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# Valerian extract characterized by high valerenic acid and low acetoxy valerenic acid contents demonstrates anxiolytic activity

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ABSTRACT

Valerian is one of the most commonly used herbal remedies for the treatment of insomnia and anxiety. Valerian extracts allosterically modulate GABAA receptors, an action related to valerenic acid, which is one of the active compounds determined from pharmacological studies. Derivatives of valerenic acid, i.e. acetoxy valerenic acid or hydroxy valerenic acid, do not allosterically modulate GABAA receptors, but they bind to identical binding sites. Therefore, the question arises whether they might interfere with the effects of valerenic acid. Two valerian extracts were tested in the elevated plus maze test and the tail suspension test for anxiolytic and antidepressive activity, respectively. Reference substances were diazepam (1.0 mg/kg) and imipramine (30 mg/kg). The extracts were standardized to the identical total amounts of the acids (0.1; 0.5; 1.0 and 2.0 mg/kg), i.e. valerenic and acetoxy valerenic acid, but the ratio between the acids was different (12:1 and 1:1.5). The extract with the ratio 12:1 prolonged the time spent on the open arm significantly when 0.5 mg/kg was applied. Of the other extract, with the ratio 1:1.5, four times that amount was required (2.0 mg/kg). Both of the tested extracts did not show any antidepressive effect, rather the other way around, the extract with the ratio 1:1.5 prolonged the immobility phase. However, since the core body temperature was reduced by the 1.0 and 2.0 mg/kg extract dose, the prolongation may be related to the temperature phenomenon and is not indicative of a specific depressive action. In conclusion, the anxiolytic activity of the valerian extract seems rather related to valerenic acid and, moreover, standardization with respect to the total amount of valerenic acids, i.e. valerenic acid together with acetoxy valerenic acid, is misleading.



## Introduction

Valerian is one of the most commonly used herbal remedies for the treatment of insomnia and anxiety. For medical purposes *Valeriana officinalis L.* is used. Pharmacological studies identified valerenic acid (VA) as one of the active compounds. The anxiolytic potential of VA is supported by in vitro experiments on the gamma-aminobutyric acid type A receptor (GABAA) (Yuan et al. 2004; Khom et al. 2007; Sichardt et al. 2007; Trauner et al. 2008; Benke et al. 2009), as well as in vivo experiments in mice (Benke et al. 2009; Khom et al. 2010) and rats (Murphy et al. 2010). In addition, it was demonstrated in vitro that VA activity at GABAA receptors is mediated preferentially by the β3 subunits (Khom et al. 2007, 2010). This was confirmed by in vivo studies to demonstrate anxiolytic activity, since point mutation in the β3 subunit abolished the anxiolytic response to VA but not to diazepam (Benke et al. 2009).

GABA is the major inhibitory neurotransmitter in the brain and essential for the overall balance between neuronal excitation and inhibition, therefore vital to normal brain function. Any imbalance can cause disorders like depression and sedation, or anxiety, restlessness and insomnia. The GABAA receptor is a chloride-conducting receptor composed most frequently of alpha, beta, and gamma subunits assembled as a pentameric structure, forming a central pore; but other subunit compositions are also possible (Sieghart 1995; Olsen and Sieghart 2008). Each subunit has a long extracellular agonist binding domain. GABAA receptors are the site of action for a variety of pharmacologically and clinically important agents, such as benzodiazepines, barbiturates, neuroactive steroids, anaesthetics, and convulsants (Johnston 2005; Atack 2005; Rupprecht et al. 2006; Möhler 2006).

Valerian extracts (VE) allosterically modulate GABAA receptors, an activity related to valerenic acid (Trauner et al. 2008). Khom reported that GABAA receptors containing β2 or β3 subunits are

*Abbreviations:* AVA, acetoxy valerenic acid; BCT, body core temperature; EPM, elevated plus maze; HVA, hydroxy valerenic acid; VA, valerenic acid; VE, valerian extract; TST, tail suspension test.

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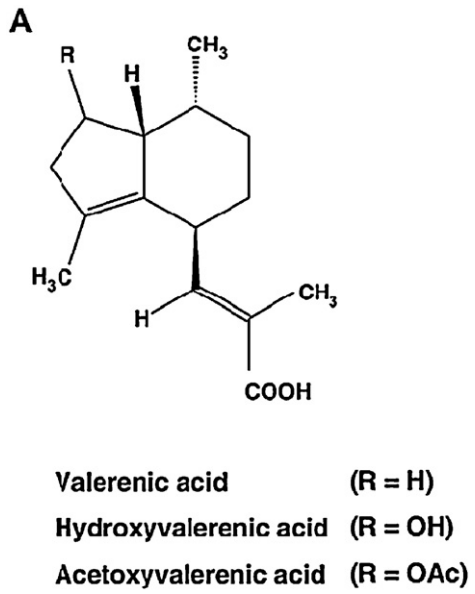


