May 2010

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

Fazal Subhan

Nasiara Karim

Anwar Gilani
Aga Khan University

Robert D. E. Sewell

PHYTOTHERAPY RESEARCH
Phytother. Res. **24**: 686–691 (2010)
Published online 26 November 2009 in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/ptr.2980

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

Fazal Subhan[1], Nasiara Karim[1], Anwarul Hassan Gilani[2] and Robert D. E. Sewell[3]*

[1]Department of Pharmacy, University of Peshawar, Peshawar, Pakistan
[2]Natural Products Research Division, Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan
[3]Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Ave., Cardiff CF10 3NB, UK

Three extracts of V*aleriana 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 wallichi; 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, Muree 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.

* Correspondence to: Dr R. D. E. Sewell, Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Ave., Cardiff CF10 3NB, UK.
Email: sewell@cardiff.ac.uk

Received 27 March 2009
Revised 3 July 2009
Accepted 5 July 2009

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 *V. 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 and temperature (≤50 C°) to a semi-solid mass of dark-brown color, yielding approximately 22.2% of aqueous-ethanol extract, 46.66 % of aqueous extract and 25% of methanolic extract. The crude 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 *V. 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 *V. 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 *V. 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 *V. 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

DOI: 10.1002/ptr

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

Test	Observation	Inferences		
		Aq-Et Ext	Me.Ext	Aq.Ext
Alkaloids:				
Extract + Draggendorff's reagent	Turbidity/precipitation	+	+	+
Saponins:				
Extract vigorously shaken in a test tube for 2 min	Frothing less than 1 cm	+	–	+
Flavonoids:				
Defatted residue of Extract + Ethanol → Filter → Filtrate + $AlCl_3$	Yellow color	+	+	–
Tannins:				
Extract + Few drops of $FeCl_3$	An immediate green precipitate formed	+	+	+
Terpenoids:				
Decolorized extract residue + Chloroform + Acetic anhydride + Conc: H_2SO_4	Brown precipitate formed	+	–	–
Anthraquinones:				
Extract + 1% HCl → Filter + Benzene + NH_4OH	No violet color	–	–	–
Coumarins:				
Extract covered with filter paper moistened with Na OH + boiling water → see under UV light	No fluorescence	–	–	–

+ = Present, – = Absent.

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 V. 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.

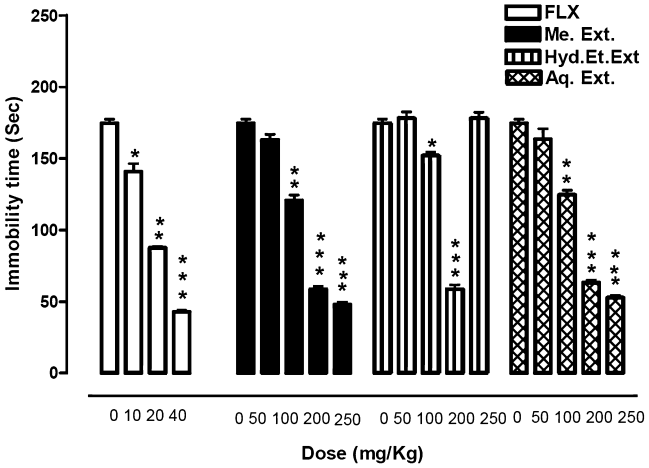

Figure 1. Immobility time of rats (Mean ± SEM, seconds) in the FST after acute treatment (1 h) with fluoxetine (FLX) administered at doses 10, 20 & 40 mg/kg, *Valeriana wallichii* rhizome methanolic extract (Me.Ext) at doses of 50, 100, 200 & 250 mg/kg, aqueous-ethanolic extract (Hyd. Et. Ext) at doses 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).

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 ($P < 0.01$) and 40 mg/kg ($P < 0.001$).

All three extracts of *Valeriana wallichii* induced statistically significant antidepressant-like activity at doses

