EVALUATION OF INVOLVEMENT OF NEURO-CHEMICAL MECHANISM IN VALERIANA WALLICHI WALLICHI INDUCED ANTIDEPRESSANT EFFECT IN MICE

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INTRODUCTION

Valeriana (V.) wallichii DC (synonym V. jatamansi; family Valerianaceae), commonly known the Indian valerian, is found among the ground flora of Himalayan regions and used as herbal medicine for CNS disorders in Indian systems of medicine. In the study, aimed to investigate involvement of neuro-chemical-systems in V. wallichii induced antidepressant effects in mice.

MATERIALS AND METHODS

Biological material

The specimens of V. wallichii were collected from the region of Bin Block (29.6154°N 80.3199°E), district Pithoragarh, Uttarakhand (India). Specimens of the plant have been identified in house and matched with the plant samples, which has cultivated in herbal garden of the Institute at field station Pithoragarh. Rhizomes/root parts of V. wallichii were dried at 40 °C temperature in oven to prepare fine powder with the help of mechanical grinder. The freshly prepared dried powder of plant material was subjected to extraction. The extract was concentrated to dryness on a rotary evaporator at 40 °C to yield a brown dry mass. The final yield was recorded 13.5% (w/w).

EVALUATION OF ANTIDEPRESSANT ACTIVITY

The antidepressant activity of aqueous alcoholic extracts of V. wallichii was measured by using Forced Swim Test and Tail Suspension Test in mice, whereas locomotor activity was observed by Actophotometer. Involvement of adrenergic, dopaminergic and serotonergic receptors in V. wallichii induced antidepressant effects in mice were also observed.

RESULTS

Sub-acute administration of V. wallichii in mice showed significant (P<0.05) increase in the antidepressant activity similar to the Imipramine, but did not show at lower doses in FST. Whereas, pretreatment of adrenergic receptor antagonist, dopaminergic receptor antagonist and serotonin synthesis inhibitor prevented V. wallichii induce anti-depressant effect in mice. Lower doses of hydroalcoholic extract of V. wallichii did not interfering the locomotor activity in mice during the sub-acute administration, but at a higher dose significantly (P<0.05) decreases the locomotor activity.

CONCLUSION

Hence, it is concluded that the antidepressant effect of V. wallichii in mice may mediate via adrenergic, dopaminergic and serotonergic systems.

Keywords: Adrenergic, Antidepressant, Dopaminergic, Serotonergic Systems and V. wallichii

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Abstract

Objective: Valeriana (V) wallichii DC is found among the ground flora of Himalayan regions and used as herbal medicine for CNS disorders in Indian systems of medicine. In the study, aimed to investigate involvement of neuro-chemical-systems in V. wallichii induced antidepressant effects in mice.

Methods: The antidepressant activity of aqueous alcoholic extracts of V. wallichii was measured by using Forced Swim Test and Tail Suspension Test in mice, whereas locomotor activity was observed by Actophotometer. Involvement of adrenergic, dopaminergic and serotonergic receptors in V. wallichii induced antidepressant effects in mice were also observed.

Results: Sub-acute administration of V. wallichii in mice showed significant (P<0.05) increase in the antidepressant activity similar to the Imipramine, but did not show at lower doses in FST. Whereas, pretreatment of adrenergic receptor antagonist, dopaminergic receptor antagonist and serotonin synthesis inhibitor prevented V. wallichii induce anti-depressant effect in mice. Lower doses of hydroalcoholic extract of V. wallichii did not interfering the locomotor activity in mice during the sub-acute administration, but at a higher dose significantly (P<0.05) decreases the locomotor activity.

Conclusion: Hence, it is concluded that the antidepressant effect of V. wallichii in mice may mediate via adrenergic, dopaminergic and serotonergic systems.

Keywords: Adrenergic, Antidepressant, Dopaminergic, Serotonergic Systems and V. wallichii

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floating or motionless more than 7 s in the water without struggling, making only minimum movement of its limb necessary to keep its head above the water. The absence of hind leg movement was recorded as immobility by stopwatch. The mice capable of swimming for 8 or more in three successive trials were selected for the study. The selected animals were divided into 12 different groups (n=6) to evaluate the antidepressant activity in the FST and investigate the involvement of possible neurochemical mechanism of *V. wallichii* induced antidepressant effect in mice.