**Fig. 1.** Structure formula of valerenic acid and their derivatives.

required for this action. Derivatives of valerenic acid (VA), i.e. acetoxy valerenic acid (AVA) or hydroxy valerenic acid (HVA) (Fig. 1), do not modulate GABAA receptors allosterically (Khom et al. 2007). Identical results were obtained with the fraction of a VE that contained AVA (Kim et al. 2008). However, AVA at higher concentrations was able to block the open GABAA channel (Khom et al. 2007) like VA, and additionally, HVA displaced $^{3}$H valerenic acid from cerebral membrane binding sites (Benke et al. 2009), indicating that both derivatives may act at the identical binding site, even if they fail in allosteric modulation.

When comparing especially prepared valerian extracts, it was revealed that the absence of valerenic acids, e.g. VA and AVA, led to a loss of activity at the GABAA receptors, whereas transformation of AVA to HVA elevated that activity caused by VA (Trauner et al. 2008). These results, obtained from in vitro experiments, could suggest that AVA is capable of inhibiting VA action, since the response was elevated after AVA removal. However, this remains to be confirmed in vivo, since it is impossible to predict in vivo results from experimental in vitro conditions (Khom et al. 2010).

The aim of the present study was to test valerian extracts distinguishable in their contents of VA and AVA in two behavioural tests, i.e. the elevated plus maze test (EPM) demonstrating anxiolytic efficacy, and the tail suspension test (TST) as an equivalent for antidepressive action. The orally administered extracts were adjusted in such a way that the applied sum of VA and AVA was identical for both extracts, however with a different ratio of VA and AVA.

## Materials and methods

In order to test the effect of VEs with different ratios of VA: AVA on GABA-related in vivo models, two different VEs were prepared. Valerian roots and rhizomes from a specifically selected species were used, as well as standard material from Poland (European Pharmacopoeia, Supplement 5.7. Valerian dry hydroalcoholic extract (1898)). A sample specimen from the selected species (HAL 115562) is preserved in the Herbarium of the Institute of Biology, Martin Luther University Halle, Germany. The dried starting material was stirred with hydro-alcohol (70%, v/v) in a ratio of 1:5 at 45 °C for 31/2 days and then separated by filtration under pressure of 2 bar. The obtained tinctures were concentrated using a rotary evaporator. Samples of both extracts were analyzed by ultra performance liquid chromatography (UPLC) (method was based on the Ph Eur monograph for valerian dry hydro-alcoholic extract [6.8./1898]). The chromatograms of both extracts are represented in Fig. 2 and quantitative data are given in Table 1. The results for the extracts with respect to their VA to AVA ratio are given in Table 2.

Male CD-1 mice (Charles River Sulzfeld, Germany) were kept under controlled laboratory conditions with a light/dark cycle of 12:12 (lights on at 06.00 a.m.), temperature 20 ± 2 °C, and air humidity 55–60%. The animals had free access to commercial pellets (ssniff R/M-H, ssniff Spezialdiäten GmbH, Soest, Germany) and tap water. The animals were housed in groups of 10 in Macrolon III cages. At the beginning of the experiments the mice were 8 weeks old. The number of animals per group was between 12 and 19. The work reported here was conducted in accordance with EC regulations and the National Act on the Use of Experimental Animals (Germany). The protocol was approved by the Saxony-Anhalt Committee on Animal Care.

VEs (0.1; 0.5; 1.0; 2.0 mg/kg, with respect to the total sum of VA and AVA), diazepam (Faustan®, AWD Pharma GmbH & Co. KG, Dresden, Germany), and imipramine (Sigma, Düsseldorf, Germany) as references were administered orally using a mouse gavage feeding needle (24 gauge, FST, Heidelberg, Germany). As control a 0.9%

