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Research Article
Adaptogenic Activity of WS® 1375, a Proprietary Dry Extract from *Rhodiola rosea* Roots and Rhizomes

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Abstract

Background and Objectives: *Rhodiola rosea* L. has been widely used in folk medicine to improve endurance and work productivity and to relief symptoms of mental and physical stress. Thus, the present study was undertaken to evaluate the stress adaptogenic activity of a proprietary extract from roots and rhizomes of *Rhodiola rosea* L. (WS® 1375) and to elucidate its possible mechanism of action. **Methodology:** Rats were subjected to electrical foot shocks for 1 h by means of a grid floor in a standard conditioning chamber for 14 consecutive days to produce a state of chronic stress. WS® 1375 was orally administered in parallel at doses of 50, 150 or 450 mg kg^{-1} once daily 1 h before the induction of stress. Experiments were conducted 1 h after the last stress procedure on day 14. Gastric ulceration as well as weight of adrenal glands and spleens were observed to assess the intensity of stress. Stress induced behavioural perturbations were analyzed by behavioural despair test, learned helplessness test and inhibition of male sexual behaviour. Stress induced cognitive dysfunction was quantified using active and passive avoidance tests. Animals were sacrificed immediately after the last stress regimen and blood was withdrawn for corticosterone estimation. Brain samples of rats were collected and monoamine concentrations were measured. General neuropharmacological screening of WS® 1375 was also performed. **Results:** The investigations reveal that WS® 1375 has pronounced stress-preventive activity and significantly normalized the levels of brain nor-epinephrine, 5-HT and dopamine in a dose-dependent manner. Plasma corticosterone levels in animals treated with WS® 1375 (150 and 450 mg kg^{-1}) were significantly lower when compared to vehicle-treated rats. While WS® 1375 exhibited CNS stimulant activity, it did not cause muscle ataxia and failed to significantly improve stress-induced cognitive impairment. **Conclusion:** Based on these observations, it is concluded that WS® 1375 has a great potential for therapeutic use as an adaptogen.

Key words: Adaptogen, stress, CNS, corticosterone, monoamines, *Rhodiola rosea*, crassulaceae

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Roots and rhizomes of *Rhodiola rosea* L., also known as "golden root", "rose root" or "arctic root", have been widely used in traditional medicinal systems in Eastern Europe and Asia to increase physical endurance and work productivity as well as treatment of altitude sickness, chronic fatigue, depression, anemia, impotence, gastrointestinal disorders and diseases of the nervous system. Actually, the medicinal use of *Rhodiola* can be traced back to Roman times when it was mentioned by Pedanios Dioskurides (c. 40-90 AD) in his De Materia Medica as a headache remedy. Since the 18th century, the plant has gained increasing popularity in Scandinavia and some other Western European countries due to its proposed ability to increase resistance to stress and to protect against damage from environmental factors[1-4]. Accordingly, *Rhodiola rosea* has been defined as an adaptogen (EMEA/HMPC/102655/2007). This term was originally coined by Lazarev and is used to define herbal preparations that non-specially increase resistance of the organism against adverse biological, chemical, physical and psychological factors by normalizing the function of different organ systems[5]. In contrast to stimulants, adaptogens are supposed to cause an increased work-capacity that is not followed by a phase of exhaustion whereas tonics are considered to attenuate states of weakness within the entire organism or in individual organ system (EMEA/HMPC/102655/2007).

Phytochemical analyses revealed the presence of six major group of constituents in *R. rosea* roots and rhizomes, e.g., phenylethanoloids, phenylpropanoids, flavonoids, monoterpenes, triterpenes and phenolic acids. The phenylethanol glycoside salidroside has generally be considered to be a major pharmacologically active constituents of *Rhodiola* extracts. However, salidroside is not a specific ingredient of *R. rosea* but is found in several species of the *Rhodiola* genus. In contrast, several cinnamic alcohol glycosides (e.g., rosavin, rosin, rosarin) have been found to be characteristic for *R. rosea*. This class of compounds is also subsumed under the term 'rosavins' and are mainly represented by the phenylpropanoid rosavin[1,3]. Both classes of compounds are now considered to contribute to the therapeutic efficacy of extracts from *R. rosea* roots and rhizomes[4].

