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EXPERIMENTAL PAPER

Influence of salidroside, a neuroactive compound of *Rhodiola rosea* L., on alcohol tolerance development in rats

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Summary

Introduction: In recent years, the search for potential neuroprotective properties of salidroside and its ability to influence the activity of nervous system become the subject of intense studies of many research groups. None of these studies, however, include an attempt to determine the effect of salidroside on the course of alcohol tolerance *in vivo*.

Objective: The aim of this study was to investigate the ability of salidroside to inhibit the development of alcohol tolerance in rats, determining whether the effect of its action may occur in a dose-dependent manner, reducing both metabolic and central tolerance without affecting body temperature in control rats.

Methods: Male Wistar rats were injected daily with ethanol at a dose of 3 g/kg for 9 consecutive days to produce ethanol tolerance. Salidroside in two doses (4.5 mg/kg and 45 mg/kg *b.w.*) or *vehiculum* was administered orally. On the 1st, 3rd, 5th and 8th day a hypothermic effect of ethanol was measured, while the loss of righting reflex procedure was performed on the 2nd, 4th, 6th and 7th day. On the 9th day rats were treated with salidroside, sacrificed 1 h after ethanol injections and blood was collected for blood-ethanol concentration measurement.

Results: Salidroside at a dose of 45 mg/kg inhibited the development of tolerance to hypothermic and sedative effects of ethanol, whereas insignificant elevation of blood-ethanol concentration was observed. The dose of 4.5 mg/kg *b.w.* had minimal effect, only small inhibition of tolerance to hypothermic action was observed. Salidroside affected neither body mass growth nor body temperature in non-alcoholic (control) rats.

Conclusions: Results of the study indicate that salidroside at a dose of 45 mg/kg inhibited the development of tolerance to the hypothermic effect of ethanol. Observed inhibition of tolerance to the sedative effect of ethanol seems to be associated with salidroside influence on the central nervous system. A comprehensive explanation of the abovementioned observations requires further pharmacological and pharmacodynamic studies.

Key words: *salidroside, alcohol tolerance, rats, dose-dependent manner, ethanol-induced hypothermia, sedative effect*

INTRODUCTION

In the pharmacological treatment of alcoholism, there are only a few clinically effective synthetic drugs, i.e. acamprostate, naltrexone and recently nalmefene being known to reduce craving and relapse in patients addicted to alcohol [1-3]. Alcohol abuse represents worldwide an important risk factor for death and disability [4, 5]. The typology of alcoholism, among other things, consists in drinking large amounts of alcohol for a long time, but also on the occurrence of the phenomenon of tolerance to alcohol. It is believed that this phenomenon of tolerance precedes alcohol addiction. Since alcoholism is one of the most prevalent neuropsychiatric diseases, having an enormous health and socioeconomic impact [5], therefore studies on new active substances with potential antialcoholic effects seem to be a high priority [6, 7].

Pre-clinical and clinical data using plant-derived medicines suggest that novel pharmacological approaches for treatment of alcoholism and alcohol abuse may with usage of natural substances and/or their bioactive compounds. Up to date, kudzu [*Pueraria lobata* (Willd.) Ohwi], St. John's wort (*Hypericum perforatum*

L.), danshen (*Salvia miltiorrhiza* Bunge), ginseng (*Panax ginseng* C.A. Mey.), Japanese raisin tree (*Hovenia dulcis* Thunb.), ibogaine (*Tabernanthe iboga* H. Bn.), evening primrose (*Oenothera biennis* L.), prickly pear fruit (*Opuntia ficus-indica* (L.) Mill.), purple passionflower (*Passiflora incarnata* L.), thyme (*Thymus vulgaris* L.), fenugreek seed (*Trigonella foenum-graecum* L.), ginger (*Zingiber officinale* Roscoe) and others drew the attention of researchers [6, 8-12].

In recent years, the search for potential neuroprotective properties of salidroside (Sal) (*p*-hydroxyphenethyl- β -D-glucoside, phenylethanoid; a potent antioxidant compound with the chemical structure of phenol glycosides extracted mainly from the root of *Rhodiola rosea* L. (RR) [11, 13]), its ability to influence the activity of nervous system has become the subject of intense studies of many research groups [11, 12, 14-24]. None of these studies, however, include an attempt to determine the effect of salidroside on the course of alcohol tolerance *in vivo*, nor evaluated the molecular mechanism of its action in the mesolimbic structures of the brain reward system.

Therefore, the aim of this study was to investigate the ability of Sal to inhibit the development of alcohol

tolerance in rats, determination whether the effect of its action may occur in a dose-dependent manner, reducing both metabolic and central tolerance without affecting body temperature in control rats. The course of the experiment and the obtained results are presented hereafter.