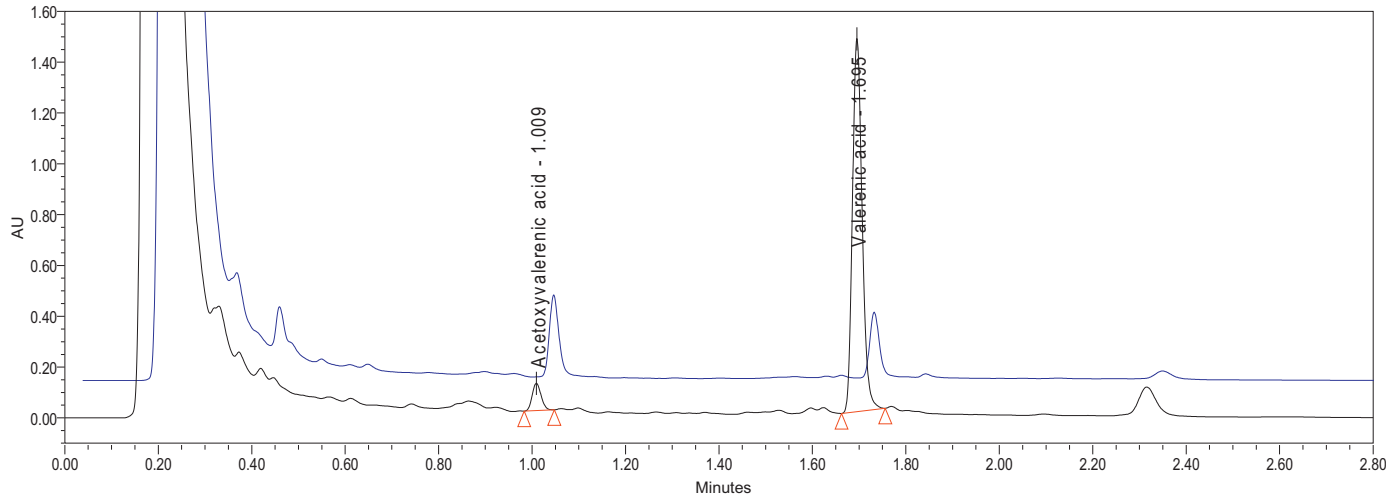


**Fig. 2.** Chromatogram of both of the valerian extracts.

**Table 1**
Content of valerenic acid as well as acetoxy-valerenic acid in both of the valerian extracts.

| Sample name | Content valerenic acid (mg/l) | Content acetoxy-valerenic acid (mg/l) | Sum of contents valerenic acid + acetoxy-valerenic acid (mg/l) | Ratio valerenic acid acetoxy-valerenic acid |
|---|---|---|---|---|
| Valerian extract 1 (VE-1) | 1165.5 | 98.3 | 1263.7 | 12: 1 |
| Valerian extract 2 (VE-2) | 199.5 | 308.8 | 508.3 | 1: 1.5 |

**Table 2**
Composition of the extracts VE-1 and VE-2 regarding their content of VA and AVA as well as the per os administered amount of the extracts.

| Content VA + AVA (mg/kg) | VE-1 | | | VE-2 | | |
|---|---|---|---|---|---|---|
| | VA | AVA | Amount (ml/kg) p.o. | VA | AVA | Amount (ml/kg) p.o. |
| 0.1 | 0.0922 | 0.00776 | 0.16 | 0.03925 | 0.06075 | 0.20 |
| 0.5 | 0.4610 | 0.0388 | 0.40 | 0.19630 | 0.30380 | 0.98 |
| 1.0 | 0.9220 | 0.0776 | 0.79 | 0.39250 | 0.60750 | 1.97 |
| 2.0 | 1.8440 | 0.1552 | 1.58 | 0.78500 | 1.21500 | 3.93 |

VA: valerenic acid; AVA: acetoxy-valerenic acid.

saline solution was administered. The given volume was 0.1 ml/10 g body weight.

*Elevated plus maze (EPM)*

Situational anxiety was measured in the elevated plus maze test (Rodgers 1997). The maze was made of black polyvinyl chloride and had two open and two closed arms (50 cm × 10 cm × 40 cm) mounted 50 cm above the floor. The floor of the arms was smooth. Light intensity was 30 lux. A mouse was placed on the central platform of the apparatus facing a closed arm. A camera on the ceiling of the test room was used to score and tape the animals' behaviours from an adjacent room for a period of 7 min. The number of entries into open arms, time spent on open arms, and the time spent on closed arms were recorded. To evaluate the anxiolytic effect, the time spent on the open arm was determined, which is prolonged by anxiolytic substances. In addition, total changes in movements were also analyzed, which reflect locomotor activity. The maze was cleaned after each trial.

*Tail suspension test (TST)*

The antidepressant-like effect of VE was measured using the tail suspension test (TST), according to Steru et al. (1985). The test was carried out immediately after measuring anxiety in the elevated plus maze (Fig. 3). The mice were suspended by their tails using adhesive tape placed approximately 1 cm from the tip of the tail and hung approximately 30 cm above the table. The animals were suspended for 6 min, and the duration of immobility was scored manually during the test. Immobility is reduced with antidepressant drugs. Immobility was defined as the absence of any limb or body movements, with the exception of those caused by respiration, when the mice hung passively and completely motionless.