Although finished herbal drugs are almost always perceived through the medicinal plant which is used as raw material for their manufacturing, the composition of an herbal extract as the active ingredient of phytotherapeutics can be highly variable depending on factors such as growth conditions, production process, extraction solvent, drug to extract ratio etc. Even more important, as *R. rosea* is still harvested in the wild to a large extend it needs to be ensured that mix-up with closely related *Rhodiola* species with a different pattern of constituents is excluded. As a consequence, reproducible and consistent therapeutic effects can only be achieved if repetitious and strictly standardized processes and conditions are employed which guarantee a well-defined herbal medicinal product. This also means that pharmacological investigations and results of clinical studies with respect to efficacy and tolerability are specific for a particular product and cannot be transferred to other preparations. Taken these points into consideration the current investigations were scheduled to comprehensively characterize the stress adaptogenic potential of WS® 1375. This is a proprietary dry extract from roots and rhizomes of *R. rosea* that has been approved recently as an herbal medicinal product in Germany and several other European countries for the relief of mental and physical symptoms of stress and overwork including fatigue, exhaustion, burnout, irritability and tenseness. Besides establishing a product specific pattern of pharmacological activity, this assessment was also perceived worthwhile in a broader context as a comprehensive evaluation of the adaptogenic action of *R. rosea* is missing although the plant has been intensively investigated for more than 50 years.

MATERIALS AND METHODS

Animals: Adult Charles foster albino rats (150±10 g) and Wistar mice (20±5 g) of either sex were used in the present study. Animals were housed in groups of six in polypropylene cages at an ambient temperature of 25±1°C and 45-55% relative humidity with a 12:12 h light/dark cycle. They were provided with commercial food pellets and water *ad libitum* unless stated otherwise. Animals were acclimatized for at least 1 week before using them for the experiments and exposed only once to every experiment. Body weight, food and water consumption of animals were monitored at regular intervals during the course of the study. 'Principles of laboratory animal care' (NIH publication number No. 85-23, revised 1985) guidelines were followed. The approval of Institutional Animal Ethics Committee (IAEC) of Banaras Hindu University was obtained on 21 December, 2010 under the number Dean/10-11/351.

Plant material and extraction: WS® 1375 is a proprietary dry extract from *Rhodiola rosea* L. rhizomes and roots obtained by extraction with 60% (w/w) aqueous ethanol (drug/extract ratio 1.5-5:1) and a specified content of 3.0 - 8% rosavins (i.d., rosavin, rosin and rosarin) and >1% salidroside. A single batch of the extract (PSC1995/WS1375/TNCh. 004) was used for all investigations.

Drug treatments: WS® 1375 was orally administered by gavage as 0.3% carboxymethyl cellulose (CMC) suspension (5 mL kg^{-1}) once daily for 14 consecutive days 45 min before the general neuropharmacological screening or 1 h before induction of stress at doses of 50, 150 or 450 mg kg^{-1}. Behavioral experiments were always performed between 09.00 and 14.00 h. On day 14 behavioral tests were conducted 1 h after the last stress procedure and 2 h after drug or vehicle administration. Control animals were treated with an equal volume of vehicle (0.3% CMC suspension). Immediately after the last stress regimen, animals were sacrificed by decapitation and blood was collected in EDTA coated tubes kept on ice and centrifuged at 1,000×g for 20 min at 4°C. Plasma was separated and aliquots were stored at -70°C until analysis. In addition, brain samples were collected and the adrenal glands as well as spleens were removed and weighed.

General neuropharmacological screening: Potentiation of pentobarbital-induced sleeping time: Pentobarbital sodium was purchased from Merck (Darmstadt, Germany) and was administered (40 mg kg^{-1}, i.p. on day 14) to control and drug treated mice. Onset of sleep (loss of righting reflex) was noted and duration of sleep was measured as the period between the loss of righting reflex and its return[6]. WS® 1375 (50, 150 and 450 mg kg^{-1}, p.o. for 14 days) and diazepam (3 mg kg^{-1}, p.o. on day 14) were administered 45 min prior to pentobarbital injection.

Locomotor activity: The spontaneous locomotor activity was assessed with the help of a photoactometer[7]. Each animal was observed for a period of 10 min in a square closed field arena (30×30×30 cm) equipped with 6 photocells in the outer wall. Interruptions of photocell beams (locomotor activity) were recorded by means of a 6 digits resettable counter. One animal was observed for locomotor activity in the apparatus at a time.