MATERIAL AND METHODS

Animals

Male Wistar rats were kept in plastic cages (dimensions 50x30x20 cm), five animals per one cage. *Ad libitum* access to fresh water and standard laboratory feed were provided. During the experiments, constant temperature (20±2°C), humidity (60–65%) and reversed 12 h light/dark cycle were maintained. All procedures were performed during the dark phase, which is a natural time of activity of these animals.

Reagents

Solutions of Sal at a concentration of 1 mg/ml and 10 mg/ml were used. Solutions were made using 65% salidroside obtained from Hangzhou Sage Chemicals Co., Ltd. (United Kingdom). Other reagents used in the experiment were of analytical grade and obtained from approved sellers.

Experimental protocol

The experiment was performed in accordance with Polish law and Local Ethics Committee in Poznań

(decision No. 87/2015). Tolerance was established by a daily intraperitoneal administration of ethanol (EtOH) at a dose of 3g/kg *b.w.* for 9 consecutive days, according to the model proposed by Crabbe [25].

Rats were randomly divided into 6 groups (tab. 1). At the beginning of the experiment, animals were weighted for calculate a dosage of reagents (EtOH and Sal). In following days, body weighting was repeated in order to correct doses. Rats were assigned to groups with lower Sal dose (4.5 mg/kg – 4.5S; 1 mg/ml Sal solution, intragastrically) and groups with higher Sal dose (45 mg/kg – 45S; 10 mg/ml Sal solution, intragastrically). Control groups were intragastrically treated with 0.5% methylcellulose (MC) in complementary volumes. One hour after the substance treatment rats were intraperitoneally injected with 30% EtOH at a dose of 3 g/kg *b.w.* or with water at complementary volume, respectively. Such schedule was repeated for 9 consecutive days in order to develop the alcohol tolerance in animals. At the 1st, 3rd, 5th and 8th day, body temperature was measured and hypothermic action of ethanol was evaluated. At 2nd, 4th, 6th, and 7th day, a sedative action of EtOH was evaluated, respectively. At 9th day of the experiment, animals were sacrificed by decapitation and peripheral blood was immediately collected for future analysis.

Temperature measurement

In selected days, body temperature of rats was measured once before treatment (t_0) and at three time points after the administration of EtOH (30, 60 and 90 minutes after EtOH injection). Measurements were performed using calibrated rectal

Table 1.
Groups assignment in the experiment according to administered drugs

Group	<i>n</i>	MC, i.g.	4.5S i.g.	45S i.g.	H ₂ O i.p.	EtOH i.p.
MC+H ₂ O	7	+			+	
MC+EtOH	20	+				+
4.5S+H ₂ O	7		+		+	
4.5S+EtOH	14		+			+
45S+H ₂ O	7			+	+	
45S+EtOH	16			+		+

n – number of rats in each group; i.g. – intragastric route of administration; i.p. – intraperitoneal route of administration; 4.5S i.g. – salidroside i.g. at a dose of 4.5 mg/kg *b.w.*; 45S i.g. – salidroside i.g. at a dose of 45 mg/kg *b.w.*; MC i.g. – 0.5% methylcellulose at complementary volume; EtOH i.p. – ethanol at the dose of 3g/kg *b.w.*; H₂O i.p. – water i.p. at complementary volume.

thermometer. Probe of the apparatus was inserted into rectum for 20 seconds, what allowed to establish the temperature value on the scale.

Sedative effect

Sedative effect of EtOH was evaluated on the number of rats in each ethanol group that lost righting reflex (LRR), its duration was measured as well. At selected days, after administration of EtOH, rats were separately placed in the cages. Immediately after LRR they were situated in supine position and the time until return to normal position was noted. Time of sleeping was defined as a difference between time of LRR and time of recovery.

Measurement of ethanol concentration in blood

On the 9th day of experiment, the last dose of Sal administration was given and rats were sacrificed 1 h after ethanol administration and blood samples were immediately collected. 100 μ l of each sample was mixed with 500 μ l of 0.015% propionitrile (internal standard) and analyzed using Gas Chromatography (GC) with Headspace. Sampling was performed with Perkin Elmer AutoSystem XL GC with Headspace Sampler Turbomatrix 40, equipped with two capillary columns Elite BAC-1, BAC-2 (Perkin Elmer, length: 30 m, diameter: 0.32 mm, film

thickness: 1.8 μ m, software: Totalchrom Workstation ver. 6.2.0) [26].

Statistical analysis

Statistical analysis were performed using Statistica 6.0 Software (StatSoft, Poland). Data are presented as the mean \pm SEM value. Analysis of variance (ANOVA) with repetitions was used for body mass and ethanol-induced hypothermia data to evaluate the differences between groups. Least Significant Difference (LSD) test was used for post-hoc analysis. ANOVA with repetitions and Pearson's chi-square test were used for LRR analysis.

