Nunavik *Rhodiola rosea* Attenuates Expression of Fear-Potentiated Startle

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Abstract

Rhodiola rosea is a plant with adaptogenic qualities used by Inuit populations of Nunavik, Quebec (Canada) for general mental and physical rejuvenation. Previous studies have demonstrated that the Canadian populations of *R. rosea* significantly attenuate the expression of learned fear and anxiety-like behaviors in rodent models. In order to further characterize the anxiolytic activity of Nunavik *R. rosea,* experiments were conducted to assess the effects of oral administration of the plant extract on both the fear-potentiated startle response and corticosterone levels. Findings suggest that oral administration of *R. rosea* ethanolic extract (75 mg/kg) significantly attenuated fear-potentiated startle, but did not produce any effects on stress-induced secretion of corticosterone.

Key words
Rhodiola rosea · Crassulaceae · anxiolytic · anxiety · PTSD · glucocorticoid · neuropharmacology · behavior

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Rhodiola rosea L. (Crassulaceae) is a flowering perennial native to central Europe and Asia, historically used for the treatment of various mental and physical conditions [1–3]. Research suggests it may have adaptogenic, antidepressant, and/or anxiolytic qualities [4]. The roots of European and Asian specimens may have potential as an intervention for anxiety- and stress-related disorders [5–9].

Recent research has identified isolated populations of *R. rosea* in Nunavik, Quebec (Northern Canada), where it is used for its medicinal properties by local indigenous Inuit populations. Cayer et al. [10] found that phytochemicals present in Nunavik *R. rosea* include those also found in European plant populations (salidroside, tyrosol, rosarin, rosavin, and rosin), and that oral administration of Nunavik *R. rosea* significantly attenuated expression of conditioned fear in animal models.

With this in mind, our research sought to further explore the effects of Nunavik *R. rosea* on the expression and extinction of fear memory using fear-potentiated startle (FPS), an animal model of anxiety-related behaviors (anxiolytic compounds decreases the FPS response in rodents; see Supporting Information for rodent information and FPS apparatus details; [11]). Additionally, some anxiolytics have significant effects on the release of stress-related glucocorticoid hormones [12, 13]. Extracts of European populations of *R. rosea* inhibit stress-induced cortisol secretion [14, 15], and plant adaptogens increase stress resistance and reduce the expression of stress-related biomarkers [16–18]. An exploratory analysis was therefore also conducted to assess the effects of *R. rosea* on stress-induced corticosterone secretion.

For fear expression on Day 4, ANOVA results revealed a significant condition by treatment interaction, $F(2,25) = 12.55$, $p < 0.001$ (see Supporting Information for statistical methodology). Follow-up analyses indicated that within the tone + noise condition, animals that received the plant extract had a significantly lower startle amplitude than those who received vehicle alone on the testing day, $p < 0.05$. Animals who received diazepam also exhibited a significantly lower startle potentiation than the controls in the tone + noise condition, $p < 0.05$.

ANOVA results also revealed a significant main effect of the treatment group for percent potentiation (see **Fig. 1 b**), $F(2,25) = 13.13$, $p < 0.001$. Games-Howell post hoc analyses indicated that the animals who received the plant extract exhibited significantly lower percent potentiation than the controls, $p < 0.01$. Animals who received diazepam also exhibited significantly lower potentiation, $p < 0.05$.

On Day 5, in the absence of *R. rosea* extract administration, there were no significant main effects or interactions in terms of mean startle amplitude (see **Fig. 2 a**). ANOVA also revealed no significant group difference in terms of percent potentiation (see **Fig. 2 b**), suggesting the effects of *R. rosea* were acute and had no prolonged effects on fear memory extinction.

In the second experiment, mixed measures ANOVA revealed no significant main effects or interaction effects (see **Table 1**).

The results support the notion that oral administration of *R. rosea* significantly attenuates the expression of fear-potentiated startle, suggesting it may have anxiolytic properties. Mean startle potentiation was significantly lower on Day 4 for rats who had received the plant extract versus the controls (indeed, the *Rhodiola* effects were slightly more pronounced than those of diazepam). These findings support those presented by Cayer et al. [10], the only other study targeting the anxiolytic properties of Nunavik *R. rosea*.

