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Fazal Subhan

Nasiara Karim

Anwar Gilani
Aga Khan University

Robert D. E. Sewell

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Terpenoid Content of *Valeriana wallichii* Extracts and Antidepressant-like Response Profiles

Fazal Subhan¹, Nasira Karim¹, Anwarul Hassan Gilani² and Robert D. E. Sewell³*
¹Department of Pharmacy, University of Peshawar, Peshawar, Pakistan
²Natural Products Research Division, Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan
³Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Ave., Cardiff CF10 3NB, UK

Three extracts of *Valeriana wallichii* DC (Valerianaceae) rhizome and fluoxetine were studied for antidepressant-like activity in two behavioral models, namely the forced swim test (FST) and the tail suspension test (TST). Fluoxetine as well as methanolic and aqueous extracts of *V. wallichii* induced monophasic dose-related decrements in immobility times in both tests. However, the aqueous-ethanolic fraction induced a biphasic dose-response profile since it produced a graded effect up to 200 mg/kg but the highest dose (250 mg/kg) was inactive in the FST. This extract also exhibited significantly reduced activity at 200 mg/kg compared to lower doses in the TST. The highest doses of aqueous-ethanolic extract also reduced locomotor activity which will have led to a negative functional interaction with antidepressant-like effects. Qualitative phytochemical analysis revealed that the aqueous-ethanolic extract of *V. wallichii* was the only separated rhizome fraction containing terpenoids. Furthermore, since the methanolic and aqueous extracts were active in the tests, it is suggested that the antidepressant-like action of this herbal plant is not contingent upon its terpenoid constituents. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: *Valeriana wallichii*; rhizome; aqueous alcoholic extracts; antidepressant; terpenoids; fluoxetine.

INTRODUCTION

Depressive disorder is a common affliction with an estimated lifetime prevalence of 15% (Reynolds, 2003). Therapeutic agents currently available for treating depression are successful in about 65–70% of patients, but serious side effects may limit treatment strategies (Keith and Matthew, 1993). Second-generation antidepressants such as selective serotonin reuptake inhibitors (SSRIs) reduce the risks of side effects in comparison to tricyclic (TCA) antidepressant drugs, but have not improved the overall effectiveness of treatment for depression (Moller and Volz, 1996). Identification of new antidepressant therapeutic modalities may therefore be of considerable clinical interest, especially with regard to enhanced efficacy and patient compliance whilst reducing the risks of adverse outcomes. It is also important that potential therapeutic agents are detectable in behavioral models of depression as a prelude to their clinical use.

In a recent clinical study, a plant extract of *Valeriana wallichii* DC (Valerianaceae) has been shown not only to attenuate stress and anxiety significantly, but also to improve symptoms of depression (Bhattacharyya et al., 2007). This plant is a small perennial herb of 14–45 cm height, with rootstock, a thick branching stem, sharply pointed leaves, white or pink flowers in clusters and hairy fruit. It occurs in Kashmir, Murree hills, Punjab, and northern areas of Pakistan (Baquar, 1989; Nadkarni, 1976). The plant is traditionally used for epilepsy, insomnia, neurosis, sciatica (Nadkarni, 1976; Marder et al., 2003) and as an analgesic (Vohora and Dandiya, 1992). It is also a remedy for habitual constipation (Baquar, 1989) and possesses antispasmodic (Gilani et al., 2005) as well as cytotoxic (Bos et al., 1998) activity. Moreover, it has been employed for anxiety and depression either alone or in combination with other herbs, specifically *Hypericum perforatum* (St John’s wort) (PaniJel, 1985; Ron et al., 2000), which has recently been shown to ameliorate physical signs of opioid withdrawal (Subhan et al., 2009).