DOI: 10.1002/ptr

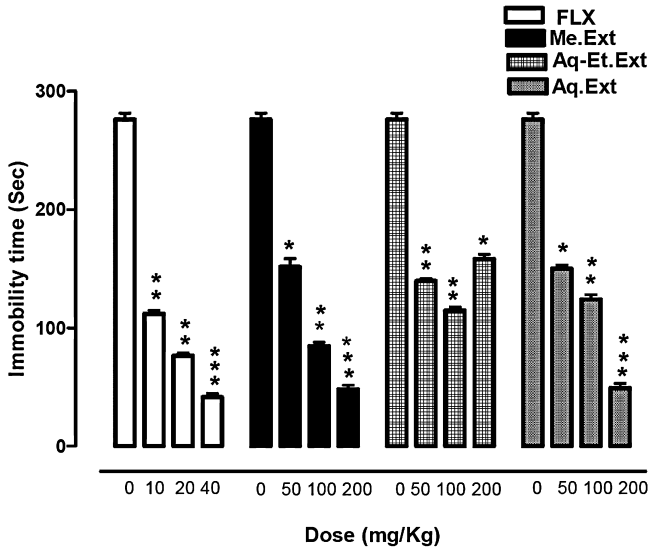

Figure 2. Immobility time of mice (mean ± SEM, seconds) in the TST after acute treatment (1 h) with fluoxetine (FLX) administered at doses of 10, 20 and 40 mg/kg. *V. wallichii* rhizome methanolic extract (Me.Et) at doses 50, 100 & 200 mg/kg, aqueous-ethanolic extract (Aq.Et.Ext) at doses 50, 100 & 200 mg/kg, and aqueous extract (Aq.Ext) at doses of 50, 100 and 200 mg/kg, intraperitoneally.* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; treatment was significantly different compared to saline vehicle treated group. (n = 8).

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).

Table 2. Locomotor activity of aqueous-ethanolic extract (Aq.Et.Ext) of *Valeriana wallichii* rhizome in rats and mice

	Treatment groups	Line-crossing counts (Mean ± sem)
Rats (n = 8)	Saline vehicle	25.5 ± 5.0
	V. wallichii (Aq.Et.Ext 200 mg/kg)	7.8 ± 1.9**
	V. wallichii (Aq.Et.Ext 250 mg/kg)	5.3 ± 0.9***
Mice (n = 5)	Saline vehicle	38.2 ± 4.2
	V. wallichii (Aq.Et.Ext 100 mg/kg)	25.0 ± 10.1
	V. wallichii (Aq.Et.Ext 200 mg/kg)	12.0 ± 2.4*

ANOVA with post hoc Dunnett's test: * $P < 0.05$ ($F_{2, 14} = 4.17$) *vs* saline vehicle in mice.
** $P < 0.01$, *** $P < 0.001$ ($F_{2, 21} = 10.84$) *vs* saline vehicle in rats.

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-methylapigenin (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

DOI: 10.1002/ptr

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 $GABA_A$ receptors (Cavadas *et al.,* 1995) along with 6-methylarpigenin 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). $GABA_A$ 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 $GABA_A$ 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 $GABA_A$-mediated myorelaxant (motor impairment) or sedative (terpenoid and 2S(-) hesperidin, flavonoid) interaction.

Since the methanolic and aqueous fractions of *V. wallichi* 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. wallichi*.

REFERENCES

Baquar SR. 1989. *Medicinal and poisonous plants of Pakistan*. Printas: Karachi; 479–480.

Bhattacharyya D, Jana U, Debnath PK, Sur TK. 2007. Initial exploratory observational pharmacology of *Valeriana wallichii* on stress management: a clinical report. *Nepal Medical College Journal* **9**: 36–39.

Bos R, Hendriks H, Scheffer JJC, Woerdenbag HJ. 1998. Cytotoxic potential of valeriana constituents, and valerian tinctures. *Phytomedicine* **5**: 219–225.

Bos R, Woerdenbag HJ, Hendriks H, Smit HF, Wikström HV, Scheffer JJC. 1997. Composition of the essential oil from roots and rhizomes of Valeriana wallichii DC. *Flavour Fragrance J* **12**: 123–131.

Cavadas C, Araújo I, Cotrim MD, Amaral T, Cunha AP, Macedo T, Ribeiro CF. 1995. In vitro study on the interaction of Valeriana officinalis L. extracts and their amino acids on GABAA receptor in rat brain. *Arzneimittelforschung* **45**: 753–755.

Chatterjee SS, Bhattacharya SK, Wonnermann M, Singer A, MIler WE. 1998. Hyperforin as a possible antidepressant component of hypericum. *Life Sci* **63**: 499–510.

Detke MJ, Rickels M, Lucki I. 1995. Active behavior in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacol* **121**: 66–72.

Gilani AH, Khan AU, Jabeen Q, Subhan F, Ghafar R. 2005. Antispasmodic and blood pressure lowering effects of *Valeriana wallichii* are mediated through K^+ channel activation. *J Ethnopharmacol* **14**: 347–352.

