Valeriana wallichii root extract improves sleep quality and modulates brain monoamine level in rats

**ARTICLE** in *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology* · July 2012

Impact Factor: 2.88 · DOI: 10.1016/j.phymed.2012.05.005 · Source: PubMed

<table>
<thead>
<tr>
<th>CITATIONS</th>
<th>DOWNLOADS</th>
<th>VIEWS</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>240</td>
<td>548</td>
</tr>
</tbody>
</table>

**8 AUTHORS, INCLUDING:**

- **Surajit Sahu**
  - Institut de neurobiologie de la méditerrané...
  - 7 PUBLICATIONS  21 CITATIONS

- **Shilpa Gupta**
  - 1 PUBLICATION  4 CITATIONS
  - SEE PROFILE

- **Hina Kauser**
  - Defence Research and Development Organis...
  - 7 PUBLICATIONS  24 CITATIONS
  - SEE PROFILE

- **Kshipra Misra**
  - Defence Research and Development Organis...
  - 23 PUBLICATIONS  97 CITATIONS
  - SEE PROFILE

Available from: Surajit Sahu
Retrieved on: 14 August 2015
This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier’s archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright
Valeriana wallichii root extract improves sleep quality and modulates brain monoamine level in rats

Surajit Sahu, Koushik Ray, M.S. Yogendra Kumar, Shilpa Gupta, Hina Kauser, Sanjeev Kumar, Kshipra Mishra, Usha Panjwani

Defence Institute of Physiology and Allied Sciences (DIPAS), Defence Research and Developmental Organization (DRDO), Lucknow Road, Timarpur, Delhi 54, India

A R T I C L E   I N F O

Keywords:
Valeriana wallichii
Telemetric EEG
Delta activity
Monoamine
Sleep–wake profile
Hesperidin

A B S T R A C T

The present study was performed to investigate the effects of Valeriana wallichii (VW) aqueous root extract on sleep–wake profile and level of brain monoamines on Sprague-Dawley rats. Electrodes and transmitters were implanted to record EEG and EMG in freely moving condition and the changes were recorded telemetrically after oral administration of VW in the doses of 100, 200 and 300 mg/kg body weight. Sleep latency was decreased and duration of non-rapid eye movement (NREM) sleep was increased in a dose dependent manner. A significant decrease of sleep latency and duration of wakefulness were observed with VW at doses of 200 and 300 mg/kg. Duration of NREM sleep as well as duration of total sleep was increased significantly after treatment with VW at the doses of 200 and 300 mg/kg. VW also increased EEG slow wave activity during NREM sleep at the doses of 200 and 300 mg/kg. Level of norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT) and hydroxy indole acetic acid (HIAA) were measured in frontal cortex and brain stem after VW treatment at the dose of 200 mg/kg. NE and 5-HT level were decreased significantly in both frontal cortex and brain stem. DA and HIAA level significantly decreased only in cortex. DOPAC level was not changed in any brain region studied. In conclusion it can be said that VW water extract has a sleep quality improving effect which may be dependent upon levels of monoamines in cortex and brainstem.

© 2012 Elsevier GmbH. All rights reserved.

