 1 min rest period and 3 min after the test. During the test, oxygen uptake (VO_2) was continuously measured using breath-by-breath ergospirometry system START 2000 (MES, Cracow, Poland), which was calibrated prior to each experiment using gas mixtures of known composition. During the test, heart rate (HR) was continuously registered (Sport-Tester Polar Team System, OY-Electro, Finland). The test was discontinued, and time to exhaustion (TTE) and peak oxygen uptake (VO_{2peak}) were recorded, when any 2 of the maximal criteria occurred as previously described.[22] Additionally, power and HR at the 4 mmol/L lactate threshold (P_{LT} and HR_{LT}, respectively) were evaluated.

2.4. Blood samples

Blood samples were drawn from a fingertip and ulnar vein (into tubes containing heparin), before the exercise test (rest), 3 min after the test (post), and following 24 h recovery period (24 h). Plasma was obtained by centrifugation at 3000 rpm for 10 min at 4°C. Erythrocyte fraction was resuspended and washed 3 times with cold isotonic saline solution. Plasma and washed erythrocytes were frozen and stored at −70°C until analysis.

2.5. Biochemical analyses

Capillary blood was assayed for the concentration of lactate (LA) with a ready diagnostic cuvette kit (Dr. Lange, catalogue No. LKM 140, Dormitz, Germany) and Miniphotometer Plus LP 20 (Hach Lange, Dormitz, Germany) as well as for hemoglobin concentration and hematocrit—with the use of an automated analyzer (OMNI-C analyzer, Roche Diagnostics, Vienna, Austria).

Activity of superoxide dismutase (SOD) was determined for erythrocytes (expressed in U/gHb), whereas blood plasma was analyzed for: activity of CK, concentration of lipid hydroperoxides (LHs) and total antioxidant capacity (TAC), and

concentrations of testosterone (T), cortisol (C), and growth hormone (GH). Activity of CK was assayed with the use of Alpha Diagnostics kit (Alpha Diagnostic Int., San Antonio, TX, USA). Plasma LHs were determined colorimetrically using a commercial kit (OXIS Internatl., Portland, OR, USA), whereas Randox diagnostic kits (Randox, Crumlin, UK) were used to measure TAC and SOD. To determine T, C, and GH levels, we used immunoenzymatic methods based on the ready-to-use sets by ELISA (IBL; International GmbH, Hamburg, Germany) and hGH-EASIA BIOSOURCE (Biosource Diagnostics, Solingen, Germany). All post-exercise samples were corrected for plasma volume shift according to the method of Dill and Costill.[23]

2.6. Statistical analysis

Statistical analysis was performed using R Statistical Software.[24] All data are presented as mean ± SD. Normally-distributed parameters (age, height, body mass, *etc.*) were compared using the unpaired *t* tests. In the case of other not normally distributed variables (based on the results of the Shapiro–Wilk test and visual inspection—quartile distribution plots, confirmed by Bartlett's test), we used non-parametric tests for comparative analysis. The data within each group were analyzed by Wilcoxon test, and Mann–Whitney test was used to compare mean values between 2 groups. The accepted level of significance was defined as $p < 0.05$.

3. Results

The anthropometric characteristics of participants in the 2 groups were similar (age: 20.9 ± 0.2 and 20.5 ± 0.3 years, height: 184.7 ± 2.1 and 182.1 ± 2.2 cm, body mass: 81.1 ± 3.0 and 79.1 ± 2.8 kg; for PL and RR groups, respectively).

The total content of phenolic compounds in ethanol solution of *R. rosea* tablet was 47 mg/tablet, whereas in the case of water solution it was 25 mg/tablet (as determined by the Folin–Ciocalteu method[19]). The content of salidroside in *R. rosea* tablet was found to average 4.7 mg of salidroside per tablet (1.1% of tablet weight on average). Taking into account that 1 tablet contained 200 mg of *R. rosea* extract, salidroside content was 2.35% of dry weight of the extract. The percentage of compliant was 100% for both RR and PL groups.

3.1. Psychomotor tests

The results of simple reaction (A1–3) and choice reaction (B1–3) testing are summarized in Table 1 and in Fig. 2.

Either prior to the experiment (Term I) or after its completion (Term II), no significant differences were observed between PL and RR groups in analyzed parameters of simple and choice reaction (Table 1). However, significant differences within the RR group were noted in the case of 3 parameters (A1, A3, and B1), when relative changes of studied characteristics (i.e., between Terms I and II) were considered (Fig. 2).