Vehicle (5% Tween 80, Oral) and Imipramine (15 mg/kg, IP) were administered in group 1 (control) and 2 (positive control) of mice respectively for 21 successive d. Four doses (20, 50, 125 and 300 mg/kg) of an aqueous alcoholic extract of *V. wallichii* were given to the animals’ group no 3, 4, 5 and 6 by the oral route for 21 d. All animals were tested by Forced Swim Test (FST) 30 min post administration of doses for the evaluation of antidepressant activity. Before 24 h of experiment, mice were placed in the water pool for 15 min and removed to dry in a heated enclosure (32 °C) before returning to their home cages. During experiments, animals were placed in the water pool and total duration of immobility was measured for next 5 min.

To investigate the involvement of possible adrenergic neurochemical mechanism in *V. wallichii* induced antidepressant effect in mice, 45 min post administration of adrenergic antagonist Prazosin (1 mg/kg, IP); vehicle (5% Tween 80 solution, oral) and *V. wallichii* extract (50 mg/kg, oral) were orally administered in group no 7 and 8 respectively.

While, two separate groups (Group no 9 and 10) of mice pretreated with dopaminergic antagonist Haloperidol (0.2 mg/kg, IP); 45 min post treated by vehicle (5% Tween 80 solution, oral) and *V. wallichii* extract (50 mg/kg, oral) respectively to evaluate possible involvement of dopaminergic neurochemical in *V. wallichii* induced antidepressant effect in mice.

To assess the possible involvement of serotoninergic neurochemical mechanism in *V. wallichii* induced antidepressant effects; two other separate groups (Group no 11 and 12) of mice were taken. After 45 min administration of serotonin synthesis inhibitor p-CPA (100 mg/kg, IP, 4 consecutive days, once in a d), vehicle (5% Tween 80 solution) and *V. wallichii* extract (50 mg/kg) were orally administered in group no 11 and 12, respectively. Control and positive control groups of mice were administered only vehicle (5% Tween 80 solution) and *V. wallichii* extract (50 mg/kg) respectively. Thirty min post treatment; all animals were tested in the forced swimming test for the evaluation of antidepressant activity.

**Antidepressant activity by Tail Suspension Test (TST)**

Tail Suspension Test (TST) is simple, rapid and reliable method for screening antidepressant effect in mice. It is considered for measuring mood level assay immobility time, which indicates changes in mood. TST induced immobility in animals is reduced by a large no of clinically active and atypical antidepressant effect [22]. The TST was performed in Swiss Albino Mice weighing 20-25 g according to the method [23] with slight modifications. Mice were suspended on the edge of the table 40 cm above the floor by adhesive tape placed approximately 2 cm from the tip of the tail. The total immobility time was observed for an overall period of 6 min as described by Steru et al. (1985) [23]. Mice were considered immobile only when they hung passively and completely motionless. Another 72 selected mice were divided into 12 different groups (n=6) to evaluate the antidepressant activity in TST. In groups 1*<sup>a</sup> and 2*<sup>b</sup>, Vehicle (5% Tween 80, oral) and Imipramine (15 mg/kg, IP) were administered for 21 successive d respectively. While, in groups no 3*<sup>a</sup>, 4*<sup>a</sup>, 5*<sup>a</sup> and 6*<sup>a</sup> *V. wallichii* extract (20% alcohol) at dose rate 20, 50, 125 and 300 mg/kg were administered by oral route for 21 successive d respectively. In groups no 7*<sup>a</sup> and 8*<sup>a</sup> Vehicle (5% Tween 80) and 50 mg/kg dose of *V. wallichii* extract (20% alcohol) were orally administered 45 min post treatment of adrenergic antagonist Prazosin (1 mg/kg, IP) respectively. In group no 9*<sup>a</sup> and 10*<sup>a</sup> Vehicle (5% Tween 80) and 50 mg/kg dose of *V. wallichii* extract (20% alcohol) was orally administered 45 min post treatment of dopaminergic antagonist Haloperidol (0.2 mg/kg, IP) respectively. In groups no 11*<sup>a</sup> and 12*<sup>a</sup> Vehicle (5% Tween 80) and 50 mg/kg dose of *V. wallichii* extract (20% alcohol) were orally administered 45 min post treatment of serotonin synthesis inhibitor p-CPA (100 mg/kg, IP, 4 consecutive d, once in a d) respectively.

After 30 min of treatment, all animals were tested by TST for the evaluation of antidepressant activity and investigate the involvement of possible neurochemical mechanism in *V. wallichii* induced antidepressant effect in mice. During experiments, duration of immobility of mice was measured for next 5 min in TST.