Effect on muscle grip performance of mice: Effect on motor co-ordination was examined on a rota-rod apparatus. Each animal was placed on a rotating rod (20 rpm) in a pre-test session and only those animals, which stayed on the rod for not less than 3 min were selected for the test session. The test session was performed on the same day as the pre-test session. Fall-off time (when the mouse falls from the rotating rod) for each animal was noted before and after drug administration[8]. WS® 1375 (50, 150 and 450 mg kg^{-1}, p.o.) and diazepam (3 mg kg^{-1}, p.o.) were administered 45 min before test session.

Tests on stress induced behavioural perturbations: The method of Porsolt *et al.*[9] was followed to induce chronic stress. Rats were randomly assigned to the unstressed control, stress and drug treated stress groups. Those assigned to the vehicle or drug treated groups were subjected daily (including Sundays) to 1 h of foot shock through a grid floor in a standard conditioning chamber with the escape route closed. The duration of each shock (2 mA) and the intervals between the shocks were randomly programmed between 3 and 5 sec and 10 and 110 sec, respectively in order to make them unpredictable. Animals were sacrificed on day 14, 1 h after the last shock procedure on completion of the test procedure involved. The following methods were used to assess behavioural depression:

Stress-induced 'behavioural despair' test: Rats were forced to swim individually in a polypropylene vessel (45×40×30 cm) with a water level of 20 cm, which ensured that the rat's feet did not touch the floor of the vessel and that it could not climb out of it. The rat was allowed to swim for 10 min. Thereafter, during the next 5 min, the total period of immobility, characterized by complete cessation of swimming with the head floating above water level was noted. This immobility period, after initial frenzied attempts to escape, was postulated to represent 'behavioural despair' as an experimental model of endogenous depression[10].

Learned helplessness test: On day 12 of the investigation, rats were subjected to foot shock (60 scrambled shocks, 15 sec duration, 0.8 mA and every min) in a two compartment jumping box (Techno) with the escape door to the adjoining unelectrified compartment closed. The exercise continued for 1 h. On day 14, 48 h later, the rats were subjected to avoidance training, using the same apparatus but keeping the escape route to the unelectrified chamber open. During this avoidance training the rats were placed in the electrified chamber and allowed to acclimatize for 5 min before being subjected to 30 avoidance trials, with an inter-trial interval of 30 sec. During the first 3 sec of the trial, a buzzer stimulus (conditioned stimulus, CS) was present followed by electroshock (unconditioned stimulus, UCS) (0.8 mA) delivered via the grid floor for the next 3 sec. The avoidance response was characterized by escape to the adjoining 'safe' chamber during CS. Failure to escape during UCS within 15 sec was assessed as 'escape' failure which was postulated to indicate despair or depression[11].

Stress-induced inhibition of male sexual behaviour: A male rat was placed in a cage in a dimly room for 10 min with 2 oestrinised (sequentially treated with estradiol valerate 5 mg/rat s.c., followed 48 h later by hydroxyprogesterone 1.5 mg/rat, s.c.) female rats. The total number of mounts was counted[12].

Tests on stress induced cognitive dysfunction: The following parameters were used to assess the effect of stress on retention of a learned task as memory.

Active avoidance test: Rats were trained for an active avoidance task before subjecting them to stress. During training, a rat was placed in the right electrified compartment of a shuttle box (Techno) and allowed to acclimatize for 5 min. Thereafter, the rat was subjected to 15 sec of a buzzer stimulus (CS) which was followed by electric shock (1 mA, 50 Hz) given through the grid floor (UCS). The rats were given at least 10 trials, with an inter-trial interval of 60 min, until they reached the criterion of 100% avoidance response of jumping to the unelectrified left chamber of the shuttle box during CS. The test was repeated on day 14 in order to assess the retention of the active avoidance learning[13].

Passive avoidance test: The test apparatus was a rectangular box (45×30×40 cm) with an electrified grid floor. An 8 cm high platform (17×12 cm) was fixed to the centre of the floor. A rat was placed on the platform and allowed to step down. After 24 h later, on day 1 of the experiment, the rat was again placed on the platform and on stepping down, received foot shock (0.75 mA, 2 sec) through the grid floor. The rat was given 3 more trials until the latency of step down had stabilized. The test was repeated on day 14 and retention of learning as memory parameter for each rat was recorded[14].

Assessment of stress intensity: Gastric ulceration and adrenal cortex and spleen weight were used as indicator for assessment of intensity of stress.