Although there was a significant reduction in startle potentiation on Day 4, testing in the absence of drug administration revealed some fear reinstatement. On Day 5, there were no significant group differences. Although the plant extract may have anxiolytic properties, its effect is likely limited to mediation of fear expression alone [the plant seemed to have no (or limited) long-term effects on fear memory or extinction]. Nunavik *R. rosea*-related biomarkers are detectable in urine 8 h after administration [19], indicating that active compounds may be excreted via the renal pathway within 8 h of treatment. This, along with our findings, suggests the effects of Nunavik *R. rosea* are likely limited to the period during which they are biologically active, producing no long-term effects on memory following excretion. Diazepam similarly showed no prolonged effects on fear.

Fear memory and expression are thought to be controlled by distinct amygdala microcircuits, with the central nucleus playing a greater role in fear expression [20]. Evidence suggests GABAergic transmission within the basolateral amygdala mediates fear learning [21]. GABAergic signalling also plays a role in the mediation of fear expression within the central nucleus and activity-

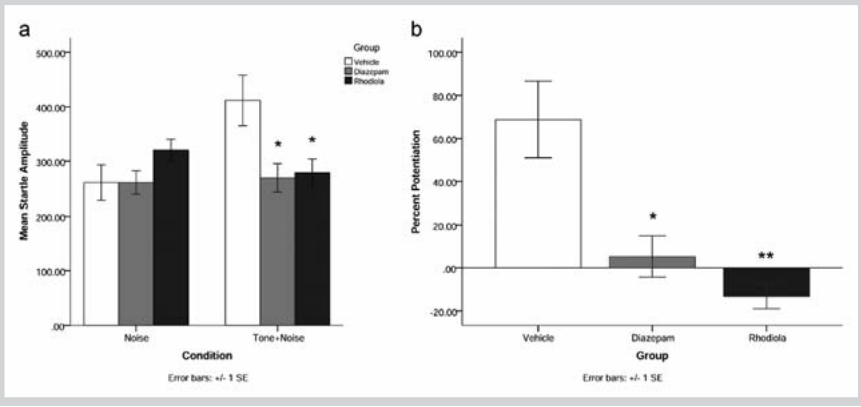

Fig. 1 Extract of Nunavik *R. rosea* and diazepam yielded significant suppression of mean startle amplitude (**a**) and percent startle potentiation on Day 4 (**b**).

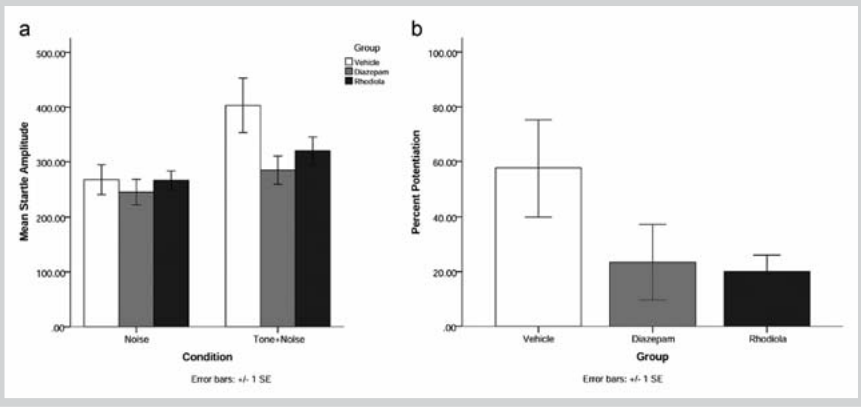

Fig. 2 Neither *R. rosea* extract nor diazepam yielded significant differences in startle amplitude (**a**) or percent startle potentiation on Day 5 (**b**).

dependent plasticity in the lateral nucleus [20–24]. However, although many anxiolytics (e.g., diazepam) bind to $GABA_A$ receptors, Nunavik *R. rosea* extract and its constituent phytochemicals have low binding affinity for the $GABA_A$ benzodiazepine receptor [10].

While benzodiazepines act as potent anxiolytics, prolonged use causes dependence [25–27], which highlights the need for anxiolytics that act through other mechanisms. Although the Cayer et al. [10] findings do not necessarily exclude the possibility of some GABAergic modulation, it suggests the primary mode of action of *R. rosea* is likely not GABAergic in nature.

Some alternative mechanisms have been suggested to explain the anxiolytic activity of *R. rosea.* For example, *R. rosea* may act as an MOA inhibitor [28]. It has also been shown to interact with both glucocorticoid [29] and neuropeptide Y (NPY) receptors [30]. However, our findings suggested that Nunavik *R. rosea* extract yielded no significant effects on endogenous secretion corticosterone secretion.

