ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF RHODIOLA ROSEA L. EXTRACT IN RATS

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ABSTRACT

BACKGROUND: Rhodiola rosea (golden root) is a unique phytoadaptagen with immunomodulatory, antioxidant, anti-inflammatory and antinociceptive activity.

AIM: The aim of this study was to evaluate the antinociceptive and anti-inflammatory effects of the alcohol/water extract of Rhodiola rosea roots in rats.

MATERIALS AND METHODS: Thirty male Wistar rats were used in the study. They were divided in 3 groups (n = 10), treated respectively with saline (controls), Rhodiola rosea extract 50 mg/kg bw and 100 mg/kg bw orally. The antinociceptive effect was evaluated using the hot-plate test, Randall-Sellito test and the formalin test. The hot-plate test evaluates the reaction time of rats which are dropped on a heated surface. The analgesy-meter test exerts a force increased at constant rate. In the formalin test we measured the total time spent in licking the injected paw during the early (0-10 min) and late phase (20-30 min) of test. To study anti-inflammatory effect the carrageenan-induced paw edema was used. The paw volume was measured plethysmometrically at 2, 3 and 4 hours.

RESULTS: In the hot-plate test Rhodiola rosea increased in both doses the latency reaction compared with that in the controls. In analgesy-meter test Rhodiola rosea in a dose of 50 mg/kg showed a significant increase of pressure reaction compared with the controls. In the formalin test Rhodiola rosea in a dose of 100 mg/kg significantly decreased the paw licking time during the first phase. In the plethysmometer test Rhodiola rosea extract significantly reduced carrageenan-induced paw edema when compared with the saline-induced edema.

CONCLUSION: The studied extract of Rhodiola rosea exhibited significant analgesic activity in all the pain models used – inhibition of thermal pain, mechanical hyperalgesia and formalin-induced pain behavior. Significant anti-inflammatory activity was observed from Rhodiola rosea extract in carrageenan induced paw edema in rats.

Key words: Rhodiola rosea, nociception, inflammation, rats

INTRODUCTION

Rhodiola rosea (Golden Root) is a unique phytoadaptogen that grows in high-altitude regions. Recently it has gained medical attention because of its various therapeutic properties.1 Rhodiola rosea L. is one of the most popular adaptogens and an antistress plant in European and Asiatic traditional medicine.2 Studies have found that Rhodiola preparations exhibit adaptogenic effect including neuroprotective, cardioprotective, anti-fatigue, antidepressive, anxiolytic, nootropic, life-span increasing effects and CNS stimulating activity.3 Its pharmacological properties appear to depend on its ability to modulate the activation of several components of the complex stress-response system.4

AIM

The aim of the study was to evaluate the antinociceptive and anti-inflammatory effects of the alcohol/water extract of Rhodiola rosea roots in rats.

MATERIALS AND METHODS

Male Wistar rats weighing 170-210 g were divided into 3 groups (n = 10). The rats were kept under standard laboratory conditions in a 08:00-20:00 h light/dark cycle and were provided with food and water ad libitum. The test substance was administered by oral gavage. The following experimental groups were used: group 1 received saline (0.1 ml/100 g b.w.); group 2 - 50 mg/kg of Rhodiola rosea; group 3 - 100 mg/kg of Rhodiola rosea.

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The extract of Rhodiola rosea we studied was evaluated by the research group of Phytochemistry Division in the Institute of Botany, Bulgarian Academy of Science.\(^5,6\) The method used was HPLC on Agilent 1100 Series HPLC System with MWD UV-Vis detector. The extract was 80/20% water/alcohol, as described previously. The natural origin voucher specimens and all botanical information were obtained.

**HOT-PLATE TEST**

The original hot plate set up (Ugo Basile, Italy) was used. A transparent glass cylinder was used to keep the rat on the heated surface of the plate. The temperature of the hot plate was set to 55 ± 0.5 °C. Latency time was defined as the time between the zero point when the animal was placed on the hot plate surface and the time when the animal licked its hind paw or jumped off to avoid thermal pain. The accepted “zero time” in this study started 30 min after drug administration. To minimize tissue damage, a cut-off time of 30 sec was adopted. The latencies of both forepaws licking or jumping were measured for each animal at 0, 60, 120 and 180 minutes.

**NOICEPTIVE TEST**

The original analgesy-meter set up (Ugo Basile, Italy) was used. The antinociceptive effect of Rhodiola rosea was assessed using a mechanical noxious stimulus as previously described by Randall & Selitto.\(^7\) Nociceptive threshold, expressed in grams, was measured in centimeters by applying an increasing pressure to the right hind paw of unrestrained rats until the rat squeaked and/or a struggle was obtained. The rats were tested at 0, 1, 2 and 3 hours after p.o. administration of the test substance.