**Locomotor activity**

To evaluate the possible effects of *V. wallichii* on locomotor activity, the mice were placed in digital photoactometer (M/S Orchid Scientifics, India). Locomotor activity was measured by the interceptions in the photobeams in the instrument. Locomotor activity of mice was recorded during the next 5 min of a total 7 min test at the end of the experiment on the last day. The locomotor activity was expressed in terms of total photo beam counts (X+Y scores), 5 min per animal [24]. For locomotor activity, 36 mice were randomly divided into six different groups (n=6) for the present study.

Vehicle (5% Tween 80) and Imipramine (15 mg/kg) were administered orally for 21 successive d in the control group (Group no 1) and positive control group (Group no 2) respectively. *V. wallichii* was administered at four doses of 20, 50, 125 and 300 mg/kg body weight, orally for 21 d in four different groups (Group no 3, 4, 5 and 6) respectively. Imipramine was taken a positive control group for comparing the locomotor activity of *V. wallichii*. Thirty min after the last administration, all animals were tested in the photoactometer for their locomotor activity.

**Statistical analysis**

The results of the study were expressed as mean±SD Statistical analysis of the results were done by using one way ANOVA followed by post-hoc analysis of Dunnett's 't' test for all groups compared vs. control group and Student-Newman-Keuls Test for comparing all pairs of groups. *p* values<0.05 significant value vs. control group and *p* values<0.05 significant value vs. VW-50 (*V. wallichii*, 50 mg/kg) treated group.

**RESULTS AND DISCUSSION**

**Acute toxicity study**

In acute toxicity studies, no significant changes in food intake, body weight, general, physical and psychological behaviour of treated animals were observed as well as there were no signs of toxicity and mortality found in oral doses of ethanolic extract of *V. wallichii* up to 2 g/kg body weight. Hence it may be categorized as non-toxic and safe.

**Effect of Imipramine and *V. wallichii* on immobility time of mice**

Both FST and TST tests have been used to evaluate the anti-stress, mood elevator and anti-fatigue effects of various compounds [22, 25, 26]. Both tests are quite sensitive and specific to all major classes of antidepressant drugs [22, 27]. Above induces a state of behavioral condition similar to human depression [22, 28]. Fig. 1 and 2 depicts the effect of oral doses of ethanolic extract of *V. wallichii* (20, 50, 125 and 300 mg/kg) and positive control drug Imipramine (15 mg/kg) on immobility time of mice in FST and TST. Reduction in immobility time of animals during a test, indicates neuromuscular anti-fatigue (skeletal muscle performance activity) and antidepressant effects in animals.

Administration of Imipramine (15 mg/kg) in mice was significantly decreased the immobility time in both tests. Similarly to Imipramine, Oral administration of different dosage [50, 125 and 300 mg/kg body wt] of *V. wallichii* for 21 d in mice were showed significant (P<0.05) decrease in immobility time in both tests, but lowest doses (20 mg/kg) did not exhibit in FST as compared to control group. *V. wallichii* at the dosage 20, 50, 125 and 300 mg/kg in mice showed decreased immobility time a dose related graded manner in the FST and TST in mice, whereas the effect at the highest dose (300 mg/kg)
exhibit decreased immobility time but did not in a dose related graded manner in FST. Hence, *V. wallichii* at the dosage 20, 50, 125 and 300 mg/kg in mice showed dose dependent manner decrements the immobility time in both tests. Similarly, Subhan et al. (2010) [29] has reported that fluoxetine as well as methanolic and aqueous extracts of *V. wallichii* induced monophasic dose-related decrements in immobility times in FST and TST tests. While, 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. The results of the present study revealed that oral administration of ethanolic extract of *V. wallichii* (50, 125 and 300 mg/kg body weight) significantly (P<0.05) enhances neuromuscular endurance and antidepressant effects in mice. Ahmad et al. (1998) [30] have reported that a gem-containing Unani formulation with *V. wallichii* has anti-stress activity against the physical stress of forced swimming. The present study is also well supported by Subhan et al. (2009) [14] who have reported that terpenoid content of *V. wallichii* extracts has antidepressant response. Hence, from the study it concluded that *V. wallichii* extracts have antidepressant effects in mice.

Effect of prazosin on *V. wallichii* induced reduction in immobility time of mice

Etiology of depression is well known that abnormalities of noradrenergic, GABAergic, dopaminergic and serotonergic transmission are known to play a key role in the depression. Monoamine neurotransmitters like serotonin, dopamine, norepinephrine and epinephrine in the central nervous system have important roles in the regulation of mood, emotion, cognition, fatigue etc. In the study, we aimed was investigated the association of neurochemical mechanism with antidepressant effects of *V. wallichii* in mice. Fig. 3 and 4 depicts the effect of alpha-1 adrenergic antagonist prazosin (1 mg/kg, IP) alone and in combination with aqueous alcoholic extract of *V. wallichii* (50 mg/kg) on immobility time in mice during FST and TST tests.