RESULTS

Body mass change analysis

Significant differences among groups in mean body masses could be observed (fig. 1). Two-way ANOVA with replication revealed significant main effects of treatment and time ($F(5,46)=19.38$; $F(1,46)=45.61$; both $p=0.0000$). The interaction between factors was also significant ($F(9,102)=8.11$, $p=0.0000$). The post-hoc analysis revealed that ethanol injection inhibited body mass growth in group MC+EtOH in comparison with MC+H₂O group ($p=0.0000$). The 4.5S+H₂O and 45S+H₂O groups did not differ from

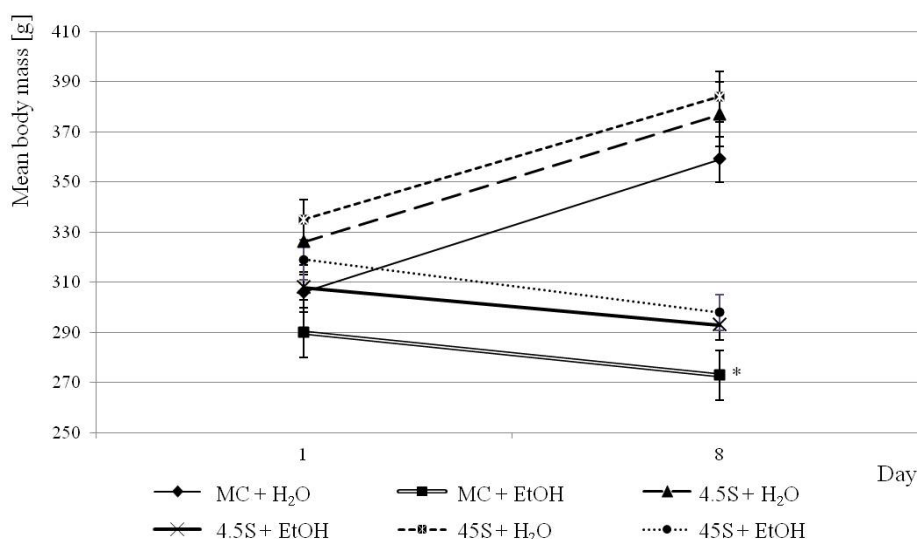


Figure 1.

Effect of salidroside (S) given in two doses (4.5 and 45 mg/kg) and ethanol (EtOH) administration on rats' mean body mass changes data from 1st and 8th day of the experiment, details included in the text

mean values \pm SEM; * - $p=0.0000$, difference from the value in the MC+H₂O group

MC+H₂O group neither on 1st nor 8th day of the experiment. The 4.5S+EtOH and 45S+EtOH groups did not differ from MC+EtOH group neither on the 1st nor 8th day as well.

Ethanol-induced hypothermia

Experimental groups differed in mean body temperatures at each time point (details in fig. 2–4). During whole experiment, mean body temperatures at time points T30, T60 and T90 in MC+EtOH group were significantly lower in comparison with MC+H₂O group ($p<0.05$). From 1st to 5th day, mean body temperature in MC+EtOH group was falling. This trend reversed on the 8th day, when mean body temperature raised (however, insignificantly) at time points T30 and T60 and significantly at time point T90 ($p=0.0000$; different from value of 5th day). In the 4.5S+EtOH group, body temperature of rats changes followed MC+EtOH group. There were no significant differences between these group except last measurement, on the 8th day, when rats from group 4.5S+EtOH reached significantly lower mean body temperature than group MC+EtOH. As

for 45S+EtOH rats, mean body temperatures did not differ significantly from group MC+EtOH on the 1st, 3rd and 5th day of the experiment. On 8th day we observed further body temperature decrease at all three time points. Consequently, the 45S+EtOH group had significantly lower mean body temperature than MC+EtOH group ($p<0.01$) on that day. Groups 4.5S+H₂O and 45S+H₂O did not differ significantly from MC+H₂O group each day.

Sedative effect

The development of tolerance to sedative action of EtOH was evaluated on the basis of number of rats in each group which LRR and on its duration after intraperitoneal administration of EtOH at the dose of 3.0 g/kg (fig. 5, 6). On the 2nd day, most rats injected with EtOH lost their righting reflex, there was no significant difference between groups in the number of sleeping rats. Situation repeated on the 4th day, when more than 90% of animals in each group showed LRR. Sedative action of EtOH has weakened in the group MC+EtOH on the 6th and 7th day of the experiment (62% and 33% of

