

Elucidation of possible mechanism of analgesic action of *Valeriana wallichii* DC chemotype (patchouli alcohol) in experimental animal models

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Valeriana wallichii (Family Valerianaceae), popularly named as Indian valerian, exists as three chemotypes. Aim of the study was to evaluate the effect of *V. wallichii* chemotype (patchouli alcohol) extract (DCME) and essential oil (VP AEO) on experimental models of nociception and to elucidate its possible mechanism of action. Analgesic effect was evaluated using acetic acid induced writhing and tail flick model. DCME and VP AEO (40 and 80 mg/kg, p.o.) significantly inhibited the number of writhings as compared to vehicle treated group. None of the doses of DCME and VP AEO exhibited any effect in tail flick model suggesting only peripheral analgesic activity. When studied for mechanism of action in acetic acid induced writhing, subeffective dose of essential oil significantly potentiated the effect of aspirin while no potentiation was seen in case of extract. These data suggest that essential oil VP AEO exerted peripheral analgesic via inhibition of prostaglandin synthesis.

Keywords: Analgesic, Essential oil, Iridoids, *Valeriana wallichii*

Valeriana (Valerianaceae) originated from the Latin word “*Valere*” meaning ‘to be in good health’, a source of important phytomedicines, have been used for curing nervous unrest, emotional troubles (as tranquillizer/sedative), epilepsy, insanity, snake-poisoning, eye-trouble, skin-diseases, as a relaxant, carminative and for complexion improvement^{1,2}. Valerian continues to be a safe sedative/hypnotic choice for patients with mild to moderate insomnia.

Valeriana wallichii DC (Indian Valerian) grows wild in the temperate Himalaya at an altitude of 1500-3000 m and is an ingredient of herbal medicines in Indian systems of medicine³. *Valeriana wallichii* is the counter part of European *V. officinalis* L. the official drug in the British and European Pharmacopeias⁴. The roots and rhizomes are highly aromatic and contain valepotriates and essential oils, both perhaps contributing towards its bioactivity. In addition, sesquiterpenoids, monoterpenoids, lignans and alkaloids with pharmacological properties have

been reported in valerian⁵⁻¹⁰. The *V. wallichii* essential oil has been the subject of previous studies which established the existence of three distinct chemotypes⁶⁻⁹. Isolation of valepotriates from *Valeriana wallichii* and their bioactivity studies have also been reported¹¹⁻¹⁵. There is a preliminary report showing analgesic effect of dried leaves of *V. jatamansi* at a dose of 2 mg¹⁶. In the present study the analgesic effect of patchouli alcohol chemotype of *V. wallichii* which is commercially used as substitute for the official drug has been reported.

Materials and Methods

Plant material and its extraction—Roots and rhizomes of *V. wallichii* were collected from Kumaun region of India and authenticated from Botanical Survey of India, Dehradun (Voucher specimen number Chem/DST/V.II). The plant material was dried in shady place and subjected to dichloromethane extraction. The extract was concentrated to dryness with rotary evaporator at 35°C to yield a brown dry mass (DCME) and the yield was 2% w/w. Similarly the essential oil (VP AEO) was extracted from the roots and rhizomes by steam distillation and the yield was 0.6% (v/w).

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Animals—Laca mice of either sex (20-40 g) bred in Central Animal House facility of Panjab University, Chandigarh, were housed in cages with food and water *ad libitum* and maintained on a natural light and dark cycle. The experimental protocols were approved by the Panjab University Animal Ethical Committee.

Drugs—Aspirin was procured from Panacea Biotech, Lalru, India. To the test drugs (DCME and VPAEO) one drop of Tween-80 was added and then volume was made up with distilled water. Solution of aspirin was also prepared using Tween-80.

HPLC Analysis—The extract (DCME) was analysed using Water's 440 HPLC coupled with RI detector in C-18 (25 cm × 4.6 mm i.d.) column and acetonitrile:water (80:20) solvent system and compared with the reference compounds.

Essential oil analysis—The oil VPAEO was analysed on Nucon 5765 GC (30m × 0.32mm, FID) with split ratio 1:48, N₂ flow of 4.0 kg/cm². GC/MS was done on thermoquest trace GC-2000 interfaced with Finnigen MAT Polaries-Q ion trap mass spectrometer fitted with RTX-5MS (Restek Corp.) fused silica capillary column (30 × 0.25 mm, 0.25 μm film coating). The oven temperature was programmed from 60-210° C at 3°C/min using He as carrier gas at 1.0 ml/min. The injector temperature was 210°C, injection volume was 0.1 μl prepared in hexane, split ratio 1:40. MS were taken at 70 ev (EI) with mass scan range of m/z 40-450 amu with mass scan time 4seconds. Identification of the constituents was done on the basis of retention index, library mass search database (NIST & Wiley) and by comparing with the mass spectral data.