![Fig. 1: Effect of *V. wallichii* and Imipramine on immobility time of mice in FST, [Values expressed in time (s) as mean±SD from six animals in each group at respective time-points. VW-20, VW-50, VW-125, and VW-300: Valeriana wallichii (20, 50, 125 and 300 mg/kg body wt.])](image1)

![Fig. 2: Effect of *V. wallichii* and Imipramine on immobility time of mice in TST, [Values expressed in time (s) as mean±SD from six animals in each group at respective time-points. VW-20, VW-50, VW-125, and VW-300: Valeriana wallichii (20, 50, 125 and 300 mg/kg body wt.])](image2)

![Fig. 3: Effect of prazosin on *V. wallichii* induced reduction in immobility time in FST, [Values expressed in time (s) as mean±SD from six animals in each group at respective time-points. VW-50: Valeriana wallichii (50 mg/kg body wt.)](image3)
Prazosin is a sympatholytic drug which acts as alpha-1 adrenergic neuron antagonist (inverse agonist) and it is used to treat high blood pressure, anxiety, stress disorder etc. Administration of aqueous alcoholic extract of *V. wallichii* (50 mg/kg) in mice were showed significant (P<0.05) decrease in immobility time as compared to control group. Whereas, *V. wallichii* (50 mg/kg) induced reduction in immobility time in mice was significantly prevented by pretreatment of alpha-1 adrenergic antagonist Prazosin (1 mg/kg, IP) in FST. Similar to FST test, *V. wallichii* (50 mg/kg) induced reduction in immobility time in mice was significantly prevented by pretreatment of alpha-1 adrenergic antagonist Prazosin (1 mg/kg, IP) in TST test. While, alone treatment of Prazosin (1 mg/kg, IP) did not show significant change in immobility time in mice in both FST and TST tests. Similarly, Sah et al. (2011) [31] have reported that prolong the treatment of *V. wallichii* significantly increases the levels of norepinephrine in forebrain of a mouse. He has also reported that the antidepressant effect of essential oil (20 mg/kg) of *V. wallichii* was prevented by pretreatment of mice with l-arginine (750 mg/kg IP) and sildenafil (5 mg/kg IP) [31]. Hence, the present study revealed that *V. wallichii* induced anti-fatigue and antidepressant effects in mice is significant (P<0.05) blocked when pretreated with an alpha-1 adrenergic antagonist Prazosin in TST and FST tests. However, it reflects from the study that the adrenergic neuronal system may have involved in the *V. wallichii* induced anti-fatigue and antidepressant effect in mice.

**Effect of haloperidol on *V. wallichii* induced reduction in immobility time of mice**

Fig. 5 and 6 depicts the effect of dopaminergic antagonist Haloperidol (0.2 mg/kg, IP) alone and in combination with aqueous alcoholic extract of *V. wallichii* (50 mg/kg) on immobility time of mice in the FST and TST.

Haloperidol is a typical antipsychotic drug which acts as non-selective D-2 dopamine receptor antagonist and it is used to treat schizophrenia, mania, psychosis etc. disorders. In FST and TST tests, *V. wallichii* (50 mg/kg) induced decrements in immobility time of mice was significantly prevented by pretreatment of D-2 dopamine non selective receptor antagonistic Haloperidol (0.2 mg/kg, IP). Whereas, alone treatment of Haloperidol (0.2 mg/kg, IP) did not show significant change in immobility time in above both tests. Hence, the findings of the present study revealed that *V. wallichii* induced reduction in immobility time was significantly (P<0.05) blocked by pretreatment of a D-2 dopamine receptor antagonistic Haloperidol. Our study is supported by Sah et al. (2011) [31] who...
have reported that prolong the treatment of V. wallichii significantly increase the levels of dopamine in forebrain of mouse. Hence, the present study revealed that V. wallichii induced anti-fatigue and antidepressant effects in mice are significant (P<0.05) blocked when pretreated with a Haloperidol. However, in the present study concluded that the dopaminergic neuronal system may have interaction in V. wallichii induced anti-fatigue and antidepressant effects in mice.