Gastric ulceration: The stomach was removed and split open along the greater curvature. The numbers of discrete ulcers were noted with the help of a magnifying glass. The severity of the ulcers was scored after histological confirmations: 0 = No ulcers, 1 = Changes limited to superficial layers of the mucosa with no congestion, 2 = Half the mucosal thickness showing necrotic changes and congestion, 3 = More than two-third of mucosal thickness showing necrotic changes and congestion and 4 = Complete destruction of the mucosa with marked hemorrhage. Thereafter, the ulcer severity score was calculated[15].

Adrenal cortex and spleen weights: The adrenal glands and spleens were removed and weighed[16,17].

Biochemical investigations: Estimation of brain monoamines: Brain monoamine concentrations [norepinephrine (NA), dopamine (DA) and serotonin (5-HT)] were measured by a validated spectrofluorometric methods as described by Welch and Welch[18]. Standard curves were prepared using standard chemicals from Sigma Aldrich.

Estimation of corticosterone: Plasma corticosterone level were quantified using an ELISA kit (Assay Design, U.S.A.) and following manufacturer's instructions.

Statistical analysis: The values are expressed as Mean±SEM. Instat (Graphpad) was used for statistical evaluation. Significance of the differences between control and treated groups was calculated by one way analysis of variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons test. The $p<0.05$ was considered to be statistically significant.

RESULTS

General neuropharmacological screening: Potentiation of pentobarbitone-induced sleeping time: WS® 1375 dose-dependently increased the onset of sleep and markedly decreased the duration of pentobarbitone-induced sleeping. The effect of WS® 1375 on sleep onset was statistically significant at all doses applied while that on total duration of sleep was significant only at doses of 150 and 450 mg kg^{-1} when compared to vehicle treated animals. In contrast, diazepam (3 mg kg^{-1}, p.o.) shortened sleep onset and prolonged sleep duration (Table 1).

Locomotor activity in mice: WS® 1375 (150 and 450 mg kg^{-1}) significantly increased the locomotor activity compared to vehicle treated animals. Diazepam treatment (3 mg kg^{-1}) caused a significant decrease in locomotor activity counts as assessed using a photoactometer (Table 2).

Table 1: Effect of WS® 1375 and diazepam on pentobarbitone-induced sleeping

Treatments	Dose (mg kg^{-1})	Onset of sleep (sec)	Duration of sleep (min)
Vehicle	-	246.33±4.25	79.83±2.76
WS® 1375	50	335.50±8.99***	73.50±2.29
WS® 1375	150	511.33±8.38***	67.83±1.40*
WS® 1375	450	691.50±18.81***	57.33±2.19***
Diazepam	3	170.83±11.03***	95.50±3.77**

*p<0.05, **p<0.01, ***p<0.001 vs. vehicle, N = 6 per group

Table 2: Effect of WS® 1375 and diazepam on locomotor activity

Treatments	Dose (mg kg^{-1})	Spontaneous activity
Vehicle	-	110.30±2.32
WS® 1375	50	112.70±3.18
WS® 1375	150	124.60±1.71**
WS® 1375	450	127.80±2.89***
Diazepam	3	76.80±2.05***

**p<0.01, ***p<0.001 vs. vehicle, N = 10 per group

Table 3: Effect of WS® 1375 and diazepam on muscle grip performance in mice

		Muscle grip performance fall-off time (sec)	
Treatments	Dose (mg kg^{-1})	Before treatment	After treatment
Vehicle	-	196.50±3.35	202.17±2.52
WS® 1375	50	195.17±2.52	200.67±1.93
WS® 1375	150	194.00±2.13	192.50±1.52
WS® 1375	450	199.83±3.80	190.33±2.57
Diazepam	3	189.83±3.16	49.67±5.60***

***p<0.001 vs. vehicle, N = 6 per group

Table 4: Effect of WS® 1375 on behavioural despair test

Treatments	Dose (mg kg^{-1})	Duration of immobility (sec)
Vehicle	-	118.33±5.61
Stress+vehicle	-	134.33±4.64*
Stress+WS® 1375	50	123.17±4.79
Stress+WS® 1375	150	107.00±2.56†
Stress+WS® 1375	450	98.83±3.08***†

*p<0.05, ***p<0.01 vs. vehicle, †p<0.001 vs. stress+vehicle, N = 6 per group

Muscle grip performance in mice: In the rota-rod test, no motor incoordination was evident following treatment with WS® 1375 (50, 150 and 450 mg kg^{-1}). In contrast, diazepam (3 mg kg^{-1}) produced ataxia and significantly impaired the muscle grip performance (Table 3).