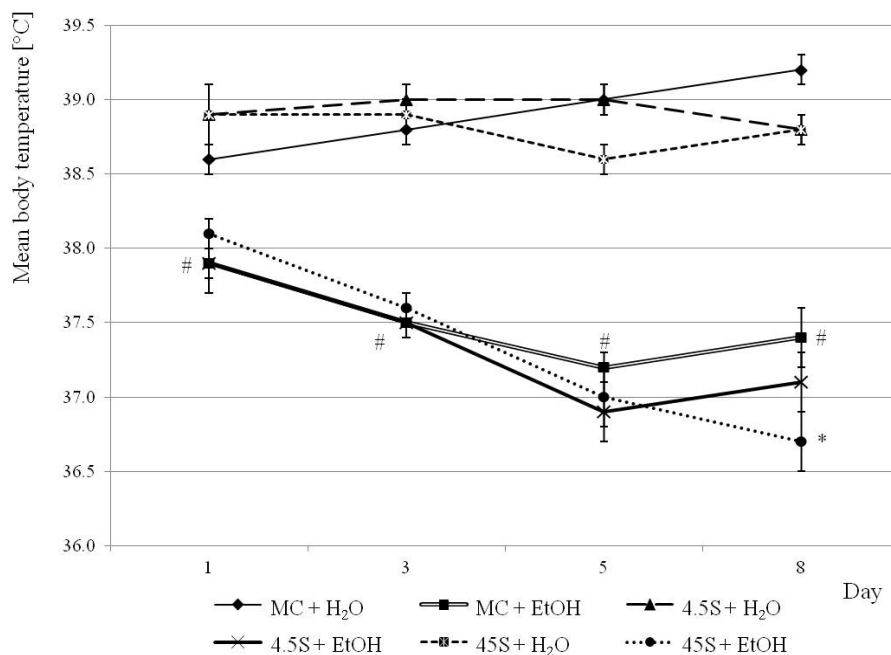


Figure 2.

Effect of salidroside (S) given in two doses (4.5 and 45 mg/kg) and ethanol (EtOH) administration on mean body temperatures of rats at time point T30

mean values \pm SEM; # - $p<0.05$, different from the value in the MC+H₂O group; * - $p<0.01$, different from the value in the MC+EtOH group. Two way ANOVA test with replication revealed significant main effects of drug treatment and time ($F(5,46)=54.4$; $F(3,138)=15.6$; both $p=0.0000$). Interaction between these two factors was also significant ($F(15,138)=5.90$, $p=0.0000$).

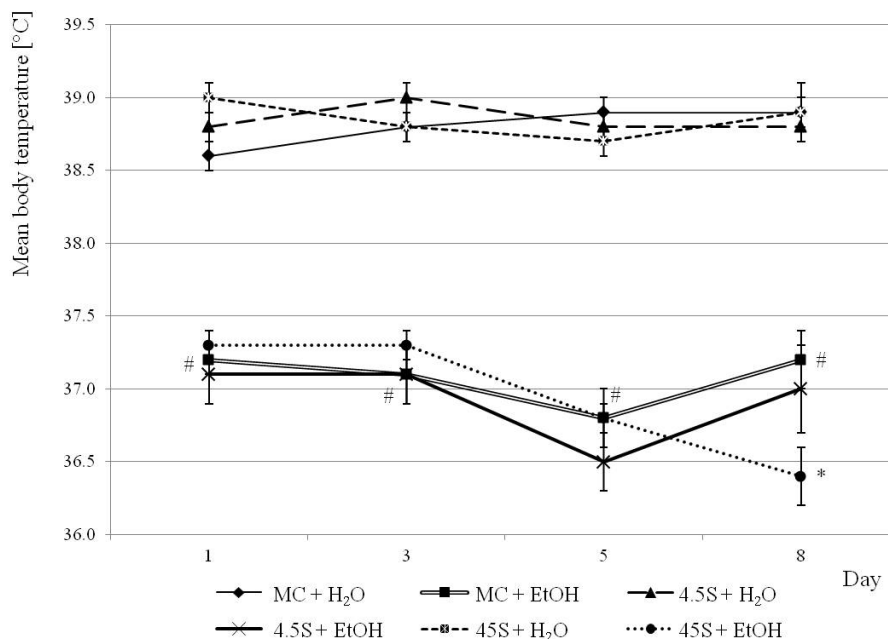


Figure 3.

Effect of salidroside (S) given in two doses (4.5 and 45 mg/kg) and ethanol (EtOH) on mean body temperatures of rats at time point T60

mean values \pm SEM; # - $p < 0.05$, different from the value in the MC+H₂O group; * - $p < 0.01$, different from the value in the MC+EtOH group. Two way ANOVA test with replication revealed significant main effects of drug treatment and time ($F(5,46)=59.9$; $F(3,138)=3.45$; respectively, both $p < 0.05$). Interaction between these two factors was also significant ($F(15,138)=2.47$, $p < 0.01$)