Introduction

Valeriana wallichii (VW) or Indian Valerian is one of the several valerian species indigenous to the temperate Himalayas found in greater parts of India (Nadkarni 2001). The plant is widely known for its use in anxiety, insomnia, epilepsy, and hysteria (Nadkarni 1976, 2001). It is considered useful as a potent tranquilizer (Nadkarni 1976), antispasmodic and hypotensive (Gilani et al. 2005). Antidepressant activity of VW extract was also reported recently (Subhan et al. 2010). Roots and rhizomes of VW contain a volatile oil (valerianic oil) containing valereneic acid, isovalerenic acid, and terpineol (Arora and Arora 1963; Nadkarni 1976). The volatile components of rhizomes of VW consist of sesquiterpenes (>89.3%), kanokonyl acetate (42.4%), gamma-curcumene (10.7%), ar-curcumene (7.2%), (Z)-beta-farnesene (3.2%), xanthorrhizol (4.1%), 7-epi-alpha-selinene (2.2%), valeronone (2.0%) and curcuphenol (1.4%) (Mathela et al. 2009). Other active constituents of VW are sesquiterpenoids (Ron et al. 2000), 6-methylapigenin (Wasowski et al. 2002) and hesperidin (Marder et al. 2003). Valeronic acid, isolated from VW decreases the breakdown of GABA in the brain (Houghton 1999) and acts as GABA receptor substrate (Benke et al. 2009) resulting in its sedative and anxiolytic actions. Hesperidin and 6-methylapigenin isolated from VW acts as benzodiazepine binding site ligand and has sedative and sleep-enhancing properties (Wasowski et al. 2002; Marder et al. 2003). There are numerous reports on the effect of valerian extract on sleep in both animal and humans (Houghton 1999; Stevinson and Ernst 2000; Gyllenhaal et al. 2000; Morin et al. 2005; Bent et al. 2006; Tomnaga et al. 2007). However, there is no study reported in literature on the effect of VW on sleep pattern in a dose dependent manner.

Brain monoamines play a key role in sleep regulation (Shouse et al. 2000; Leu-Semenescu et al. 2010; Monti 2010). The earlier literature lacks any explanation on the neurochemical basis underlying the action of VW, except its effect on GABA receptor modulation as mentioned in the aforesaid paragraph. Our earlier studies on sleep disturbance in hypoxia have also shown alterations in regional brain monoamines (Ray et al. 2011). So, it was thought worthwhile to investigate this aspect in the present study, in relation to VW.

* Corresponding author at: Neurophysiology Division, Defence Institute of Physiology and Allied Sciences (DIPAS), Defence Research and Developmental Organization (DRDO), Timarpur, Delhi 54, India. Tel: +91 11 23883 203; fax: +91 11 23914 790.
E-mail address: neurophysiolab_dipas@rediff.com (U. Panjwani).
0944-7113/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.phymed.2012.05.005
The present study aimed to evaluate the effect of VW root extract on sleep pattern by recording sleep–wake profile, electroencephalogram (EEG) delta activity during sleep and estimation of regional brain monoamines.

Materials and methods

Animals

Adult male Sprague-Dawley rats (n = 36; body weight, 250–300 g; bred in the animal house of the institute) were used in this study. Animals were randomly housed in pair at controlled temperature (26 ± 2 °C) and humidity (50% ± 5%) conditions with a 12 h:12 h light dark cycle. Animals were allowed access to food and water ad libitum. All procedures involving handling of animal were conducted according to standard guidelines for the care and use of animals in neuroscience and behavioral research (National Research Council, 2003) and guidelines of Institutional Animal Ethical Committee.

Plant material

VW root materials were collected by Numerouno natural herbs, Delhi, India from the hilly regions of the North-West Himalayas (the region lies between latitude 32–36° North and longitude 76–79°) in the months of October–November, where the plant grows widely under natural conditions. Plant material (Voucher specimen VW-PACT-02-2009) was characterized by ethno-botanist Dr. O.P. Chaurasia, Scientist, Defence Institute of High Altitude Research, DRDO, Leh, India.

Aqueous extract of VW roots was prepared by soaking the powdered dry roots with distilled water at room temperature (25 ± 1 °C). After 24 h, supernatant was decanted and residue was re-soaked in fresh distilled water. The process was repeated three times for complete extraction. The respective supernatants was pooled, filtered through muslin cloth and centrifuged at 5000 × g for 10 min at 4 °C. Accelerated solvent extraction system ASE 350 equipped with the solvent control unit from Dionex Corporation (USA) was used for extraction of VW root. The collected extract was lyophilized and kept at -20°C for further use (yield = 43.77% of dry weight).