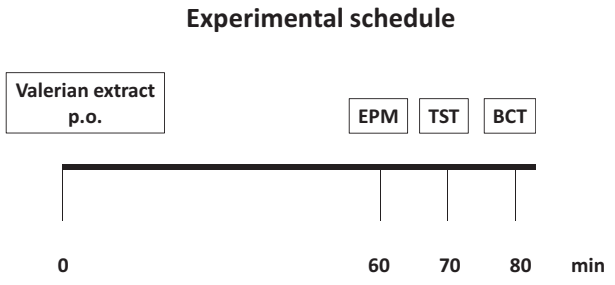


**Fig. 3.** Schedule of the experiment sequence.

Immobility was measured from minute 1 to minute 6. The values from the 2nd to the 6th minute were used to evaluate drug effects.

Following the TST, body core temperature (BCT) was measured with a digital thermometer (ama digit) manufactured by Amarell GmbH (Kreuzwertheim, Germany). For that purpose the lubricated probe (Ø 1 mm) was gently inserted 3 cm into the rectum.

All behavioural tests were performed in the light period between 8.00 a.m. and 2.00 p.m. The animals were randomly assigned for testing.

*Statistics*

Statistical evaluation was carried out using the Kruskal–Wallies *H*-test, followed by the Mann–Whitney *U*-test. The animals which exhibited exceptional hypothermia (9 out of 27 in the VE-2 group that received 2 mg) were compared using the contingency table. The threshold for significance was generally set at <0.05.

## Results

*Elevated plus maze*

The control animals (saline) spent 12.92 ± 2.49% of their time on the open arm. Animals which had received diazepam (1 mg/kg) significantly prolonged that time to 24.5 ± 2.2% ($p = 0.003$). In contrast, for animals of the imipramine group (30 mg/kg) that time was only insignificantly changed (Fig. 4).

Animals which had received VE-1 (total amount of VAs 0.5 mg/kg) exhibited also extended times on the open arm (17.62 ± 2.31%; $p = 0.032$) like the diazepam reference group. Therefore, this can be interpreted as an anxiolytic effect. The other groups (0.1, 1.0, and 2.0 mg/kg) were in the range of the saline control group.

VE-2 induced different effects in the EPM. Small doses of the VAs (0.1 and 0.5 mg/kg) reduced the time spent on the open arm indicating anxiogenic activity (5.5 ± 1.46% and 7.95 ± 2.56%; $p = 0.008$ and = 0.031). Whereas a higher dose, i.e. 2 mg VAs/kg demonstrated anxiolytic activity (27.07 ± 3.88%; $p = 0.039$).

The locomotor activity (total arm changes) was similar in all experimental groups (Fig. 5).

*Tail suspension test*

Both reference substances influenced the immobility time differently when compared to saline (92 ± 12.7 s). Whilst diazepam significantly prolonged immobility (143 ± 35.51 s), it was