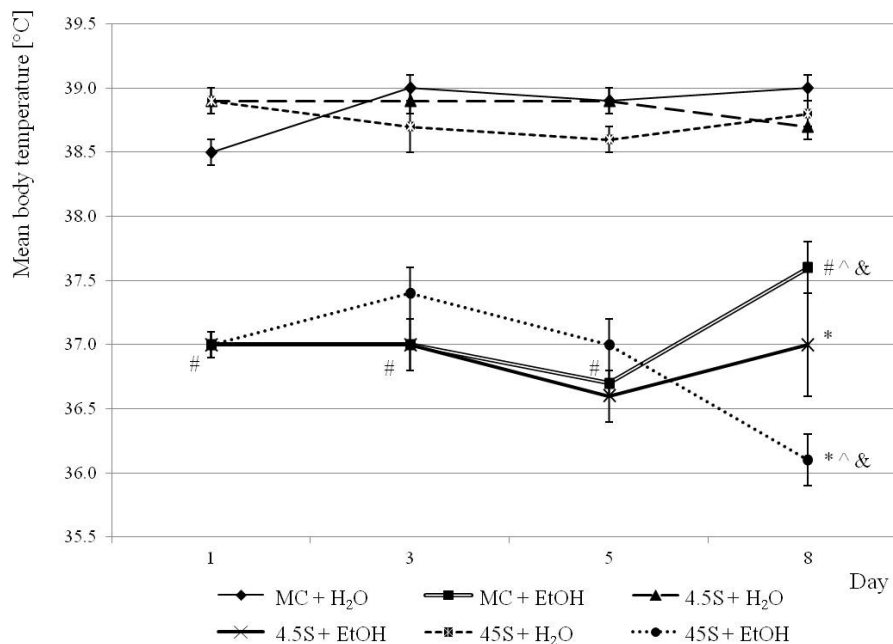


Figure 4.

Effect of salidroside (S) administered in two doses (4.5 and 45 mg/kg) and ethanol (EtOH) on mean body temperatures at time point T90

mean values \pm SEM; # - $p < 0.05$, different from the value in the MC+H₂O group; * - $p < 0.01$, different from the value in the MC+EtOH group; ^ - $p < 0.05$, different from the value on the 5th day in the group; & - $p < 0.01$, different from the value on the 1st day in the group. Two way ANOVA test with replication revealed significant main effects of drug treatment and time ($F(5,46)=54.4$; $F(3,138)=15.6$; both $p = 0.0000$). Interaction between these two factors was also significant ($F(15,138)=5.90$, $p = 0.0000$).

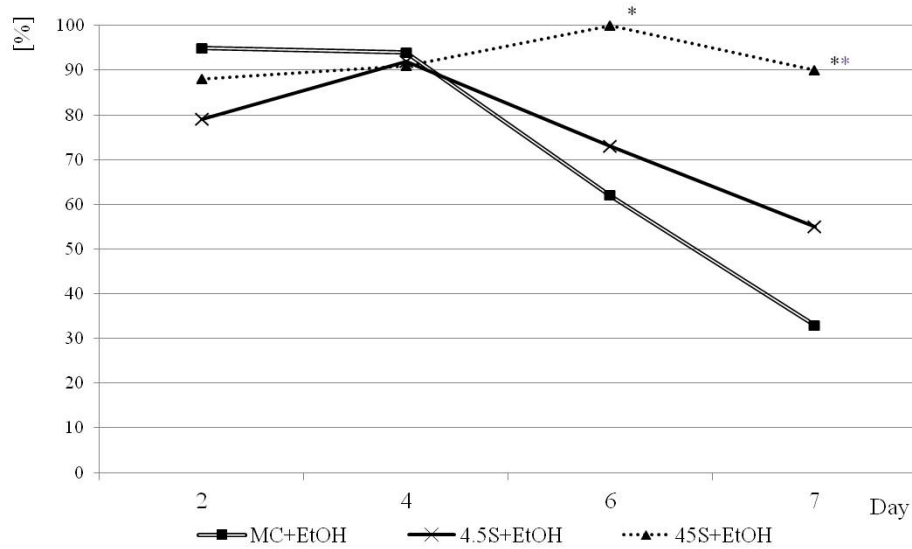


Figure 5.

Effect of solidoside (S) given in two doses (4.5 and 45 mg/kg) and ethanol (EtOH) on the number of rats that lost righting reflex (LRR).

Values expressed in percentage of rats with LRR * - $p < 0.05$, different from the value in the MC+EtOH group; * * - $p < 0.01$, different from the value in the MC+EtOH group