Phytochemical profiling of VW water extract

Determination of total phenol content

Total phenolic content of extracts was determined by the Folin–Ciocalteu method (Kriangsak et al. 2006): extract 150 μl (100 mg/ml VW), 2400 μl of nanopure water and 150 μl of 0.25 N Folin–Ciocalteu reagent were combined and mixed. The mixture was allowed to react for 3 min, 300 μl of 1N Na2CO3 solution was added and mixed. The solution was incubated at room temperature in the dark for 2 h. The absorbance was measured at 725 nm using a spectrophotometer and the results were expressed in milligram of gallic acid equivalents per 1 g of extract.

Determination of total flavonoid content

1.0 ml aliquot of appropriately diluted VW sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% NaNO2 solution. After 6 min, 0.15 ml of 10% AlCl3 solution was added and allowed to stand for 6 min; thereafter 2 ml of 4% NaOH solution was added to the mixture. The mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance was measured at 510 nm versus prepared blank (Yanping et al. 2004). Rutin was used as a standard compound for the quantification of total flavonoids. All values were expressed as milligram of rutin equivalents per 1 g of extract.

HPLC analysis

The HPLC system (Waters Corporation, USA) equipped with Waters 515 HPLC pump, Waters 717 plus auto-sampler and Waters 2487 UV detector, interfaced with an IBM Pentium 4 personal computer was used. The separation was performed on a Symmetry C18 250 mm × 4.6 mm ID; 5 μm column (Waters, USA) by maintaining the gradient flow rate at 0.75 ml/min of the mobile phase (Solution A; water:O-phosphoric acid 99.7:0.3 and Solution B; acetonitrile:methanol 75:25) and peaks were detected at 285 nm. Identification of compounds was performed on the basis of the retention time, co-injections, and spectral matching with standards. For the preparation of the calibration curve, standard stock solution of hesperidin was prepared in ethanol, filtered through 0.22 μm filters (Millipore), and appropriately diluted (0.01–100 μg/ml) to obtain the desired concentrations in the quantification range. The calibration graphs were plotted after linear regression of the peak areas versus concentrations (Fig. 1).

Drug treatment

Sleep–wake profile and EEG delta activity were studied in 4 groups (n = 6 in each group) as follows: Gr-I, saline control; Gr-II, VW 100 mg/kg body weight; Gr-III, VW 200 mg/kg body weight; Gr-IV, VW 300 mg/kg body weight. Doses were selected from a previously reported study (Subhan et al. 2010). A single dose of VW was selected for the estimation of brain monoamines, which was most appropriate according to the effects of VW on sleep architecture (200 mg/kg body weight). VW root extract was dissolved in normal saline (2 ml/kg body weight) and rats were administered VW orally by force feeding. Diazepam (5 mg/kg body weight) group was used as a positive control.

Surgery and sleep recording

The rats were anaesthetized by intraperitoneal injection with a combination of ketamine (50 mg/kg; Themis Medicare Ltd., India) and xylazine (10 mg/kg; Indian Immunologicals Ltd.) and placed in a stereotaxic set-up (Stolting, USA). Rats were implanted with a telemetric unit (4-ET magnet activated transmitter, Data Science International, USA) for recording of electroencephalogram (EEG) and electromyogram (EMG) in freely moving condition. The transmitter was implanted by creating two subcutaneous pockets 5 cm long and 3 cm wide with blunt dissection on the both sides of the back from midline. Subdural EEG was recorded from visual cortex and frontal cortex according to guidelines of a standard brain atlas (Paxinos and Watson 2007). EMG was recorded from dorsal neck muscle. Rats were allowed to recover for at least 7 days with...
proper post-operative care. Rats were positioned on a receiver plate (RPC-2, DSI, USA) within their home cages to record the signals from 4-ET transmitter. Signals collected by RPC-2 receiver were acquired on a computer through a Data Exchange Matrix (DSI, USA). The digital data were converted to an analog signal by DSI A.R.T analog software and stored. Total recording time for each experimental condition in each rat was 8–9 h (starting from 9.30 am). The raw EEG and EMG data were analyzed by Neuroscore 2.0 CNS analysis software (DSI, USA). The relative power of delta wave (0–4 Hz, from frontal cortex) in every 10 s epoch was exported to MS Excel for sorting and statistical analysis. Three sleep stages viz. Wake, NREM and REM were scored for every 10 s epoch by Neuroscore automated sleep scoring module based on EEG, EMG and spectral power band and corrected manually using standard guidelines (Tokunaga et al. 2007).