Species of Valeriana have been reported to contain fragrant monoterpenoids, sesquiterpenoids and valepotriates as active constituents (Keochanthala-Bounthanh et al., 1993; Mathela et al., 2005). *V. wallichii*, in particular, is characterized by the presence of three major terpenoids: maaliol, patchouli alcohol and 8-acetoxypatchouli alcohol (Mathela et al., 2005). Similarly, *H. perforatum* also contains terpenoids such as hyperforin which is thought to be a key antidepressant component (Chatterjee et al., 1998) since an aqueous-alcoholic *H. perforatum* extract lacking hyperforin has been shown to be devoid of antidepressant-like activity (Mennini and Gobbi, 2004). In view of these findings, the aim of this study was to examine the antidepressant-like activity of an aqueous-ethanolic extract of *V. wallichii* in comparison with terpenoid containing methanolic and aqueous extracts in order to establish any importance of terpenoid content to its antidepressant-like action.
MATERIALS AND METHODS

Animals. Adult Sprague-Dawley rats (160–220 g, n = 8 per group) were used in the forced swim test (FST) and locomotor activity studies. BALB/c mice (18–26 g) were employed in the tail suspension test (TST) (n = 8 per group) and locomotor activity studies (n = 5 per group). All animals were bred in the animal facility at Peshawar University and both males and females were used. The animals were maintained under standard laboratory conditions (light period of 12 h/day and ambient temperature 22°C ± 2°C). with access to standard laboratory chow and water ad libitum. Experimental procedures were carried out between 8.00 and 16.00 h and in compliance with the Animals (Scientific Procedures) Act UK 1986.

Plant material. Fresh rhizomes of Valeriana wallichii were collected from northern areas of Pakistan (Galyat), identified by a taxonomist and a voucher specimen (82002 pup) was deposited with the herbarium of the Department of Botany, University of Peshawar, Pakistan.

Preparation of different extracts of Valeriana wallichii. The plant rhizomes were washed, shade dried and coarsely ground. The resultant powdered material was soaked either in 70% aqueous-ethanol, water, or methanol at room temperature for 7 days with occasional shaking. The extracts were filtered through a muslin cloth and then through a filter paper. This procedure was repeated thrice and the combined filtrate was evaporated on a rotary evaporator under reduced pressure. The extracts were completely solubilized in normal saline (0.9% sodium chloride) for use in the in vivo experiments.

Chemicals and drugs. Ethanol was obtained from Khazana Sugar Mills (Peshawar, Pakistan). Methanol was purchased from the Merck (Germany) through authorized supplier, Science Centre, Peshawar, Pakistan. Fluoxetine was gratefully donated by Raza Pharmaceuticals (Pvt) Ltd (Peshawar, Pakistan). All drugs and extracts were dissolved in normal saline prior to use.

Statistical analysis. Results were analyzed by one-way analysis of variance (ANOVA) with Dunnett’s test for multiple comparisons. Effects or differences were considered significant at p < 0.05.

Qualitative phytochemical analysis. Qualitative phytochemical analysis of Valeriana wallichii was performed for alkaloids, tannins, saponins, terpenoids, coumarins and anthraquinones (Sofowora, 1993).

Antidepressant-like activity

Forced swim test (FST). The FST of Porsolt et al. (1978) is a commonly employed animal model which is sensitive to antidepressant treatments. The protocol was composed of dual phases involving a pretest session performed 24 h before the actual test. In the pretest session, rats of either sex (160–220 g, n = 8 per group) were placed individually in a transparent glass tank (height = 45 cm, width = 18 cm) filled with water (up to a height of 25 cm) and the temperature was maintained at 25°C. After 15 min, animals were removed, gently dried with a warm towel and returned to their home cages. Animals showing any signs of impairment, nose bleeding or those that sank during the pretest session were excluded from the study.

Twenty-four hours after the pretest session, saline (control), fluoxetine (positive control) or Valeriana wallichii extracts (methanolic, aqueous-ethanolic or aqueous fractions) were administered intraperitoneally. One hour after treatment, rats were re-exposed to the FST under identical experimental conditions to those described in the pretest session. The duration of immobility time in seconds was recorded for a period of 5 min as reported by Porsolt et al. (1978). An animal was considered immobile when it remained floating with all four limbs motionless in a slightly hunched but upright position or when only necessary movements were made which maintained the nose above water level. The mean percentage reduction in immobility time was calculated in relation to the control animals.