In both PL and RR groups, no significant changes in A2 were noted in Term II (Table 1). However, a significant improvement in A1 (shortening by 21.3 ms; 9.5%; $p < 0.05$) was observed in RR group, in contrast to nonsignificant change noted in PL group (Table 1). Relative change in A1 observed in RR group differed significantly ($p < 0.05$) from that detected in PL group (Fig. 2A). Similarly, the relative change in A3 significantly improved (shortening by 5.7%;

Table 1
Psychomotor performance and exercise parameters determined before (Term I) and after (Term II) 4-week supplementation with PL or RR (mean ± SD).

Variable	PL ($n = 13$)		RR ($n = 13$)	
	Term I	Term II	Term I	Term II
Psychomotor performance				
Simple reaction				
Reaction time (A1; ms)	240.4 ± 9.7	253.5 ± 11.4	247.8 ± 12.8	226.5 ± 8.3*
Movement time (A2; ms)	97.3 ± 5.6	99.0 ± 6.3	109.3 ± 4.1	112.5 ± 7.8
Total response time (A3; ms)	337.6 ± 10.9	352.5 ± 15.1	357.1 ± 14.1	339.0 ± 13.3
Choice reaction				
Number of correct responses (B1; *n*)	251.3 ± 9.8	267.9 ± 9.5*	232.5 ± 5.8	269.6 ± 9.5**
Number of incorrect responses (B2; *n*)	43.9 ± 6.9	44.8 ± 6.1	59.2 ± 5.6	50.8 ± 4.0
Response time (B3; s)	0.708 ± 0.015	0.661 ± 0.011**	0.712 ± 0.011	0.666 ± 0.016**
Exercise capacity				
LA_{rest} (mmol/L)	1.45 ± 0.05	1.49 ± 0.09	1.44 ± 0.08	1.23 ± 0.07*,#
LA_{max} (mmol/L)	14.25 ± 1.25	15.47 ± 1.24	14.25 ± 1.13	13.29 ± 0.89
HR_{rest} (beats/min)	70 ± 4	72 ± 2	69 ± 2	68 ± 4
HR_{max} (beats/min)	190 ± 2	190 ± 3	183 ± 3	184 ± 3
HR_{LT} (beats/min)	151 ± 4	147 ± 3	150 ± 4	144 ± 3
P_{max} (W)	303.5 ± 15.9	290.5 ± 15.9*	293.6 ± 13.5	307.5 ± 12.1
P_{LT} (W)	165.6 ± 6.7	170.5 ± 6.5	172.0 ± 6.7	176.2 ± 6.2
VO_{2peak} (mL/kg/min)	48.19 ± 1.97	47.33 ± 1.66	50.76 ± 1.95	49.37 ± 1.90
TTE (s)	789.1 ± 18.2	779.0 ± 22.8	800.0 ± 19.5	820.8 ± 28.0

* $p < 0.05$, **$p < 0.01$, compared to Term I (within group), Wilcoxon test.
\# $p < 0.05$, compared with Term II in PL group, Mann–Whitney test.
Abbreviations: HR_{rest} = heart rate at rest; HR_{max} = maximal heart rate; HR_{LT} = heart rate at lactate threshold; LA_{rest} = blood lactate concentration at rest; LA_{max} = blood lactate concentration after an incremental cycloergometer test; P_{max} = maximal power; P_{LT} = power at lactate threshold; PL = placebo; RR = *Rhodiola rosea*; TTE = time to exhaustion; VO_{2peak} = peak oxygen volume consumption.