Estimation of monoamines in cortex and brain stem

Animals were sacrificed by over dose of ketamine (80 mg/kg body weight)–xylazine (20 mg/kg body weight) combination after 2 h of saline or VW (200 mg/kg) treatment. The brains were excised from the skull and transferred into ice cold saline. Samples of cortex and brainstem were weighed and homogenized in ice cold phosphoric acid (0.1 M)–EDTA (0.5%) buffer. After centrifuge at 3000 rpm for 5 min, the supernatant was filtered with 0.22 μm syringe filter (Millipore) and stored at 4°C until subsequent use. Brain neurotransmitters and metabolites (NE, DA, DOPAC, 5-HT, HIAA) were estimated using HPLC-Electrochemical detector (Waters, USA) (Mohanakumar et al. 1994).

Statistical analysis

Data was presented as mean ± SEM. The effect of VW extract on sleep parameters was analyzed by one way ANOVA followed by Tukey post hoc test. To measure EEG delta activity, 10–12 ten second epoch were taken in each hour from each rat and averaged to obtain the value of one animal. For hourly EEG delta activity repeated measure ANOVA was used for each dose. To compare hourly changes from respective baseline Dunnett post hoc test was used. Student t-test was used to estimate the effect of VW on monoamine neurotransmitters and effects of diazepam on sleep and EEG delta activity. All statistical analysis was done in GraphPad InStat version 3.00 for Windows (GraphPad Software, San Diego, CA, USA). The value of p < 0.05 was considered statistically significant.

Results

Phytochemical profile

Total phenolic content (±SD of 3 samples) was 56.15 ± 1.31 mg (mg gallic acid/g of extract). Total flavonoid content (±SD of 3 samples) was 102.51 ± 3.53 mg (mg rutin/g of extract). The amount of hesperidin (Fig. 1) was 6.18 ± 0.26 mg/g of extract (±SD of 3 samples).

Sleep–wake profile

Sleep latency was significantly decreased (Fig. 2B) after VW treatment at the dose of 300 mg/kg. Duration of NREM sleep (Fig. 2E) as well as total sleep (NREM + REM, Fig. 2C) increased significantly after VW treatment at the doses of 200 mg and 300 mg. VW at the doses of 200 mg and 300 mg significantly decreased the duration of wake state (Fig. 2D). Duration of REM sleep (Fig. 2F) did not alter after VW treatment. The total duration of NREM sleep was increased significantly after diazepam treatment (Fig. 4A). There was no significant change of total duration of REM sleep after administration diazepam. Duration of wakefulness was decreased significantly after administration of diazepam as compared to saline treated group (Fig. 4A).
EEG delta activity

NREM sleep delta activity in frontal cortex was transiently increased (Fig. 3) during 1–2 h of VW post-treatment in the dose of 200 mg/kg but the change reversed to baseline at 3–8 h as depicted in Fig. 3. In the dose of 300 mg/kg VW significantly increased NREM sleep delta power, which sustained for 8 h with a peak during 5–6 h of VW post-treatment. EEG delta activity significantly decreased up to 4th hour after diazepam treatment and slowly became normal in later hours (Fig. 4B).

Monoamines profile

After 2 h of VW treatment (200 mg/kg), the levels of neurotransmitters and metabolites were decreased significantly: NE: 51.63%, DA: 58.14%, HIAA: 15.69% and 5-HT: 20.14% in the cortex (Fig. 5B). The levels also decreased significantly in brainstem region (Fig. 5C): NE: 22.03%, HIAA: 17.57% and 5-HT: 24.32%.