Neurobehavioral tests

Stress induced behavioural despair test: Chronic stress induced a significant increase in the duration of immobility in the Porsolt behavioural despair test. Statistically significant dose-dependent effects of WS® 1375 in stressed animals were observed after its two higher doses (150 and 450 mg kg^{-1}) only (Table 4).

Stress induced learned helplessness test: Results of this test are summarised in Table 4. They reveal that chronic stress induced significant increase in escape failures along with a significant decrease in avoidance responses. Treatments with WS® 1375 at doses of 150 and 450 mg kg^{-1} reversed these effects, while the outcomes at a dose of 50 mg kg^{-1} did not reach statistical significance (Table 5).

Stress induced sexual behaviour: Chronic stress significantly inhibited the male sexual response manifested as decrease in the number of mountings on oestrinised female rats. This effect was attenuated by WS® 1375 (150 and 450 mg kg^{-1}) but not by the lower dose of WS® 1375 i.e., 50 mg kg^{-1} (Table 6).

Table 5: Effect of WS® 1375 on learned helplessness test

Treatments	Dose (mg kg^{-1})	Escape failure (N)	Avoidance response (N)
Vehicle	-	13.83±1.28	5.17±0.48
Stress+vehicle	-	21.67±1.54***	1.67±0.49***
Stress+WS® 1375	50	18.83±1.25*	2.50±0.43**
Stress+WS® 1375	150	16.33±0.84†	3.83±0.48†
Stress+WS® 1375	450	16.17±0.83†	4.33±0.42†

*p<0.05, **p<0.01, ***p<0.01 vs. vehicle, †p<0.01, ††p<0.001 vs. stress+vehicle, N = 6 per group

Table 6: Effect of WS® 1375 on stress induced suppression of sexual behaviour in male rats

Treatments	Dose (mg kg^{-1})	No. of mountings
Vehicle	-	8.67±0.84
Stress+vehicle	-	1.50±0.43***
Stress+WS® 1375	50	2.50±0.50***
Stress+WS® 1375	150	4.83±0.60**†
Stress+WS® 1375	450	5.83±0.65*††

*p<0.05, **p<0.01, ***p<0.01 vs. vehicle, †p<0.01, ††p<0.001 vs. stress+vehicle, N = 6 per group

Table 7: Effect of WS® 1375 on active avoidance test

Treatments	Dose (mg kg^{-1})	Retention (%) of active avoidance learning on day 14
Vehicle	-	83.33±3.33
Stress+vehicle	-	36.67±3.33***
Stress+WS® 1375	50	38.33±4.77***
Stress+WS® 1375	150	43.33±4.22***
Stress+WS® 1375	450	45.00±3.42***

***p<0.001 vs. vehicle, N = 6 per group

Table 8: Effect of WS® 1375 on passive avoidance test

Treatments	Dose (mg kg^{-1})	Step down latency (Inflexion ratio)
Vehicle	-	8.94±1.88
Stress+Vehicle	-	3.75±0.52***
Stress+WS® 1375	50	3.94±0.58***
Stress+WS® 1375	150	4.28±0.43***
Stress+WS® 1375	450	5.20±1.63***

***p<0.001 vs. vehicle, Inflexion ratio = $(L_{14}-L_1)/L_1$ (where, L_1 and L_{14} are step down latencies in seconds on day 1 and 14, respectively), N = 6 per group

Stress-induced cognitive dysfunction: In active avoidance test, chronic stress caused a significant decrease in the retention of active avoidance learning. WS® 1375 did not alter stress-induced memory deficits (Table 7). Similarly, in a passive avoidance test model chronic stress caused a significant decrease in the retention of passive avoidance learning measured as step down latency. WS® 1375 did not cause a statistically significant increase in passive avoidance learning (Table 8).

Assessment of stress intensity

Stress induced gastric ulceration: Chronic stress markedly induced gastric ulcers, the numbers and severity of which were lesser in groups treated with WS® 1375 at the two higher doses (Table 9).