Effect of p-CPA on V. wallichii induced reduction in immobility time of mice

Fig. 7 and 8 depicts the effect of p-CPA (100 mg/kg, IP, 4 consecutive days) alone and in combination with ethanolic extract of V. wallichii (50 mg/kg) on immobility time of mice in the FST and TST.

p-CPA acts as irreversible and selective inhibitor of tryptophan hydroxylase enzymes, which is a rate limiting factor for biosynthesis of serotonin. V. wallichii (50 mg/kg) induced reduction in immobility time was significantly prevented by pretreatment of p-CPA (100 mg/kg, IP, 4 consecutive days) in FST. Similar to FST test, V. wallichii (50 mg/kg) induced reduction in immobility time was significantly blocked by pretreatment of p-CPA (100 mg/kg, IP, 4 consecutive days) in TST test also. While, alone treatment of p-CPA (100 mg/kg, IP) did not show significant change in immobility time in FST and TST tests. Hence, the findings of the present study revealed that the effect of V. wallichii on reduction in immobility time was significantly (P<0.05) blocked by pretreatment of a serotonin synthesis inhibitor p-CPA in mice.

According to the results of the present study, V. wallichii has induced anti-fatigue and antidepressant effects in mice were prevented by the pretreatment of alpha-1 adrenergic antagonist Prazosin, nonselective dopaminergic antagonist Haloperidol and serotonin synthesis inhibitor p-CPA in both FST and TST tests, which is indicative of involvement of adrenergic, dopaminergic and serotonergic systems in the enhancement of neuronal anti-fatigue and antidepressant effect of V. wallichii in mice. Hence, the results suggested that V. wallichii enhanced neuronal anti-fatigue and antidepressant effects through adrenergic, dopaminergic and serotonergic system.

Effect of V. wallichii and Imipramine on locomotor activity

Fig. 9 depicts the effect of ethanolic extract of V. wallichii (20, 50, 125 and 300 mg/kg) and positive control drug Imipramine (15 mg/kg) on locomotor activity of mice in photoactometer.

Effect of V. wallichii and Imipramine on locomotor activity in actophotometer, [Values expressed in scores as mean±SD from six animals in each group at respective time-points. VW: Valeriana wallichii, Imipramine: 15 mg/kg body wt.]
In the experiment, locomotor activity of Imipramine treated animals was not significantly affected as compared to control animals. Similar to Imipramine, 20, 50 and 125 mg/kg dosage of *V. wallichii* did not alter the locomotor activity of mice significantly. Whereas, higher dose of *V. wallichii* (300 mg/kg body weight, orally) significantly (P<0.05) decreased locomotor activity as compared to control and positive control (Imipramine) groups. Hence, this study reveals that Imipramine and *V. wallichii* has no effect on locomotor activity but higher dose of *V. wallichii* (300 mg/kg body wt) has a significant effect in decreasing the locomotor activity, similar to Subhan et al. (2010) [29] they have exhibited that the highest doses of aqueous-ethanolic extract reduced locomotor activity. However, *V. wallichii* does not interfere the motor activity of mice at lower doses; while higher doses may produce other effects-like sedative or sleep enhancing in mice. One another researcher has also reported that the presence of sedative and sleep-enhancing compound flavanone glycoside 2S (+) hesperidin (HN) in *V. officinalis* and *V. wallichii* [9]. Thus, it may be concluded that the higher doses of *V. wallichii* may have sedative or sleep-enhancing-like effect in mice.

**CONCLUSION**

Oral administration of *V. wallichii* at dosage rate (50-300 mg/kg body weight) showed significant (P<0.05) enhancement in neuronal anti-fatigue, mood elevator and antidepressant effects in mice without interfering their motor activity. While, pretreated animals with Prazosin alpha 1-adrenoceptor antagonist, Haloperidol as nonselective D-2 dopaminergic antagonist and p-CPA as serotonin synthesis inhibitor did not show significant (P>0.05) enhancement in *V. wallichii* induced anti-fatigue, mood modifying and antidepressant effects. Hence, it is concluded that the neuronal anti-fatigue, mood elevator, and antidepressant effects of *V. wallichii* may be mediated via adrenergic, dopaminergic and serotoninergic systems. No significant difference was noticed in the locomotor activity of mice, which were administered with Imipramine (15 mg/kg) and *V. wallichii* (20, 50, and 125 mg/kg). While, *V. wallichii* at the higher dose (300 mg/kg) exhibited significant (P<0.05) reduction in locomotor activity, which may be attributed to sedative or sleep enhancing-like effect in mice. Therefore, there is scope of more research on the effect of *V. wallichii* and involvement of specific parts of brain and receptor.

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**AUTHORS CONTRIBUTIONS**

Harshay Meena: Study design, conduct experiments, data collection and analysis, and preparing manuscript. Col. V K Joshi: The manuscript was reviewed and edited. The experiment was conducted under the guidance and supervision of Dr Madhu Bala, Scientist ‘G’.

**CONFLICTS OF INTERESTS**

No conflicts of interest

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