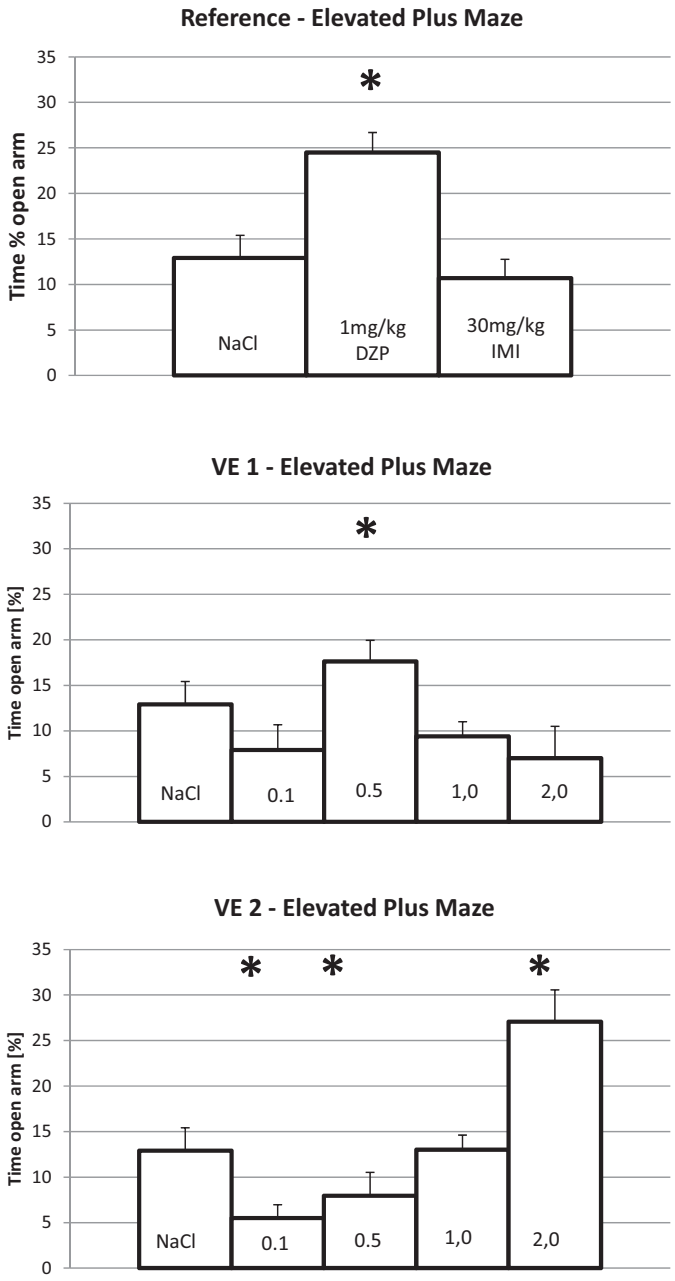


**Fig. 4.** Time spent of the open arm for controls and reference substances as well as for both of the extracts (numbers indicate applied amount as mg/kg BW).

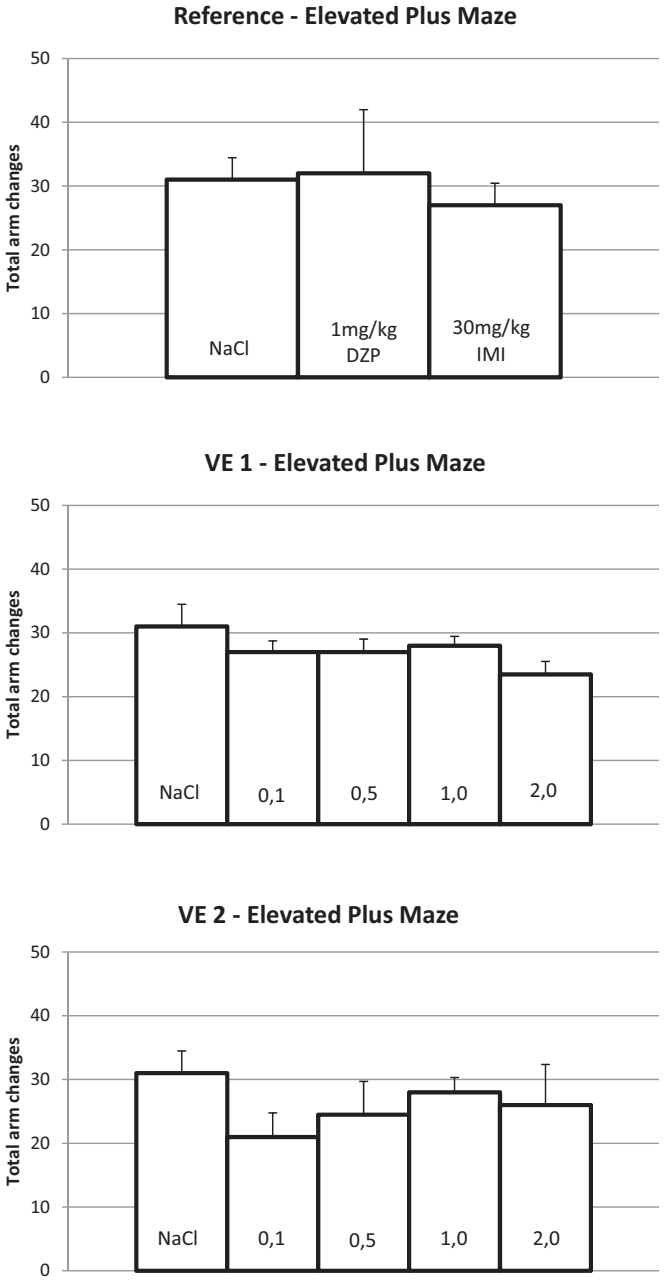


**Fig. 5.** Total arm changes within the test period for controls and reference substances as well as for both of the extracts (numbers indicate applied amount as mg/kg BW).

shortened by imipramine (48 ± 15.01 s) indicating an antidepressive effect (Fig. 6).

VE-1 did not change the immobility time. However, VE-2 in higher doses (1.0 and 2.0 mg/kg) induced prolongation of immobility (1.0: 99.9 ± 23.67 s, $p = 0.017$; 2.0: 115.5 ± 27.42 s, $p < 0.001$) indicating a depressive effect.