Karim N. 2007. *Pharmacological investigation of the indigenous plant, Valeriana wallichii for depression in animal models*. MPhil thesis, University of Peshawar, Pakistan.

Keith SJ, Matthews SM. 1993. The value of psychiatric treatment: its efficacy in severe mental disorders. *Psychopharmacol Bull* **29**: 427–430.

Keochanthala-Bounthanh C, Beck JP, Haag-Berrurier M, Anton R. 1993. Effects of two monoterpene esters, valtrate and didrovaltrate, isolated from *Valeriana wallichii*, on the ultrastructure of hepatoma cells in culture. *Phytother Res* **7**: 124–127.

Kubacka M, Librowska T, Czarnecki R, Frackowiak B, Lochyhski S. 2006. Anticonvulsant and antidepressant activity of the selected terpene GABA derivatives in experimental tests in mice. *Pharmacol Reports* **58**: 936–943.

Malick JB. 1983. Potentiation of yohimbine-induced lethality in mice: predictor of antidepressant potential. *Drug Devel Res* **3**: 357–363.

Marder M, Viola H, Wasowski C, Fernandez S, Medina JH, Paladini AC. 2003. 6-methylapigenin and hesperidin: new valeriana flavonoids with activity on the CNS. *Pharmacol Biochem Behav* **75**: 537–545.

Mathela CS, Chanotiya CS, Sammal S S, Pant AK, Pandey S. 2005. Compositional diversity of terpenoids in the himalayan *Valeriana* genera. *Chem Biodivers* **2**: 1174–1182.

Mennini T, Gobbi M. 2004. The antidepressant mechanism of Hypericum perforatum. *Life Sci* **75**: 1021–1027.

Moller H, Volz H. 1996. Drug treatment of depression in the 1990s. *Drugs* **52**: 625–638.

Nadkarni KM. 1976. *Indian Medica, 3rd ed.* Popular Prakashan: Bombay; 1260–1262.

Panijel M. 1985. Treatment of moderately severe anxiety states. *Therapiewoche* **35**: 4659–4668.

Porsolt RD. 2000. Animal models of depression: utility for transgenic research. *Neurosci Rev* **11**: 53–58.

Porsolt RD, Anton G, Deniel M, Jalfre M. 1978. Behavioral despair in rats: a new animal model sensitive to antidepressant treatments. *Eur J Pharmacol* **47**: 379–391.

Porsolt RD, Bertin A, Jalfre M. 1977. Behavioral despair in mice: A primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* **229**: 327–336.

Quinton RM. 1963. The increase in toxicity of yohimbine induced by imipramine and other drugs in mice. *Brit J Pharmacol* **21**: 51–66.

Reynolds EH. 2003. Brain and mind: a challenge for WHO. *Lancet* **361**: 1924–1925.

Ron BH, Willis CV, Bone K, Morgan M. 2000. Herbal products: active constituents, mode of action and quality control. *Nutrition Res Rev* **13**: 47–77.

Rudolph U, Crestani F, Benke D, Brünig I, Benson JA, Fritschy J-M, Martin JR, Bluethmann H, Möhler H. 1999. Benzodiazepine actions mediated by specific γ-aminobutyric acid receptor subtypes. *Nature* **401**: 796–800.

Sofowora EA. 1993. Phytochemical assays. In *Medicinal plants and traditional medicine in Africa. 3rd ed*. Spectrum Books Ltd: Nigeria; 150–153.

Steru L, Chermat R, Thierry B, Simon P. 1985. The tail suspension test: A new method for screening antidepressants in mice. *Psychopharmacol* **85**: 367–370.

Subhan F, Khan N, Sewell, RDE. 2009. Adulterant profile of illicit street heroin and reduction of its precipitated physical dependence withdrawal syndrome by extracts of St John's wort (*Hypericum perforatum*). *Phytother Res* **23**: 564–571.

Thierry B, Steru L, Simon P, Porsolt RD. 1986. The tail suspension test: ethical considerations. *Psychopharmacology* **90**: 284–285.

Vohora SB, Dandiya PC. 1992. Herbal analgesic drugs. *Fitoterapia* **63**: 195–207.

Wasowski C, Marder M, Viola H, Medina JH, Paladini AC. 2002 Isolation and identification of 6-methylarpigenin, a competitive ligand for the brain GABA(A) receptors from Valeriana wallichii. *Planta Med* **68**: 934–946.

Willner P. 1984. The validity of animal models of depression. *Psychopharmacology* **83**: 1–16.