Tail suspension test (TST). The TST was conducted using mice weighing 18–26 g (n = 8 per group) under acoustically isolated conditions. Saline (control), fluoxetine (positive control) or Valeriana wallichii extracts were administered by intraperitoneal injection. One hour following treatment, mice were suspended (at least 1 m from the nearest object) from the edge of a laboratory bench top 35 cm above the floor by adhesive tape gently placed approximately 1 cm from the extremity of the tail. The total duration of immobility was recorded in seconds for an overall period of 6 min as described by Steru et al. (1985). Mice were considered immobile only when they hung passively and completely motionless. The reduction in mean immobility time of test animals was expressed as a percentage of that displayed by the corresponding control group.

Locomotor activity

The locomotor activity arena measured 50 × 40 cm and the floor was divided by lines into 4 equal-sized rectangular zones. Rats (n = 8 per group) or mice (n = 5 per group) were habituated to laboratory conditions 90 min prior to testing. Doses of Valeriana wallichii aqueous ethanolic extract or saline vehicle were administered intraperitoneally and animals were placed in the recording apparatus 30 min later. Group mean line-crossing counts were subsequently recorded between 1 and 30 min following introduction to the arena by means of a Cat Eye camera, coupled to a remote personal computer.

RESULTS

Phytochemical analysis

Qualitative phytochemical analysis of Valeriana wallichii rhizome detected the presence of terpenoids.
together with alkaloids, saponins, tannins, and flavonoids in the aqueous-ethanolic extract; alkaloids, flavonoids plus tannins in the methanolic extract, and alkaloids, saponins together with tannins in the aqueous extract (Table 1).

**Antidepressant-like effect in the FST**

Fluoxetine as a positive control, exhibited a dose dependent antidepressant-like effect (i.e., reduced immobility time) over the dose range (10–40 mg/kg) in the FST (Fig. 1). ANOVA followed by Dunnett’s post hoc analysis revealed a significant difference between treatment groups and saline controls at dose levels of 10 mg/kg ($P < 0.05$), 20 mg/kg ($P < 0.01$), and 40 mg/kg ($P < 0.001$).

The methanolic extract of *Valeriana wallichii* was similarly tested for an antidepressant-like effect in the FST over the dose range 50–250 mg/kg and no significant decrement ($P > 0.05$) in immobility time was noted at the lowest dose (50 mg/kg) but a highly significant reduction was observed at doses of 100 ($P < 0.01$), 200 and 250 mg/kg ($P < 0.001$). Moreover, the aqueous-ethanolic extract did not show any significant FST effect ($P > 0.05$) at the lowest (50 mg/kg) and notably at the highest dose (250 mg/kg) tested, though significant dose-dependent antidepressant-like effects were observed for the mid-range doses (100–200 mg/kg) in the FST indicating that the overall dose response relationship was biphasic. Likewise, the aqueous extract also had no effect at 50 mg/kg though significant monophasic dose-dependent decreases in FST immobility time were observed for the 100 ($P < 0.01$), 200 and 250 mg/kg ($P < 0.001$) treated groups in comparison with saline vehicle controls.

**Antidepressant-like effect in the TST**

As shown in Fig. 2, fluoxetine displayed significant antidepressant-like effects in the TST over the dose range 10–40 mg/kg (i.p.). Thus, graded decreases in immobility time compared to the saline control group were recorded at fluoxetine dose levels of 10, 20 & 40 mg/kg, *Valeriana wallichii* rhizome methanolic extract (Me.Ext) at doses of 50, 100, 200 & 250 mg/kg, aqueous-ethanolic extract (Hyd. Et. Ext) at doses of 50, 100, 200 & 250 mg/kg and aqueous extract (Aq.Ext) at doses of 50, 100, 200 & 250 mg/kg, intraperitoneally. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; treatment was significantly different compared to saline group. (n = 8).