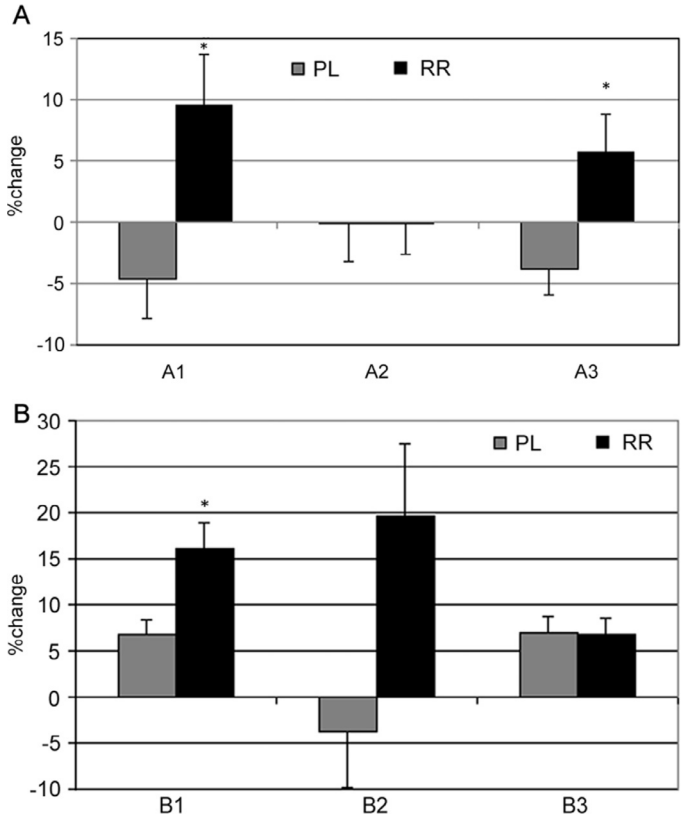

Fig. 2. Relative (%) change (mean ± SD) in the simple (A) and choice (B) reaction parameters evaluated by the Vienna Test System, after 4-week supplementation with placebo (PL) or *Rhodiola rosea* (RR). *$p < 0.05$, compared with PL group, Mann–Whitney test. A1 = reaction time; A2 = movement reaction; A3 = total response time; B1 = number of correct responses; B2 = number of incorrect responses; B3 = median of response time.

$p < 0.05$) in RR group as compared to change observed in PL group (Fig. 2A).

In both groups, significant improvements in B1 and B3 were observed in parameters of choice reaction (Table 1), while no significant change was noted in B2. Noticeably, a relative increase in B1 was significantly higher in RR than in PL group (16% *vs.* 6.6%, respectively, $p < 0.05$; Fig. 2B). No significant differences were observed between PL and RR groups with respect to B2 and B3 (Fig. 2B).

3.2. Exercise parameters

Comparative analysis of the performance parameters between Terms I and II of the study in the PL and RR groups was compiled in Table 1. Significant decrease in peak power (P_{max}) was observed in PL group in Term II as compared to Term I ($p < 0.05$). On the contrary, in RR group, P_{max} did not change significantly throughout the experimental period ($p > 0.05$). Although absolute values of P_{max} did not differ between PL and RR, slight but significant differences in relative changes in P_{max} were observed between PL and RR groups (−4.1% *vs.* 5.7%, respectively; $p < 0.05$). Moreover, 4 weeks of *R. rosea* ingestion caused a significant decrease in resting LA concentration in blood in RR group ($p < 0.05$). In Term II, resting LA concentration was significant lower in RR group, as compared to PL group ($p < 0.05$). Apart from changes in P_{max} and LA_{rest}, the other parameters (VO_{2peak}, TTE, P_{LT}, and LA_{max}, as well as HR values) did not change significantly in either group.

3.3. Redox state, muscle damage, and hormonal profile

Table 2 shows the values of oxidative stress and muscle damage biomarkers. In RR group, resting plasma TAC increased significantly in Term II as compared to Term I (by 22%; $p < 0.05$), whereas no signifiance changes were observed in PL group. In case of other parameters (SOD, LHs, CK), no significant differences between Terms I and II were observed neither in each group, nor between PL and RR groups.

In Term I, in both groups, plasma TAC increased post-exercise ($p < 0.05$) and subsequently decreased slightly after 24-h of recovery ($p < 0.05$). In Term II, plasma TAC did not change significantly in both groups. Incremental exercise caused significant increase in SOD activity in PL, but only in Term I. In RR group, no significant changes in SOD activity were observed in either term. In both terms, plasma LH concentrations increased significantly immediately after exercise ($p < 0.05$), but remained unchanged during recovery in both groups. Plasma CK activity increased post-exercise in both groups, but remained unchanged during recovery in Term I ($p < 0.05$). There were no significant changes in CK activity after the incremental exercise in Term II.

No significant changes in hormonal response (Table 2) between Terms I and II were observed in either PL or RR group. Moreover, all hormonal parameters analyzed were unaffected by *R. rosea* intake when compared to the placebo treatment.