**FORMALIN TEST**

In the formalin test formalin (0.1 ml, 2%) was injected into the plantar surface of the left hind paw. Each animal was placed in an observation chamber and nociceptive response was recorded for a period of 30 min. The sum of time (in s) spent in licking and biting the injected paw in the first 10 min and that from 20 min to 30 min was taken as an indicator of nociceptive response.\(^8\)

**ANTI-INFLAMMATORY TEST**

To study the anti-inflammatory effect the carrageenan-induced paw edema was used. Paw edema was induced by injecting 0.1 ml of 1% solution sterile carrageenan lambda in saline into the right hind paw of the rat.\(^9\) Carrageenan caused visible redness and pronounced swelling that was well developed within 4 hours and persisted for more than 48 hours. The rats received vehicle or Rhodiola rosea (p.o.) 30 min before carrageenan administration. Hind footpad thickness was measured immediately before carrageenan injection and at 2, 3 and 4 hours thereafter with a plethysmometer.

**STATISTICAL ANALYSIS**

The results obtained were expressed as mean ± SEM. The comparison between the groups was made by Student’s t-test of analysis of variance (one way ANOVA), in the INSTAT computer program. Tukey-Kramer Multiple Comparison Test was used to compare each parameter of the respective experimental group with the control group. A value P < 0.05 was considered as statistically significant.

For the anti-inflammatory test the difference between two readings was taken as the volume of edema, and the percentage of anti-inflammatory activity was calculated using the following equation:

\[
\text{Percentage of anti-inflammatory activity} = \frac{(V-V_0)}{V_0} \times 100,
\]

where V is the paw volume at 1, 2, 3 and 4 hours after the carrageenan injection and V\(_0\) is the initial paw volume.

**RESULTS**

**ANTINOCICEPTIVE ACTIVITY**

In the hot plate analgesic test the average latency of the controls was between 12 and 20 sec. The animals treated with Rhodiola rosea at both doses increased the latency reaction at 2 and 3 hours (p < 0.05) as compared with the respective control group (Fig. 1).

In the analgesy-meter test the control animals showed average pressure reaction between 10 and 13 cm. Rats treated with 50 mg/kg of Rhodiola rosea showed no change of paw licking duration during the first and second phases. The rats treated with 100 mg/kg of test substance showed an increase of pressure reaction at 1 hour (p < 0.05) as compared with the respective controls (Fig. 2).

In the formalin test the duration of paw licking reaction for the controls was 43 sec for the first phase and 31 sec for the second phase. The group treated with 50 mg/kg of Rhodiola rosea showed a significant increase (p < 0.05) of pressure reaction at 1, 2 and 3 hours as compared with the respective control group. The group treated with 100 mg/kg of test substance showed an increase of pressure reaction at 1 hour (p < 0.05) as compared with the respective controls (Fig. 2).

In the formalin test the duration of paw licking reaction for the controls was 43 sec for the first phase and 31 sec for the second phase. The group treated with 50 mg/kg of Rhodiola rosea showed no change of paw licking duration during the first and second phases. The rats treated with 100 mg/kg of test substance significantly decreased (p < 0.05)
the duration of licking during the first phase and did not change it during the second phase (Fig. 3).

**ANTI-INFLAMMATORY ACTIVITY**

Carrageenan administered in control rats caused visible redness and pronounced swelling that was well developed within 4 hours and persisted for more than 48 hours. Rats treated with 50 mg/kg of Rhodiola rosea showed inhibition of carrageenan-induced paw edema (p < 0.05) at 4 hours as compared with the respective controls. Animals treated with 100 mg/kg of test substance significantly decreased (p < 0.05) the paw volume at 3 and 4 hours (Fig. 4).
DISCUSSION

Based on the results of this study we can assume that the extract of Rhodiola rosea we studied has an antinociceptive effect because it increases the latency of reaction at 2 and 3 hours in the hot plate test. The extract of Rhodiola rosea exerts an analgesic effect on mechanical hyperalgesia assessed with the Randall-Selitto paw pressure test widely used for qualification of the thresholds of the rat high paw withdrawal reflex to noxious pressure stimulation. The extract of Rhodiola rosea does not have a well pronounced suppressive ef-

*\( p < 0.05 \) vs respective saline group

Figure 3. Effects of Rhodiola rosea L. – Formalin test.