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**Table 1. Qualitative phytochemical analysis of aqueous-ethanolic (Aq.Et.Ext), methanolic (Me.Ext) and aqueous (Aq.Ext) extracts of Valeriana wallichii rhizome**

<table>
<thead>
<tr>
<th>Test</th>
<th>Observation</th>
<th>Inferences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids:</td>
<td>Extract + Dragendorff’s reagent Turbidity/precipitation</td>
<td>+</td>
</tr>
<tr>
<td>Saponins:</td>
<td>Extract vigorously shaken in a test tube for 2 min Frothing less than 1 cm</td>
<td>−</td>
</tr>
<tr>
<td>Flavonoids:</td>
<td>Defatted residue of Extract + Ethanol → Filter → Filtrate + AlCl₃ Yellow color</td>
<td>+</td>
</tr>
<tr>
<td>Tannins:</td>
<td>Extract + Few drops of FeCl₃ An immediate green precipitate formed</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids:</td>
<td>Decolorized extract residue + Chloroform + Acetic anhydride + Conc: H₂SO₄ Brown precipitate formed</td>
<td>−</td>
</tr>
<tr>
<td>Anthraquinones:</td>
<td>Extract + 1% HCl → Filter + Benzene + NH₄OH No violet color</td>
<td>−</td>
</tr>
<tr>
<td>Coumarins:</td>
<td>Extract covered with filter paper moistened with NaOH + boiling water → see under UV light No fluorescence</td>
<td>−</td>
</tr>
</tbody>
</table>

+$=$ Present, $-$= Absent.
of 50–200 mg/kg in the TST. Accordingly, a significant difference between treatment groups and saline controls at doses of 50 (P < 0.05), 100 (P < 0.01) and 200 mg/kg (P < 0.001)) for the methanolic extract, 50 and 100, 150 (P < 0.01) and 200 mg/kg (P < 0.05) for the aqueous-ethanolic extract and 50 (P < 0.05), 100, (P < 0.01) and 200 mg/kg (P < 0.001) for the aqueous extract were observed (Fig. 2). It was noteworthy that the highest dose of the aqueous-ethanolic extract (200 mg/kg) produced a smaller shortening of immobility time than the lower doses (50 or 100 mg/kg).

**Locomotor effects of V. wallichii aqueous-ethanolic extract**

Since the highest doses of *V. wallichii* aqueous-ethanolic extract evoked apparently reduced antidepressant-like effects in the FST and TST, they were consequently tested on locomotor activity. In rats, the extract produced a graded and highly significant reduction in locomotor line crossing at 200 (P < 0.01) and 250 mg/kg (P < 0.001) shown in Table 2. Similarly, in mice, the extract yielded a statistically significant decrease (P < 0.05) in line crossing at 200 mg/kg (Table 2).

**DISCUSSION**

The FST and TST are widely used models of depression for screening new antidepressant drugs (Porsolt et al., 1978; Steru et al., 1985). These tests are somewhat sensitive and relatively specific to all major classes of antidepressants including tricyclics, SSRIs, monoamine oxidase inhibitors (MAOIs) and atypical antidepressants (Porsolt et al., 1977; Steru et al., 1985; Detke et al., 1995).

Animal immobility observed in the FST and TST is termed behavioral despair, and it is believed to model a condition similar to human depression (Willner, 1984; Steru et al., 1985). The state of behavioral despair is inhibited by several agents, which are therapeutically effective in clinical depression. In fact, a significant correlation has been observed between the efficacy of antidepressants in both FSTs and TSTs and their potency in the clinic (Porsolt, 2000).