4. Discussion

It has been suggested that *R. rosea* can exert stress resistant properties. In the present study, the last week of the experiment corresponded to the period of the examination session for all participating students. Under such circumstances, a 4-week supplementation with *R. rosea* was reflected in improved results of psychomotor tests. In the simple reaction testing, *R. rosea* ingestion resulted in significantly shorter reaction time, with resultant improvement of total reaction time, although it did not affect movement time. Surprisingly, in the case of choice reaction ability testing, significant improvement in the number of correct responses and response time was documented in both studied groups. This phenomenon can be interpreted as a "learning effect" (i.e., practicing the technique and formulating a strategy for testing procedure). Although the students in our study were given precise instructions about testing procedures prior to the first evaluation (in Term I), a possible limitation of this study is the lack of a planned pretest before the first evaluation. On the other hand, previous study[25] showed no learning effects on reaction time test as measured by the Vienna Test System. Moreover, it must be added, that in the case of the number of correct responses in our study, a significantly greater increase was observed in RR as compared to PL (16.0% *vs.* 6.8%; $p < 0.05$). Moreover, the improvement in number of incorrect responses was seen in RR, but not in

Table 2
Blood parameters of oxidative stress, muscle damage, and hormonal profile determined at rest (before incremental exercise), 3 min post-exercise, and following 24-h recovery, before (Term I) and after (Term II) 4-week supplementation with PL or RR (mean ± SD).

Variable	Time	PL ($n = 13$)		RR ($n = 13$)	
		Term I	Term II	Term I	Term II
TAC (mmol/L)	Rest	1.38 ± 0.06[a]	1.50 ± 0.04[a]	1.34 ± 0.06[a]	1.63 ± 0.06[a,*]
	Post	1.54 ± 0.05[b]	1.62 ± 0.06[a]	1.46 ± 0.02[b]	1.54 ± 0.06[a]
	24 h	1.48 ± 0.04[a]	1.50 ± 0.04[a]	1.40 ± 0.04[a]	1.63 ± 0.11[a]
SOD (U/gHb)	Rest	1143.1 ± 60.9[a]	1000.4 ± 147.1[a]	1132.1 ± 91.1[a]	1007.5 ± 80.2[a]
	Post	1395.4 ± 90.8[b]	1140.9 ± 79.9[a]	1225.0 ± 70.5[a]	1201.6 ± 98.4[a]
	24 h	1231.8 ± 95.8[a,b]	988.9 ± 107.5[a]	1266.0 ± 90.5[a]	1217.9 ± 60.6[a]
LHs (μmol/L)	Rest	2.48 ± 0.26[a]	2.36 ± 0.26[a]	2.42 ± 0.28[a]	2.46 ± 0.25[a]
	Post	3.48 ± 0.38[b]	3.40 ± 0.41[b]	3.43 ± 0.48[b]	3.44 ± 0.34[b]
	24 h	3.19 ± 0.56[a,b]	2.55 ± 0.29[a,b]	3.22 ± 0.62[a,b]	2.48 ± 0.35[a,b]
CK (U/L)	Rest	141.4 ± 17.2[a]	122.6 ± 18.5[a]	118.8 ± 12.1[a]	110.0 ± 9.2[a]
	Post	170.9 ± 18.8[b]	126.1 ± 19.6[a]	141.0 ± 16.1[b]	119.6 ± 11.0[a]
	24 h	161.0 ± 22.1[a,b]	104.1 ± 9.2[a]	123.6 ± 9.3[a,b]	106.3 ± 10.4[a]
C (nmol/L)	Rest	518.1 ± 25.2[a]	507.3 ± 25.2[a]	540.8 ± 19.3[a]	552.7 ± 25.4[a]
	Post	376.1 ± 34.5[b]	390.6 ± 35.6[b]	429.5 ± 43.7[b]	376.4 ± 55.0[b]
	24 h	480.0 ± 33.6[a]	530.0 ± 25.9[a]	484.6 ± 22.6[a,b]	524.2 ± 25.7[a]
T (nmol/L)	Rest	24.7 ± 2.1[a]	26.5 ± 2.5[a]	21.7 ± 2.2[a]	21.6 ± 1.2[a]
	Post	23.4 ± 2.3[a]	27.8 ± 2.5[a]	19.8 ± 1.9[a]	23.9 ± 1.7[a]
	24 h	24.7 ± 2.2[a]	26.3 ± 2.8[a]	22.5 ± 2.1[a]	23.7 ± 1.3[a]
T/C ratio	Rest	4.8 ± 0.5[a]	5.3 ± 0.5[a]	4.1 ± 0.5[a]	3.9 ± 0.2[a]
	Post	6.7 ± 1.1[a]	7.7 ± 1.0[b]	5.6 ± 1.1[a,b]	6.7 ± 0.9[b]
	24 h	5.3 ± 0.4[a]	5.1 ± 0.7[a]	4.7 ± 0.5[b]	4.6 ± 0.3[c]
GH (ng/mL)	Rest	0.2 ± 0.1[a]	0.2 ± 0.1[a]	0.3 ± 0.1[a]	0.2 ± 0.1[a]
	Post	13.7 ± 3.3[b]	12.7 ± 5.3[b]	14.5 ± 3.1[b]	12.4 ± 3.7[b]
	24 h	0.3 ± 0.1[a]	0.2 ± 0.1[a]	0.2 ± 0.1[a]	0.3 ± 0.1[a]