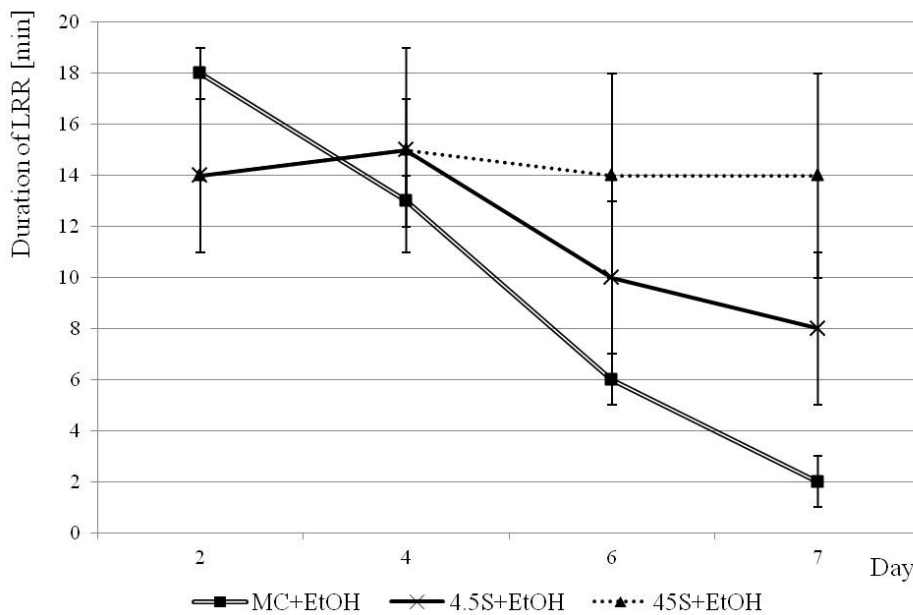


Figure 6.

Effect of solidoside (S) given in two doses (4.5 and 45 mg/kg and ethanol (EtOH) on duration of lost of righting reflex (LRR)

animals lost their righting reflex, respectively). We observed similar effect in the group 4.5S+EtOH, on the 6th day 73% and on 7th day 55% rats slept. We did not observe attenuation of EtOH action in group 45S+EtOH at any day, always more than 88% animals showed LRR. It resulted in significant difference in a number of sleeping rats in 45S+EtOH and MC+EtOH groups on the 6th day (Pearson's

chi-squared test, $p < 0.05$) and the 7th day (Pearson's chi-squared test, $p < 0.01$). As for duration of LRR, no significant differences among groups were found (ANOVA interaction $F(6,90)=1.006$; $p=0.4268$), although a trend has been observed. As experiment was moving forward, duration of LRR in group MC+EtOH shortened, while in group 45S+EtOH it stayed on fairly constant level.

Ethanol concentration in blood

Ethanol concentration in blood was measured on the 9th day of the experiment, one hour after ethanol administration (tab. 2). We found no significant difference among groups in terms of blood ethanol concentration (ANOVA $F(2,27)=0.4117$, $p=0.6666$), although a trend could be observed. The lowest mean ethanol concentration was in group MC+EtOH and the highest was in group 45S+EtOH

Table 2.

Effect of salidroside (S) administration given in two doses (4.5 and 45 mg/kg, i.g.) on blood-ethanol concentration on the 9th day, 1h after ethanol (EtOH) administration (3 g/kg, i.p.)

Group	<i>n</i>	ethanol concentration in blood [g/l]
MC+EtOH	10	2.053±0.077
4.5S+EtOH	10	2.084±0.104
45S+EtOH	10	2.170±0.103

mean values ± SEM

DISCUSSION

Organism response to given drug changing in time after repeated administration and attenuation of that response is called tolerance. One of the substances well known for such effect is ethyl alcohol. It is believed that tolerance develops mainly to aversive properties of ethanol and appearance of particular effects may vary in time (that is why acute, rapid and chronic tolerance is distinguished) [27, 28]. Appearance of tolerance to alcohol action is a strong factor in developing the addiction to alcohol [29]. Therefore, searching for a new substances that might affect development of tolerance seems to be justified. The aim of our study was to examine the influence of Sal, the active compound of *R. rosea*, on the development of alcohol tolerance in rats according to model proposed by Crabbe [25], subsequently successfully applied in our team [26]. Development of tolerance to ethanol can be evaluated on changes in the hypothermic and sedative action of ethanol. Single ethanol exposure causes decrease of body temperature and strong sedative effect in animals. However, prolonged exposure results in a development of tolerance, manifested by a less severe decrease of body temperature and weaker sedative action at the same dose of ethanol. Rats

develop tolerance to these ethanol-induced effects within a week of daily ethanol administration [25, 30]. Therefore, the measurement of body mass and temperature changes (hypothermic effect) and elimination ethanol-induced LRR has become a crucial methodological approach for the evaluation of potential anti-alcoholic xenobiotics interfering the development of tolerance to ethanol.