*Body core temperature*

Diazepam significantly reduced the BCT (37.05 ± 1.13 °C) whilst imipramine (37.8 ± 0.12 °C) did not change the body temperature when compared to saline (37.7 ± 0.12 °C) (Fig. 7). However, VE-1 (0.5: 37.0 ± 0.29 °C; 1.0: 37.2 ± 0.23 °C and 2.0: 36.2 ± 0.69 °C), as well as VE-2 (1.0: 36.1 ± 0.78 °C and 2.0: 34.8 ± 0.46 °C) significantly lowered BCT gradually in relation to the applied amounts.

Moreover, VE-2 could not be tested in doses higher than 2 mg VAs/kg. In preliminary tests it was seen that animals which had received 5 mg demonstrated a dramatic drop in BCT, and a few perished with signs of respiratory paralysis. After administration of 2 mg VAs/kg, the temperature was reduced below 34 °C in 9 out of 27 animals. The results of these animals were disregarded for evaluation. After applying VE-1, this phenomenon was only seen in one animal. This difference in temperature change between VE-1 and VE-2 is statistically significant ($p = 0.002$).

## Discussion

Valerian extract modulates GABAA receptor function after oral administration and demonstrates anxiolytic potential, since the time spent on the open arm in the elevated plus maze test was

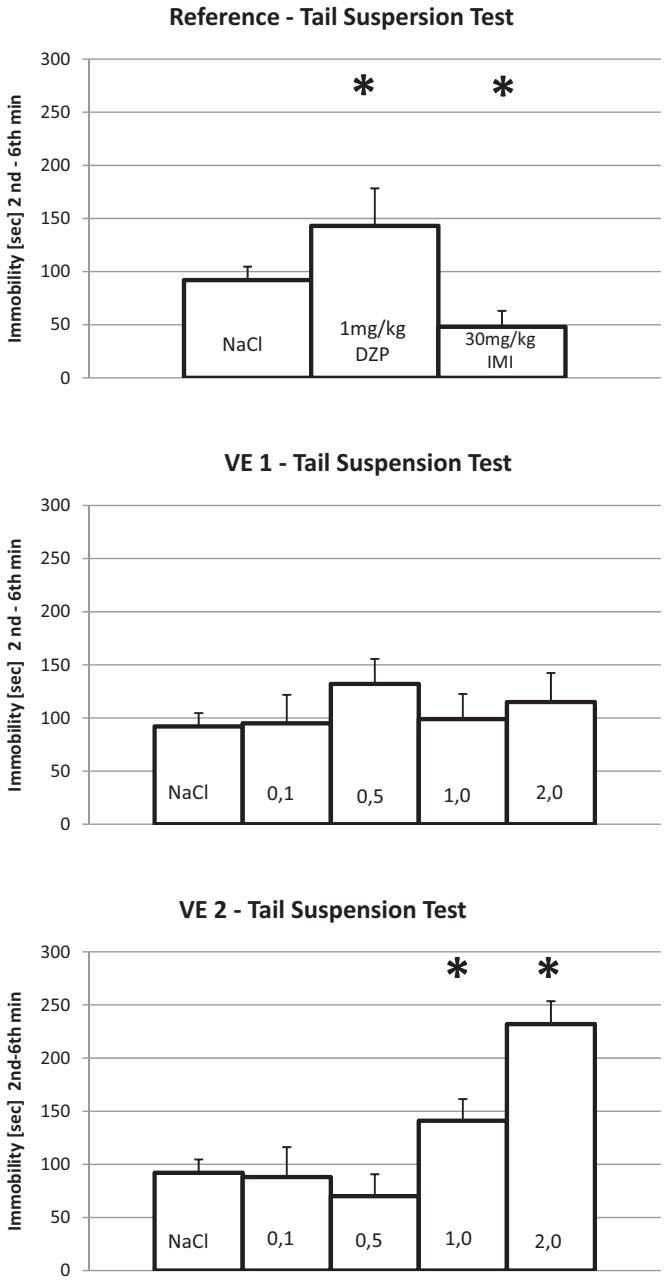


**Fig. 6.** Immobility time in the tail suspension test for controls and reference substances as well as for both of the extracts (numbers indicate applied amount as mg/kg BW).