[a,b,c] Values at rest, 3 min post-exercise, and after 24 h recovery (within the same group and at the same term) that do not have common letters are significantly different, Wilcoxon test, $p < 0.05$.
* $p < 0.05$ compared with Term I within group; Wilcoxon test.
Abbreviations: C = cortisol; CK = creatine kinase; GH = growth hormone; LHs = lipid hydroperoxides; PL = placebo; RR = *Rhodiola rosea*; SOD = superoxide dismutase; T = testosterone; TAC = total antioxidant capacity.

PL, however, these differences were not significant, maybe because of higher SD.

Similar to our results, other investigations have produced mixed results. Spasov et al.[8] investigated the effect of *R. rosea* supplementation (100 mg daily for 20 days) on mental performance in medical students. They observed a significant improvement in mental fatigue (by self-assessment) and neuromotor test (accuracy of movement *vs.* speed in maze test) compared with the control groups, but the results of neuromuscular tapping test or the correction of text tests were found to be lacking significance. No effect of acute (200 mg once) and 4-week *R. rosea* intake (200 mg daily) on speed of limb movement, reaction time, and ability to sustain attention was found in the study by De Bock et al.[9] in healthy students. Taking into account inconclusive results of the above cited studies and our results, the answer as to whether *R. rosea* ingestion improves neural or cognitive performance is problematic. Differences in results can be mediated by *R. rosea* dose and test specificity since a wide variety of tests is used in the evaluations. It must be emphasized that contrary to the above cited studies, we used the Vienna Test System, a very reliable, recognized, and validated testing system,[25,26] to evaluate mental performance in our study. Thus, the finding from this investigation is that chronic *R. rosea* ingestion can improve some parameters of mental performance like reaction time and the number of correct responses as evaluated by the Vienna Test System in young, healthy, and physically active men.

So far, several mechanisms have been proposed to be involved in improvement of the cognitive and/or neural performance following *R. rosea* ingestion. Among others, the results of animal studies indicate that the anti-fatigue effect of *Rhodiola* species may be related to changes in cortisol secretion,[27] probably as a result of neuropeptide Y activation by salidroside constituent.[28] However, no changes in salivary cortisol were observed after submaximal exercise as a result of acute *R. rosea* intake in healthy individuals.[29] Similarly, in our study we did not observe any changes in plasma cortisol following chronic *R. rosea* ingestion. It must be emphasized, however, that further study with more post-exercise time points should be conducted to confirm these findings.

Some literature data indicate that *R. rosea* may stimulate the synthesis, transport, and receptor activity of opioid receptors and peptides such as β-endorphins.[2] This mechanism may be responsible for an improvement not only in cognitive performance, but also in endurance exercise capacity, since endogenous opioid system is involved in the modulation of pain tolerance.[30] However, in our study no changes in TTE, VO_{2peak}, HR values, or anaerobic threshold were observed after *R. rosea* supplementation. It cannot be excluded that enhanced metabolization/degradation of

R. rosea, administered chronically at a relatively high dose, might occur in our study; consequently, the time elapsed between ingestion of the last dose of *R. rosea* and the incremental exercise test (1.5−2 h) might be too long to affect physical performance. On the other hand, De Bock et al.[9] observed an increase in TTE and VO_{2peak} after acute (200 mg once) but not chronic *R. rosea* ingestion at low doses (200 mg/day). Thus, *R. rosea* may exert a temporary effect that is no longer observed upon repeated intake,[9] irrespective of the dose. It has been confirmed in a recent study of Noreen et al.,[29] in which acute *R. rosea* ingestion decreased HR response to submaximal exercise and improved endurance performance. Although, in our investigation, *R. rosea* supplementation prevented a decrease in P_{max} and decreased the resting lactate concentrations, these changes were too small to state about anti-fatigue effect of *R. rosea* ingestion. Therefore, taking into account our study and previous studies,[6,18] it is unlikely that chronic *R. rosea* ingestion may enhance physical performance.