Our results are also in contradiction to other findings regarding *R. rosea* extract and glucocorticoid secretion [29]. However, Panossian et al. [29] utilized a 7-day treatment protocol, which might account for the difference in glucocorticoid effects.

Research suggests the primary mechanism of Nunavik *R. rosea* is neither glucocorticoid-based nor GABAergic (see Cayer et al. [10]). Although the results of the present investigation are positive, they are limited in that they do not address this mechanism directly. Future research should therefore explore the mechanism responsible for the anxiolytic effects of Nunavik *R. rosea*, possibly targeting the role of NPY receptors or monoamine oxidase [28, 30].

Table 1 Mean corticosterone (ng/sample) by treatment group.

	Treatment group	Mean corticosterone (ng/sample)	Standard deviation
30 Minutes	Vehicle	3864.48	2203.49
	Diazepam	3047.89	2851.58
	R. rosea	4040.05	1855.77
60 Minutes	Vehicle	1528.87	702.13
	Diazepam	1241.07	1528.35
	R. rosea	2224.99	1671.78
5 Days (Baseline)	Vehicle	1232.22	1486.07
	Diazepam	932.08	774.05
	R. rosea	1147.85	958.37

Materials and Methods

R. rosea roots were collected near Kuujjuaq, Nunavik, Quebec (Canada). Samples were identified by Alain Cuerrier (University of Montreal, Canada). A voucher specimen was deposited in the University of Ottawa Herbarium, UOH#19847. HPLC data (chromatogram of the plant extract) is available in Cayer et al. [10], and the same batch of *R. rosea* extract was used for both Cayer et al. [10] and this paper. Roots were dried in a commercial plant dryer at 35 °C and ground with a Wiley Mill (2 mm mesh size). Roots were extracted with 90% ethanol (10 m/v) and vacuum filtered through Whatmann no. 1 filter paper. The residue was re-extracted with 90% ethanol (5 m/v) twice and filtered. The filtrates

were combined, the solvent was roto-evaporated at 40 °C, and the extract lyophilized (percent yield = 7 %). The extract was stored at 4 °C and protected from light.

All experiments were conducted in accordance with the guidelines established by the Canadian Council on Animal Care and approved by the University of Ottawa Animal Care Committee (protocol approved September 26 2014, ACC-2011-006).

Rats were randomly assigned to treatment groups: *R. rosea* (75 mg/kg dose), vehicle, or diazepam (1 mg/kg dose, positive control; produced by Sandoz, original concentration 5 mg/mL in 40 % propylene glycol, 10 % alcohol, and 50 % water). *R. rosea* ethanolic extract and diazepam were suspended in 50 % sweetened condensed milk (vehicle). Controls received the vehicle alone.

Animals in the *R. rosea* group were treated over 3 days, following the procedures outlined by Cayer et al. [10], with the final dose administered 50 min prior to testing. Animals in the other groups received the vehicle alone on those days. The positive control group received an acute dose of diazepam administered 50 min prior to testing on the final day. All treatments were administered in a volume of 2 mL/kg body weight.

On Day 1, animals were placed inside the startle chamber and exposed to random bursts of white noise (95, 110, and 115 dB) for acclimatization and to obtain baseline startle amplitudes. On Day 2, animals were exposed to a conditioning paradigm where a tone was paired with subsequent foot shock (1.0 mA, 0.5 s duration) during the last 500 ms of the tone. The Day 2 conditioning consisted of seven CS-US pairings over the course of nine minutes, (average 60 s between pairings, interval lengths randomized).

Forty-eight hours later (Day 4), animals were re-exposed to the conditioning chamber. Over the course of 25 min, 20 trials of 110 dB white noise bursts (at randomized time intervals averaging 60 s) were presented, followed by 5 trials where the tone was presented along with 110 dB white noise bursts.

Forty-eight hours later (Day 5), animals were re-exposed to the conditioning chamber in the absence of any drug administration to measure long-term/extinction effects. Over 25 min, 20 trials of 110 dB white noise bursts (at randomized time intervals averaging 60 seconds) were presented, followed by 5 trials where the tone was presented with 110 dB white noise bursts.

The percentage of fear-potentiated startle was computed as [(startle amplitude on tone–noise minus noise-alone trials)/ noise-alone trials] × 100

Supporting information

Details on the apparatus, statistical analyses, and animals are available as Supporting Information.

Conflict of Interest

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The authors have read and understood Planta Medica Letters policy on the declaration of interests and declare that we have no competing interests.

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