Stress induced changes in adrenal gland and spleen weight: Chronic stress significantly increased the weight of adrenal glands while the spleen weight was significantly reduced. These stress-induced pathologies were partially, but significantly reduced by all WS® 1375 doses. However, its effect does not seem to be strictly dose dependent (Table 10).

Biochemical investigations: Chronic stress significantly reduced the level of NA, 5-HT and DA in rat brains as compared to vehicle treated non-stressed animals. Pre-treatment with WS® 1375 (150 and 450 mg kg^{-1}) significantly countered the adverse effect of chronic stress causing significant elevation in levels of NA, 5-HT and DA in brain. However, WS® 1375 (50 mg kg^{-1}) only caused an insignificant elevation of all the three monoamines in brain as compared to vehicle treated stress group (Table 11). Chronic stress induced a significant increase in plasma corticosterone level compared to the vehicle treated non-stressed group. Pre-treatment with WS® 1375 (150 and 450 mg kg^{-1}) significantly inhibited the elevation of plasma corticosterone level compared to vehicle treated animals (Table 12).

Table 9: Effect of WS® 1375 on stress induced gastric ulceration in rats

Treatments	Dose (mg kg^{-1})	Number of ulcers	Severity of ulcers
Vehicle	-	-	-
Stress+Vehicle	-	17.83±1.82	33.83±3.70
Stress+WS® 1375	50	11.50±2.46	21.33±4.46
Stress+WS® 1375	150	8.67±1.94*	16.50±3.97*
Stress+WS® 1375	450	6.83±2.23*	12.00±3.99**

*p<0.05, **p<0.01 vs. stress+vehicle, N = 6 per group

Table 10: Effect of WS® 1375 on stress induced changes in adrenal gland and spleen weight in rats

		Weight (mg/100 g)	
Treatments	Dose (mg kg^{-1})	Adrenal gland	Spleen
Vehicle	-	21.44±1.09	226.95±3.62
Stress+vehicle	-	32.88±1.22***	144.79±3.46***
Stress+WS® 1375	50	28.33±1.49**†	166.77±5.19***††
Stress+WS® 1375	150	26.13±0.93††	174.79±3.76***†††
Stress+WS® 1375	450	25.48±1.48††	178.37±3.75***†††

**p<0.01, ***p<0.01 vs. vehicle;† p<0.05, ††p<0.01, †††p<0.001 vs. stress+vehicle, N = 6 per group

Table 11: Effect of WS® 1375 on brain monoamines in rats

		Brain monoamines (ng g^{-1} of tissue)		
Treatments	Dose (mg kg^{-1})	Serotonin	Norepinephrine	Dopamine
Vehicle	-	438.21±12.05	389.82±9.89	827.23±8.67
Stress+vehicle	-	179.42±8.99	174.23±11.27	434.89±6.05
Stress+WS® 1375	50	229.14±10.52	222.75±6.93	451.26±5.30
Stress+WS® 1375	150	312.75±4.96***	268.19±10.87**	527.31±10.21**
Stress+WS® 1375	450	357.10±10.66***	319.29±10.19***	681.97±12.08***

**p<0.01, ***p<0.001 vs. stress+vehicle; N = 2 per group

Table 12: Effect of WS® 1375 on plasma corticosterone level in rats

Treatments	Dose (mg kg^{-1})	Plasma corticosterone (ng mL^{-1})
Vehicle	-	97.08±2.10
Stress+vehicle	-	159.67±3.13***
Stress+WS® 1375	50	152.67±2.98***
Stress+WS® 1375	150	126.04±4.38***†††
Stress+WS® 1375	450	109.43±1.99†††

***p<0.001 vs. Vehicle, †††p<0.001 vs. stress+vehicle, N = 6 per group

DISCUSSION

Changing environmental conditions pose a permanent challenge for any individual. This is perceived as stress, whenever such a stimulus is considered as annoying or threatening. The organism reacts to stressful conditions with a coordinated response that includes a number of systems and processes with the aim to maintain or re-establish a state of dynamic equilibrium. This stress response is usually divided into two different phases. The early "fight-or flight" reaction involves the rapid activation of the autonomic nervous system that causes the release of adrenaline and noradrenaline from the adrenal medulla. These "stress" hormones elevate metabolic rate, blood pressure, as well as respiration and increase blood flow to organs that are essential for the "fight-or-flight" response, such as the heart and skeletal muscles[19].