It has been proposed that *R. rosea* may have antioxidant properties.[2] In our study, an increase in resting plasma TAC was observed as a result of *R. rosea* supplementation. It can indirectly reflect increased bioavailability of antioxidant compounds from *R. rosea* extract since plasma levels of endogenous antioxidants such as uric acid and albumin, which contribute primarily to plasma TAC, were not affected by *R. rosea* supplementation (data not shown). Although, in present study, the exercise test induced oxidative stress and muscle damage, these parameters were not affected by *R. rosea* ingestion. Our results are in agreement with the study of Skarpanska-Stejnborn et al.,[16] in which much lower dose of *R. rosea* extract was used as compared to our study (200 *vs.* 600 mg/day, respectively). Similarly, no effects of *R. rosea* supplementation (600 mg/day) on exercise-induced muscle damage and inflammatory markers in plasma were observed in runners following a competitive marathon.[18] Finally, it is believed that salidroside and rosavins are responsible for the effects of *R. rosea* ingestion. Recently, several hypothetical mechanisms of *R. rosea* action have been proposed. They include cell response regulation, at the transcriptional level, affecting various signaling pathways associated with beneficial effects of *R. rosea* on different disorders.[31] Interestingly, the biological activity of the *R. rosea* total extract differed from the activity of the purified compounds (i.e., salidroside, triandrin, and tyrosol).[31] Therefore, it cannot be excluded that other compounds contained in the extract (including flavonoids) may also affect brain function.

The potential of flavonoids to promote memory, learning, and cognitive function has been described in a number of studies.[32−34] Aside from antioxidant activity, flavonoids may influence brain function in multiple ways, including interaction with important neuronal signaling cascades controlling long-term potentiation and memory.[35] Taking into account low bioavailability of phenolic antioxidants, their role in direct scavenging free radicals *in vivo* has even been questioned. Instead, paradoxical oxidative activation of Nrf2 (nuclear factor erythroid 2-related factor 2), the transcription factor regulating expression of genes coding phase II and some phase III enzymes (e.g., heme oxygenase-1), so called para-hormesis or xeno-hormesis, has been recently proposed to understanding of the physiological mechanism of action for plant phenols.[15] Other adaptive cellular response pathways in nerve cells may include those involving the transcription factors like nuclear factor-κB (NF-κB), hypoxia-inducible factor 1α (HIF-1α), peroxisome proliferator-activated receptors (PPARs), and forkhead box subgroup O (FOXO), as well as the production and action of trophic factors and hormones.[36] These mechanisms of action may allow flavonoids to improve not only age and neurodegenerative diseases-related decrease in mental performance, but also enhancing "normal" mental performance.[37] Interestingly, flavonoids supplementation in healthy students has been found to improve cognitive function and test scores on university exams.[38] Thus, in our study, despite no influence of *R. rosea* supplementation on oxidative stress parameters, it cannot be excluded that phenolic compounds of *R. rosea* extract, including flavonoids, via influencing brain function, may be responsible for improvements observed in mental performance parameters. Thus, more studies are needed to understand the exact mechanisms of action of *R. rosea* extract in healthy population.

In conclusion, we found that chronic *R. rosea* ingestion can improve some parameters of psychomotor performance in young, healthy, and physically active men. However, these effects seem not to be related to changes in cortisol release or antioxidant activity of *R. rosea* extract. Thus, the exact mechanisms responsible for the effects of chronic *R. rosea* ingestion in healthy persons require further investigations.

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Authors' contributions

EJ conceived of the study, participated in its design and coordination, carried out the biochemical studies and interpretation of data, and drafted the manuscript; JS conceived of the study; BD carried out the physiological studies and helped interpret the data; DG carried out the psychomotor studies and helped draft the manuscript; BO carried out the hormonal measurements and interpretation of data; IC designed and performed the statistical analyses. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

The authors declare that they have no competing interests.

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