*\( p < 0.05 \) vs respective control

Figure 4. Antiinflammatory effect of Rhodiola rosea L. extract on carageenin oedema in plethysmometer test.
fect in the formalin test. Only the number of paw licking during the early phase decreased when the higher dose of the substance was used. The formalin test is considered to be the most predictive of acute pain and is believed to be a more valid model for clinical pain. Our results also showed that oral administration of Rhodiola rosea extract inhibited carrageenan-induced inflammatory pain. Carrageenan-induced inflammatory pain is well-known to involve inflammatory mediators like cyclooxygenase products (PG E2), leucotriennes, mast sells products (histamine, 5-HT), nitric oxide, etc. which are released as a result of tissue injury. Pooja et al. studied the anti-inflammatory activity of the tincture extract of Rhodiola rosea roots through carrageenan-induced paw edema, formaldehyde-induced arthritis and nystatin-induced paw edema in a rat model. They found that the tincture extract exhibited an inhibitory effect against acute and subacute inflammation at a dose of 250 mg/kg body weight. Inhibition of nystatin-induced edema was also observed in a dose-dependent manner. The in vitro inhibitory effects of the tincture extract from Rhodiola rosea roots was evaluated against the enzymes relating to inflammation. The enzymes include cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and phospholipase A2 (PLA2). The extract showed varying inhibitory activities against these enzymes depending on the concentrations. A potent inhibition was observed against COX-2 and PLA-2. Inhibition of nystatin-induced edema and phospholipase A2 suggested that membrane stabilization could be the most probable mechanism of action of Rhodiola rosea in anti-inflammation.

The findings in many studies suggest that Rhodiola rosea root extract can be used in the treatment of inflammatory conditions. Several mechanisms of action possibly contributing to the clinical effect have been identified for Rhodiola extracts. They include interactions with HPA-system (cortisol-reducing), protein kinases p-JNK, nitric oxide, and defense mechanism proteins (e. g. heat shock proteins Hsp 70 and FoxO/DAF-16). Rhodiola rosea was shown to have high potential for singlet oxygen scavenging, hydrogen peroxide scavenging, ferric reducing, ferrous chelating and protein thiol protection. Rhodioloside and Rhodiola rosea were the most active inhibitors of stress-induced p-SAPK/p-JNK. Our results are in support of such suggestion.

CONCLUSIONS

The studied extract of Rhodiola rosea exhibited significant analgesic activity in all the pain models used – inhibition of thermal pain, mechanical hyperalgesia and formalin-induced pain behavior. Significant anti-inflammatory activity was observed from Rhodiola rosea extract in carrageenan induced paw edema in rats. The antinociceptive effect is probably induced by inhibition of COX enzyme and PLA2, and the anti-inflammatory effect – by influencing HPA-system, nitric oxide and some other defense proteins.

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ИССЛЕДОВАНИЕ АНТИНОЦИЦЕПТИВНО-ГО И ПРОТИВОВОСПАЛИТЕЛЬНОГО ЭФФЕКТОВ ЭКСТРАКТА RHODIOLA ROSEA L. НА КРЫС

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РЕЗЮМЕ

ВВЕДЕНИЕ: Rhodiola rosea /золотой корень/ представляет уникальный фитоадаптоген с иммуномодуляторным, антиоксидантным, противовоспалительным и антипейцицептивным свойствами.

ЦЕЛЬ: Данное исследование ставит себе цель оценить антицинцицептивное и противовоспалительное действия спиртового/водяного экстракта Rhodiola rosea.

МАТЕРИАЛЫ И МЕТОДЫ: На мужских крыс породы Wistar, разделенных на 3 группы (n = 10), воздействовали физиологическим раствором/контрольную группу/, а также экстрактом Rh. rosea 50 мг/кг массы тела и 100мг/кг массы тела орально/вторая и третья группы/. В целях исследования анальгетического действия использованы 3 теста: тест «горячая плита», тест с механическим давлением на лапку/аналгезиометр/ и формалиновый тест. Аналгетический тест «горячая плита» измеряет реакцию крыс, поставленных на «горячую плиту». Аналогиометрический тест показывает нарастающую с постоянной скоростью силу давления. При формалиновом тесте подсчитано время /в сек./ облизывания лапки после инъекции во время ранней фазы (0-10 мин.) и поздней фазы (20-30 мин.). Для измерения противовоспалительного эффекта был использован каррагинан-индукционный отёк задней лапки крысы. Объём лапки измерен плетисмометром на 2-й, 3-й и 4-й ч.

РЕЗУЛЬТАТЫ: При тесте «горячая плита» крысы, подвергнутые воздействию обеих доз Rh. Rosea, увеличивают статистически значимо латентное время реакции по сравнению с временем реакции крыс контрольной группы. При тесте анальгетического эффекта Rh. rosea в дозе 50 мг/кг крысы проявляют значительное увеличение реакции на давление по сравнению с контрольной группой. При формалиновом тесте доза в 100мл/кг статистически достоверно уменьшает время облизывания лапки только во время первой фазы. При тесте плетисмометр экстракт Rh. rosea синхронизированно ингибирует каррагинан-индукционный отёк лапы по сравнению с физиологическим раствором.

ЗАКЛЮЧЕНИЕ: Изученный экстракт Rh. rosea проявляет значительный анальгетический эффект во всех использованных моделях боли /подавляет термальную боль, уменьшает механическую гиперAINS/ и индуцированное формалином болевое поведение/. Значимо достоверный противовоспалительный эффект наблюдается при каррагинан-индукционном отёке лапки.

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