In this study, fluoxetine was tested as a positive control antidepressant and it exhibited dose-dependent (10–40 mg/kg) antidepressant-like activity (reduction of immobility time) thus validating both behavioral models and experimental conditions (Figs 1 and 2). The aqueous, methanolic and aqueous-ethanolic extracts of *V. wallichii* also induced significant antidepressant-like activity in the FST (Fig. 1) and TST (Fig. 2), there being graded decrements in immobility times compared with vehicle-treated controls. The methanolic and aqueous fractions produced monophasic dose-related antidepressant-like effects in both tests. In contrast, the aqueous-ethanolic extract yielded a biphasic (‘bell-shaped’) dose response relationship since at the highest dose, there was no significant effect in the FST and a markedly reduced activity in the TST. It has been contended that since the TST is less stressful than the FST, it possesses greater pharmacological sensitivity to lower doses of antidepressant (Thierry et al., 1986) Furthermore, an identical bell-shaped dose-response profile for an aqueous-ethanolic *V. wallichii* extract and matching monophasic dose-response patterns for aqueous or methanolic extracts have also been reported (Karim, 2007) in the yohimbine potentiation test in mice (Quinton, 1963) which is also a useful predictor of antidepressant potential (Malick, 1983).

*V. wallichii* has previously been shown to contain flavonoids such as an anxiolytic benzodiazepine binding site ligand, 6-methylpapigenin (Wasowski et al., 2002; Marder et al., 2003) and a sedative compound, namely 2S(-) hesperidin (Marder et al., 2003). The plant rhizome also contains essential oils (α-santalene, ar-cucumene etc.) along with a high content of triterpenoids (Marder et al., 2003). The rhizome and rhizome extract (10 mg/kg) evoked antidepressant-like activity in the FST and TST (Porsolt et al., 1978). In the present study, the aqueous, methanolic and aqueous-ethanolic extracts of *V. wallichii* rhizome also evoked antidepressant-like activity in both tests. In contrast, the aqueous-ethanolic extract yielded a biphasic “bell-shaped” dose response profile since at the highest dose, there was no significant effect in the FST and a markedly reduced activity in the TST. It has been contended that since the TST is less stressful than the FST, it possesses greater pharmacological sensitivity to lower doses of antidepressant (Thierry et al., 1986). Furthermore, an identical bell-shaped dose-response profile for an aqueous-ethanolic *V. wallichii* extract and matching monophasic dose-response patterns for aqueous or methanolic extracts have also been reported (Karim, 2007) in the yohimbine potentiation test in mice (Quinton, 1963) which is also a useful predictor of antidepressant potential (Malick, 1983).
REFERENCES


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and xanthorrhizol) (Bos et al., 1997), and a range of active terpenoids including valtrate, didrovaltrate, maaliol, patchouli alcohol and 8-acetoxypatchouli alcohol (Keochanthala-Bounthanh et al., 1993; Mathela et al., 2005). The aqueous-ethanolic extract of V. wallichii in the current study, was the only separated fraction of the plant rhizome in which terpenoids were qualitatively detected and it displayed either inactivity or a reduced antidepressant-like effect at the highest doses studied in the TST or FST respectively. The data also indicated that there was a considerable degree of antilocomotor activity at the higher doses of the aqueous ethanolic extract. The onset of this activity at higher doses would probably generate a negative functional interaction with the antidepressant-like activity at these doses and may also associate the terpenoids with the sedative component of V. wallichii.

Previously, an aqueous-ethanolic extract from V. officinalis has been shown to bind GABA<sub>A</sub> receptors (Cavadas et al., 1995) along with 6-methylapigenin found in V. wallichii (Wasowski et al., 2002). Furthermore, in this context, several terpenoid GABA derivatives are known to induce inherent anti-immobility effects in the FST (Kubacka et al., 2006). GABA<sub>A</sub> receptors are molecular substrates for the regulation of vigilance, anxiety, muscle tension, epileptogenic activity and memory function (Rudolph et al., 1999). It might therefore be hypothesized that the terpenoid containing V. wallichii aqueous-ethanolic extract activates the GABA<sub>A</sub> receptor complex at higher doses. If this is the case, then the lack of anti-immobility effect observed at the highest dose might derive from a GABA<sub>A</sub>-mediated myorelaxant (motor impairment) or sedative (terpe

Since the methanolic and aqueous fractions of V. wallichii were devoid of qualitative terpenoid content, it would appear that the antidepressant-like activity of these herbal rhizome extracts is not contingent upon its specific terpenoid constituents. This finding warrants further study in order to establish which other constituents contribute to the antidepressant-like action of V. wallichii.