The usage of Sal in our experiment was based on current scientific reports on *R. rosea* preparations and this compound itself [11, 12, 14-16, 18, 20, 22-24, 31-33]. It has been found, for example, that hydroalcoholic extract from *R. rosea* had the ability to prevent establishing of conditioned place preference (CPP) in mice and was effective in facilitating extinction of morphine-induced CPP [34]. Moreover, Sal itself prevented the acquisition and reinstatement of nicotine-induced CPP in mice [35]. The results of other studies, both *in vitro* and *in vivo*, also indicate the ability of Sal to affect the activity of nervous system. Zhao *et al.* [36], for example, revealed that Sal was able to make rat mesenchymal stem cells (MSCs) to differentiate into dopaminergic neurons. Focal cerebral ischemia-reperfusion injury in rat model was protected by Sal pretreatment possibly involving its ability to reduce the permeability of blood brain barrier [15, 20]. Following experimental traumatic brain injury in mice, Sal administration produced behavioral improvement and decreased apoptosis via modulation of PI3K/Akt signaling. In $A\beta$ (25–35)-induced SH-SY5Y human neuroblastoma cells, it reduced the oxidative stress via enhancing the activity of antioxidant enzymes, decreased also the level of Bax and increased the B-cell lymphoma-extra large [Bcl-X(L)] level [17]. This compound revealed also a neuroprotective effect against hydrogen peroxide-induced apoptosis and markedly attenuated H_2O_2 -induced cell viability loss and apoptotic cell death in a dose-dependent manner [37]. Furthermore, Sal attenuated β -amyloid-induced cognitive deficits via modulating oxidative stress and inflammatory mediators in rat hippocampus [16], also its action on memory improvement, anxiolytic and antidepressant activities in mice were observed [38]. In addition, this compound influenced the SIRT1/NF- κ B signal pathway in the hippocampus in Alzheimer's disease animal model [23]. It also induced neurogenesis in dentate gyrus of the hippocampus [39] and prevented death of dopaminergic neurons induced by 6-OHDA (6-hydroxydopamine) [22]. Recent studies also showed neuroprotective potential of Sal metabolite – *p*-tyrosol – after global cerebral ischemia in rats [20] or its protective

effect against dopaminergic neuronal cell death in *in vitro* model of Parkinson's disease [40]. None of these studies, however, did include an attempt to determine the effect of salidroside on the course of alcohol tolerance *in vivo*.

Based on above, we hypothesized that Sal can affect the alcohol action in model organisms. Doses of the compound were established on the basis of our previous pilot study aimed on extract from *R. rosea* influence on alcohol tolerance development [unpublished data], in which rats were administered intragastrically with the extract (the content of Sal was calculated on body weight and resulted with concentration 0.45 mg/kg). Due to the report that bioavailability of some compounds from extracts is higher as compared with administration in pure form (e.g. 10 times more potent action of daidzin in extract than in pure form), we decided to multiply the dose 10 times in our study [41]. To examine the potential dose-dependency, we have applied a higher dose of 45 mg/kg *b.w.*, as well.

In our study, repeated injections of EtOH at a dose of 3 g/kg *b.w.* resulted in a significant inhibition of body mass growth in rats, as compared with control animals. Such effect is in agreement with general consensus about anorexic properties of alcohol [42]. Treatment with Sal neither influenced body mass changes in groups administered with EtOH, nor groups receiving water. Furthermore, Sal did not influence body temperature in rats in normal conditions (control rats). In the MC+EtOH group we observed significant decrease of mean body temperature in comparison with MC+H₂O group at time T30, T60 and T90 points during whole experiment. On the 8th day, mean body temperature raised in this group in comparison with 5th day, most clearly at T90 when difference reached the level of significance. We concluded that during these days the tolerance to hypothermic action of ethanol started to develop. This result differs from observations by Okulicz-Kozaryn *et al.* [30] when such tolerance appeared on the 3rd day. The 4.5S+EtOH and 45S+EtOH groups did not differ significantly from MC+EtOH group on the 1st, 3rd and 5th day. On the 8th day, rats from 45S+EtOH group reached significantly lower mean body temperatures at each time point as compared with MC+EtOH group. The group administered with a lower Sal dosage (4.5S+EtOH) reached significantly lower temperature only at T90 time point. Therefore, it means that Sal inhibited the development of tolerance to hypothermic action of ethanol and such effect was dose-dependent. In MC+EtOH group we

also observed the development of tolerance to sedative action of EtOH, since on the 6th and 7th day less rats showed LRR than on preceding days and trend towards shortening duration of LRR was also observed. This observation is also in agreement with abovementioned study [30]. In 45S+EtOH group, the number of rats that showed LRR stood over 88% during whole experiment, which significantly distinguished this group from MC+EtOH group on the 6th and 7th day. There was no trend towards shortening the duration of LRR in this group as well. We did not observe the influence of Sal in lower dose (4.5S+EtOH group) on ethanol-induced LRR.