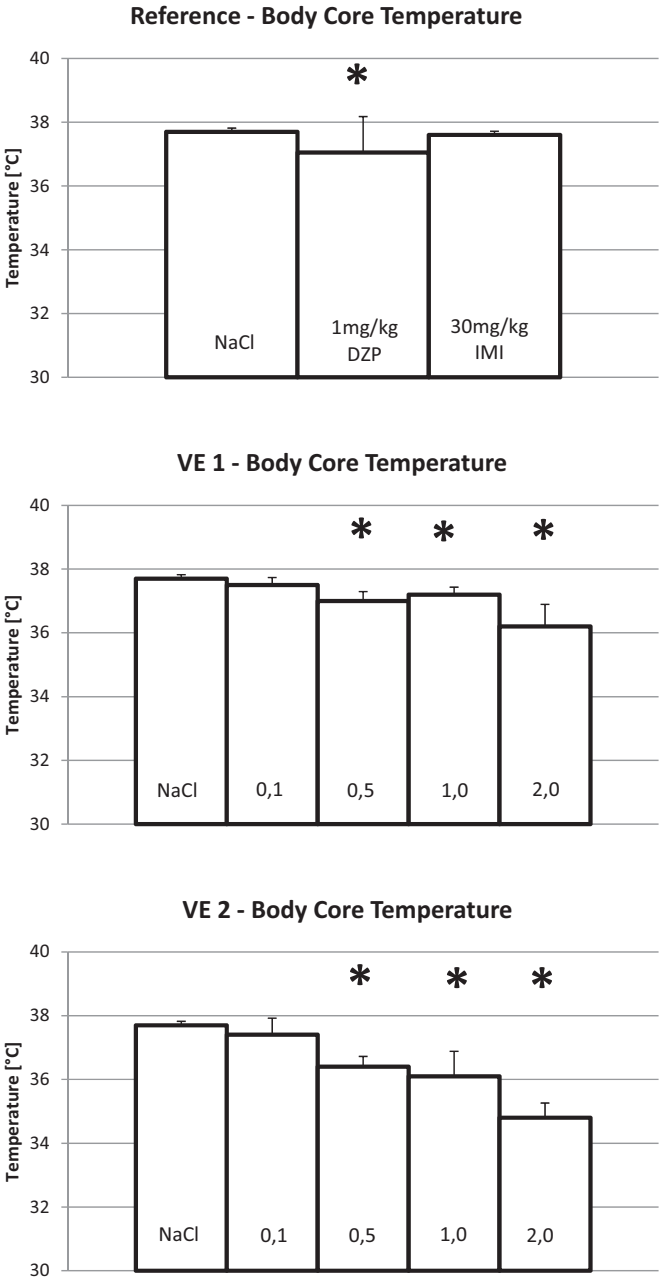


**Fig. 7.** Body core temperature for controls and reference substances as well as for both of the extracts (numbers indicate applied amount as mg/kg BW).

prolonged without reducing the frequency of changing positions. The results are restricted to the animals used within the described conditions. However the experimental approach is usually used for detection of new entities which might be effective in anxiolyse. Next step in the development process, therefore, has to be a clinical trial in subjects suffering from anxiety. This is very important since the dosage which was efficient in the rodents cannot easily be transferred to human beings.

These reported results confirm earlier findings by other groups (Hattesohl et al. 2008; Benke et al. 2009; Khom et al. 2010; Murphy et al. 2010). However, the necessary amounts of both tested extracts to induce such an effect were different. Of the VE-2 four times (2.0 mg/kg) the amount of VA + AVA was needed than that of the VA-1 extract (0.5 mg/kg) to prolong the time spent on the open arm to a comparable extent. However, with this regime the body temperature in the animals which had received VE-2 had already been remarkably reduced, which could indicate overdosing (Haffner 1929). Therefore, whether or not this action induced by VE-2 reflects a genuine anxiolytic effect remains questionable. It may be worth mentioning that 0.5 mg of total valerian acids were contained in 0.4 ml VE-1, whereas the amount of 2.0 mg total valerian acids in VE-2 required 3.9 ml, nearly ten times more.

The most important difference between both VEs was the proportion between VA and AVA. In the VE-1 the relationship was nearly 12: 1 whereas in VE-2 the relationship was 1:1.5. So far only for VA, but not for AVA, an allosteric modulation of the GABAA channel has been reported. In these in vitro experiments the chloride currents through GABAA receptors were measured in response to different concentrations of valerenic acid as well as azetoxy valerenic acid and hydroxy valerenic acid. In low concentration (0.1–100 μM) only valerenic acid modulates allosterically

the GABAA receptor. In higher concentration valerenic acid blocked the open GABAA channel as did azetoxy valerenic acid both with an IC50 value around 190 μM (Khom et al. 2007). Moreover, HPLC-based activity profiling of an EtOAc extract of the roots of *Valeriana officinalis* for GABAA ligands revealed that only the fraction which contains valerenic acid is active whilst the fraction which contains azetoxy valerenic acid failed (Kim et al. 2008). Additionally, Benke et al. (2009) demonstrated that hydroxy valerenic acid inhibited binding of valerenic acid at rat brain membranes (IC50 value 61 ± 19 μM). Taken these results together an allosteric action only for valerenic acid is underlined. Azetoxy valerenic acid as well as hydroxy valerenic acid seem capable of binding at the valerenic binding site and also block open GABAA channel in higher concentrations similar to pure valerenic acid.