Discussion and conclusion

In the present study, it was found that VW root extract significantly decreased sleep latency and increased duration of total sleep as well as NREM sleep. As shown in Fig. 2, VW extract in the doses of 200 mg and 300 mg significantly increased sleep duration (total and NREM sleep) and decreased duration of wakefulness compared to saline treated group. However there was no significant difference observed between the two effective doses (200 mg and 300 mg/kg) of VW. EEG delta activity during NREM sleep was studied as slow wave activity during NREM sleep is an indicator of sleep quality (Tokunaga et al. 2007). VW transiently increased delta activity in the dose of 200 mg/kg in first 2 h and returned to baseline in subsequent hour but in the dose of 300 mg/kg, it increased delta activity for much longer duration. The delta activity tended to return to baseline after 6 h indicating that the quality of sleep in 300 mg VW treated group was better than 200 mg VW treated group though duration of sleep was almost the same.

The alteration in sleep profile and EEG observed in the present study is likely to be a multi-factorial effect. Hesperidin has been isolated as one of the active components by this group as well as other researchers (Marder et al. 2003). Hesperidin is likely to contribute to the sedative effect of VW, by acting synergistically with other valepotriates and 6-methylapigenin.

Other species of valerian have been studied previously for their sleep inducing properties (Stevinson and Ernst 2000; Shinomiya et al. 2005; Tokunaga et al. 2007) but unlike the present study other species of Valerian did not show any improvement in EEG delta activity. The difference in results may be due to different experimental designs, dosages and species. Diversified chemical constituents of different valerian species (Mathela et al. 2003) may attribute to the variation of efficacy.

In order to investigate the mechanism involved in the sleep inducing effects of VW, the regional brain monoamine neurotransmitters were evaluated. VW in the dose of 200 mg/kg significantly decreased NE level in both cortex and brainstem. Role of NE in central arousal system is well known (Berridge, 2008; Mitchell and Weinschenker, 2010). It is tempting to suggest that VW may increase sleep duration by inhibiting noradrenergic wake promoting regions of brain. VW also decreased 5HT level in both cortex and brainstem and DA level in cortex. It is currently accepted that 5HT and DA function to promote waking (Leu-Semenescu et al. 2010; Monti 2010). Hence, decreased levels of DA and 5HT may have a role in sleep inducing effects of VW. As shown in Fig. 5, VW treatment also significantly decreased levels of HIAA indicating decreased synthesis of 5HT rather than increased utilization. This aspect needs further investigation.

The EEG spectral analysis has been widely used to identify the nature of pharmacological action of drugs on modulation of brain neurotransmitters (Dimpfel 2003). Studies revealed that serotonin increases EEG desynchronization and produces an increase in vigilance level by tonic activation of 5HT2 receptors. Ritanserin, a 5HT2 receptor antagonist caused an immediate transient increase in delta activity (Kantor et al. 2002). Dopamine D1 receptor antagonism also alter the properties of neural networks which generate delta and spindle waveforms through the upstream modulation of GABA\_A receptor activity (Eder et al. 2003). Decreased level of 5HT and DA in the present study may have played a role in the modulation of delta activity after VW treatment.

In conclusion, the VW root extract may serve as an herbal therapeutic intervention to improve sleep quality, with an increase in deep sleep as demonstrated by an increase in EEG delta power. It has a potential application in amelioration of the effect of sleep disturbances. The effect on sleep quality may be dependent upon modulation of regional brain monoamines.
Conflict of interest

The authors have declared that there is no actual or potential conflict of interest.

Acknowledgements

This study was fully supported by Defence Research and Development Organization (DRDO), Ministry of Defence, India. Authors are also thankful to Dr. O.P. Chaurasia, Scientist at the Defence Institute of High Altitude Research, DRDO, Leh, India for identification of the plant specimen.

References