At a later stage, the hypothalamic-pituitary-adrenal (HPA) axis is activated. This part of the neuroendocrine system coordinates emotional, cognitive, neuroendocrine and autonomic inputs in order to adjust the extend and specificity of the individual behavioral, neural and hormonal reaction. Mediated is this response by glucocorticoids (GC), which primarily regulate the expression of target genes by transactivation or transrepression. However, faster, non-genomic effects of glucocorticoids have also been

described after binding to putative membrane-bound receptors. The activation of the HPA axis is induced by the release of corticotropin-releasing hormone (CRH) from the paraventricular nucleus (PVN) that stimulates synthesis and secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland and subsequently the formation and releases GCs from the adrenal cortex. Regulated is this process by a negative feedback loop after GC binding to high-affinity mineralocorticoid (MR) and lower affinity glucocorticoid receptors (GR). Whereas acute and short-term stress is generally considered to be beneficial and adaptive, chronic stress may cause an imbalance between MR and GR or their down-regulation, which can alter the HPA feedback mechanism and may result in overexposure of the brain and peripheral tissues to these highly bioactive steroids[19].

It is generally accepted that living in complex and demanding modern industrializes societies is associated with a constant and steadily increasing frequency of pressing and burdening situations in everyday life. Thus, it is not surprising that there is a high demand for well-tolerated and effective products to successfully cope with stressful mental or physical challenges. Adaptogenic substances that have the capacity to normalize body functions, which are compromised by stress, would ideally suit this requirement. However, it is difficult to reconcile the concept of an adaptogen with modern drug development programs, which aims to design selective ligands that act on a single disease target. Instead, herbal medicines whose therapeutic efficacy is generally considered to be caused the combined action of a mixture of constituents may be more promising to mediate an increased resistance against a broad spectrum of harmful factors of different physical, chemical or biological nature.

It was the aim of the present study to establish a detailed product specific pharmacological pattern with respect to the claimed adaptogenic activity. Although the plant has been intensively investigated for more than 50 years and an actual search in PubMed, using the keywords "(Rhodiola rosea) AND adaptog*" delivered 114 hits (<https://www.ncbi.nlm.nih.gov/pubmed?term= (Rhodiola%> 20rosea)%20 AND%20 adaptog*; May 15, 2018), a comprehensive evaluation of the adaptogenic action of extracts from R. rosea is still missing.

Using a broad battery of animal models of stress-induced behavioural perturbations, WS® 1375 was observed to dispose of a pronounced stress-preventive activity as it dose-dependently ameliorates the behavior disturbances induced by chronic application of unpredictable foot shocks. This stress-relieving potential was confirmed by an impairment of morphological changes in selected organs, e.g., a reduction in the numbers and severity of gastric ulcers as well as counterregulation stress-induced weight changes of adrenal glands and spleen. Likewise, the dose-dependent normalization of plasma corticosterone concentrations and the brain levels of norepinephrine, 5-HT and dopamine support the adaptogenic activity of WS® 1375.

It may also be concluded that WS® 1375 has a CNS stimulant type of action, which is evident from its activity profile in photoactometer and pentobarbitone-induced hypnosis testing. However, the extract appears not to dispose of strong cognitive function altering potentials. WS® 1375 was well tolerated in the doses studied and general neuropharmacological screening revealed that it is devoid of any adverse effect on muscle coordination.

These results are in line with previous investigations which demonstrated that a hydroalcoholic *R. rosea* extract containing 3% rosavin and 1% salidroside prevented chronic mild stress-induced alteration in female rats[20] and reduced acute stress- and CRF-mediated anorexia[4]. Based on these observations, it conclude that WS® 1375 has a great potential for therapeutic use as an adaptogen.

SIGNIFICANCE STATEMENT

The present study provides a comprehensive evaluation of the adaptogenic action of WS® 1375, a proprietary dry extract from *Rhodiola rosea* roots and rhizomes, in chronically stressed rats. Following oral administration of human-equivalent doses, pronounced stress-preventive activity was demonstrates in a broad set of neuropharmacological test models. The beneficial effect on behavioural disturbances was accompanied by an impairment of stress-induced morphological changes in selected organs (e.g., gastric ulceration as well as weight changes of adrenal glands and spleen) and a dose-dependent normalization of plasma corticosterone concentrations and brain levels of norepinephrine, 5-HT and dopamine. Based on these observations, it can be conclude that WS® 1375 has a compelling potential for therapeutic use as an adaptogen.

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