Moreover, only for valerian extracts and for valerenic acid anxiolytic activities were shown in rodents (Benke et al. 2009, Murphy et al. 2010). There are no reports available regarding AVA and anxiety. AVA binds to the GABAA receptor without inducing allosteric modulation. However, after transformation of AVA to HAV by applying KOH (Trauner et al. 2008) the allosteric effect of VE in the in vitro model was elevated. From these results the conclusion can be drawn that AVA could inhibit the anxiolytic activity of VA in the extract rather than amplify it. Unfortunately, the composition of the extracts before and after treatment with KOH was not given so that this assumption is tentative.

Different tests are used to assess potential antidepressant activity. Among them is the tail suspension test (Cryan et al. 2005). VE-1 was inactive in this test system, whereas VE-2 prolonged the inactivity in a dose dependent manner. The latter effect would implicate an intensification of the depression. However, that prolongation is accompanied by a reduction in body core temperature, which is similarly seen in the diazepam paradigm. Possibly both effects, i.e. prolonged inactivity and temperature reduction, could be caused by a central phenomenon. This phenomenon may be related to the increased extract amount (0.4 vs. 3.9 ml/kg) or the AVA amount in the VE-2. However, this proposition has to be verified separately. On the other hand, immobility together with hypothermia are signs of valerian extract overdose (Haffner 1929).

For a special valerian extract (1000 mg/kg; phytofin 389) no changes in locomotor activity were reported within 120 min after administration (Hattesohl et al. 2008), which could be comparable with the results reported here for VE-1 and VE-2. However, the immobility observed in the forced swim test, which was used to asses antidepressive activity, was reduced by this special extract after 2× 125 mg/kg were administered daily for 16 days. However, in the present experiments the observation time was restricted to a 6 min period 70 min after acute administration. A dose related immobility prolongation was found for the VE-2 which is contrary to the findings by Hattesol. Nevertheless, it should be kept in mind that VE-2 was acutely administered and also that the lowered body core temperature may indicate a completely different phenomenon.

Benzodiazepines mainly used for anxiolysis are burdened by sedation, decreased attention span, memory deficits, addiction, and abuse potential. Moreover, these compounds possess a relatively narrow therapeutic window between doses that produce anxiolysis and those that cause sedation. Consequently selective drugs, particularly non-sedating in nature, are in the focus of pharmacological research and development (Rupprecht et al. 2006, Da Settino et al. 2007, Ator et al. 2010; Atack 2011). Benzodiazepines reside at the α + γ-interface (Da Settino et al. 2007; Olsen and Sieghart 2008), whereas VE and respectively VA are active at the β3 subunit of the GABAA receptor (Khom et al. 2007; Benke et al. 2009). Ernst et al. (2005) reported on a so far unknown drug binding pocket. More recently, the GABAA receptor α + β-interface was reported to have binding sites with a much broader action than benzodiazepines, and therefore may become important clinically for the development of new treatment possibilities (Sieghart et al. 2011; Ramerstorfer et al. 2011). Possibly, VA could become a potent candidate in this respect; first results have already been reported (Khom et al. 2010), however, obtained from in vitro experiments.

Another target for VE is insomnia. Here, the lignans contained in the extracts are thought to be important for an effect that is mediated by adenosine receptors in the frontal cortex (Brattström 2007). In this context it may be of interest that a particular human GABAA receptor mutation on β3 was found in a patient with chronic insomnia. This identical mutation was found in members of the patient's family (Buhr et al. 2002). Moreover, an experiment with mice with a targeted mutation on the beta 3 subunit, peripheral administration of oleamide, which usually decreases sleep latency and wake time whilst increasing non-rapid eye movement and total sleep time, failed completely to change sleep parameters (Laposky et al. 2001). Therefore, the allosterical modulation induced by VA at the GABAA receptor may contribute to overcome sleep problems, in addition to lignans that act at adenosine receptors.

In conclusion, in the elevated plus maze test a valerian extract with a high VA and low AVA content was more effective than extracts with lower VA and higher AVA contents. Both of the tested extracts did not show antidepressive activity in the tail suspension test. More generally spoken, standardization of extracts from *Valeriana officinalis* with respect to the sum of VA and AVA is misleading, since the antianxiety effect induced by VA may be, at least in part, inhibited by AVA. This could also explain why the outcome of many trials using VA-extracts is not satisfactory (Bent et al. 2006; Navarrete et al. 2006). Furthermore, the aim of extract development has to focus on plants with a high VA content, since chemical manipulation of the extracts to reduce AVA (Trauner et al. 2008) may additionally change extract composition by influencing also other compounds.

## Conflict of interest

No conflict to disclose

## Acknowledgment

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