Taken together, these results indicate that Sal at dose 45 mg/kg *b.w.* inhibited the development of tolerance to sedative action of ethanol. Mechanism of its action remains largely unknown. Groups did not differ significantly in terms of blood-ethanol concentrations, however, a trend could be seen – the lowest concentration on the 9th day was in MC+EtOH group and the highest in 45S+EtOH group. Hence, Sal impact on ethanol pharmacokinetics could contribute to observed effects. On the other hand there is an evidence of enhancement of pentobarbital sedative action caused by Sal [43]. Barbiturates and ethanol are well known for similarities in action on GABA-ergic neurotransmission and a cross-tolerance between pentobarbital and ethanol has been already confirmed [44]. These facts may indicate that observed in our study interaction between Sal and EtOH may be of pharmacodynamic character. The compound at the dose of 4.5 mg/kg *b.w.* revealed much smaller impact on alcohol tolerance than that with a higher dose. Inhibition of development of tolerance to hypothermic action of EtOH was seen only at the last measurement point on the 8th day (T90) and no influence on sedative action of EtOH was observed. It is possible that 4.5 mg/kg *b.w.* dose of Sal is too low to effectively trigger a significant pharmacological action. Also a potential rapid metabolism of that compound could not allow to reach significant concentration in blood with small oral doses [18].

CONCLUSIONS

Results of our study indicate that daily exposure to EtOH at the dose of 3 g/kg *b.w.* resulted in the development of tolerance to hypothermic and sedative effects of ethanol in Wistar rats. Sal at a dose of 45 mg/kg inhibited the development of tolerance to hypothermic effect of EtOH, while insignificantly

elevated the ethanol concentration in blood. Observed inhibition of tolerance to sedative effect of EtOH seems to be associated with Sal influence on central nervous system. The dose of 4.5 mg/kg had minimal effect with only a slight inhibition of tolerance to hypothermic effect. EtOH impaired body mass growth whereas Sal did not affect this parameter neither in rats receiving EtOH nor control groups, and did not change the body temperature in non-alcoholic rats.

The complex explanation of abovementioned issues requires therefore more comprehensive pharmacological and pharmacodynamic studies supported by high-throughput molecular analyses of molecular and biochemical orchestra in the brain mesolimbic structures of the reward system under the influence of Sal and/or other bioactive compounds from *R. rosea* extracts.

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Conflict of interest: Authors declare no conflict of interest.

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Wpływ salidrozydu, neuroaktywnego związku *Rhodiola rosea* L. na rozwój tolerancji alkoholu u szczurów

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Streszczenie

Wstęp: W ostatnich latach poszukiwanie potencjalnych właściwości neuroprotektoryjnych salidrozydu i jego zdolności do wpływania na aktywność układu nerwowego stało się przedmiotem intensywnych badań wielu grup naukowców. Żadne z tych badań nie obejmowało jednak próby określenia działania tego związku na przebieg tolerancji alkoholowej *in vivo*.

Cel: Celem doświadczenia była ocena zdolności salidrozydu do hamowania rozwoju tolerancji na alkohol u szczurów oraz ustalenie czy efekt jego działania może wystąpić w sposób zależny od dawki, czy może znosić tolerancję metaboliczną i centralną bez wpływu na temperaturę ciała u zwierząt.

Metody: Samcom szczurów szczepu Wistar codziennie podawano etanol w dawce 3 g/kg m.c. i.p. przez 9 kolejnych dni w celu uzyskania tolerancji jego obecności. Salidrozyd w dwóch dawkach (4,5 mg/kg i 45 mg/kg m.c.) lub *vehiculum* podawano i.g. W kolejnych dniach mierzono hipotermiczny efekt działania etanolu oraz utratę odruchu utrzymania postawy ciała. Ostatniego dnia po dekapitacji zwierząt pobrano krew w celu pomiaru stężenia we krwi etanolu.

Wyniki: Salidrozyd w dawce 45 mg/kg hamował rozwój tolerancji objawiający się hipotermią i uspokajającym działaniem etanolu, podczas gdy obserwowano nieznaczne zwiększenie stężenia etanolu we krwi.

Dawka 4,5 mg/kg m.c. wykazała minimalny efekt, obserwowano jedynie niewielkie zahamowanie tolerancji na działanie hipotermiczne. Salidrozyd nie wpływał ani na przyrost masy ciała ani na temperaturę ciała u szczurów nie otrzymujących alkoholu (kontrolnych).

Wnioski: W badanym układzie doświadczalnym z wykorzystaniem eksperymentalnego modelu tolerancji potwierdzono hamujący wpływ salidrozydu w dawce 45 mg/kg m.c. na rozwój tego procesu. Hamowanie tolerancji działania sedacyjnego etanolu wydaje się być związane z wpływem salidrozydu na ośrodkowy układ nerwowy. Kompleksowe wyjaśnienie powyższych obserwacji wymaga dalszych badań farmakologicznych i farmakodynamicznych.

Słowa kluczowe: : *salidrozyd, tolerancja alkoholu, szczury, efekt zależny od dawki, hipotermia wywołana etanolem, działanie sedacyjne*