Effect of valerian extract and oil in acetic acid-induced writhing—Acetic acid (1%) was injected i.p. in mice and the writhing response characterized by abdominal constriction and hind limb stretching was counted for 10 min¹⁷. VPAEO and DCME (20, 40 and 80 mg/kg, p.o.), aspirin (100 mg/kg, p.o.) as the standard drug, were administered orally to mice 1 hr before the test. Vehicle treated group served as control. The number of writhings in these groups were recorded and compared with control and standard groups.

Effect of valerian extract and oil on tail-flick latency—Analgesia was assessed with analgesiometer (Techno Electronics, India). Basal reaction time of animals to radiant heat was recorded by placing the tip (last 1-2 cm) of the tail on the radiant heat source.

The tail withdrawal from the heat (flicking response) was taken as the end point. The animals, which showed flicking response within 3-5 sec, were selected for the study. A cut off period of 12 sec was observed to avoid damage to the tail¹⁷. The measurement of tail flick latency was recorded at 15, 30, 60, 120 and 180 min after administration of test drugs and aspirin.

Effect of valerian extract and oil in combination with aspirin in acetic acid induced writhing—For mechanistic study sub-effective dose i.e 20 mg/kg dose of DCME and VPAEO was combined with 5 mg/kg dose of aspirin and was then studied in acetic acid induced writhing. Aspirin was given intraperitoneally 30 min after the administration of test drugs and acetic acid was then injected 30 min after aspirin. *Per se* effect of 5 mg/kg aspirin was also studied in acetic acid induced writhing.

Statistical analysis—All data were expressed as mean ± SEM of 6 animals. Results were analysed statistically by One-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison using sigma stat software. Values of *P* < 0.05 were considered statistically significant.

Results

The results of the GC-MS analysis of essential oil (VPAEO) showed patchouli alcohol (40%) as the major constituent followed by the presence of δ-guaiene (10%), seychellene (8%), acetoxyl patchouli alcohol (5%) and viridiflorol (5%) (Table 1). The HPLC analysis of DCME extract demonstrated the presence of valepotriates like valtrate, isovalerohydroxy-dihydrovaltrate, 1α-acevaltrate, and didrovaltrate. So, the result demonstrated that out of the three reported chemotypes, it is patchouli alcohol containing chemotype of *V. wallichii*.

Oral administration of essential oil VPAEO at doses ranging from 10-1000 mg/kg mg/kg did not

Table 1—Chemical constituents of DCME and VPAEO

	Major constituents
DCME	Valtrate
(Valepotriates)	Isovalerohydroxydihydrovaltrate 1α-Acevaltrate Didrovaltrate
VPAEO	Patchouli alcohol (40%)
(Terpenes)	Viridiflorol (5%) 8-Acetoxy patchouli alcohol (5%) Seychellene (8%) δ -Guaiene (10%)

Table 2—Effect of different doses of VP AEO and DCME in tail flick latency in mice

[Values are mean \pm SE of 6 independent observations in each group.]

Group (mg/kg)	Tail flick latency in seconds					
	Pre-drug reaction	0.25 h	0.5 h	1 h	2 h	3 h
VP AEO (20)	2.89 \pm 0.13	3.31 \pm 0.15	2.79 \pm 0.41	2.99 \pm 0.36	3.32 \pm 0.3	3.0 \pm 0.31
VP AEO (40)	2.23 \pm 0.27	2.49 \pm 0.18	3.15 \pm 0.3	3.29 \pm 0.4	3.2 \pm 0.4	2.3 \pm 0.4
VP AEO (80)	2.74 \pm 0.19	2.8 \pm 0.07	3.24 \pm 0.3	2.9 \pm 0.29	3.6 \pm 0.4	3.3 \pm 0.195
DCME (20)	2.04 \pm 0.1	1.75 \pm 0.6	2.11 \pm 0.2	2.4 \pm 0.01	2.5 \pm 0.4	2.15 \pm 0.4
DCME (40)	2.08 \pm 0.4	2.44 \pm 0.3	2.46 \pm 0.2	3.48 \pm 0.6	3.88 \pm 0.8	3.57 \pm 0.7
DCME (80)	2.22 \pm 0.3	1.89 \pm 0.1	2.18 \pm 0.5	3.00 \pm 0.5	2.70 \pm 0.3	2.28 \pm 0.6
Aspirin (100)	3.79 \pm 0.2	3.94 \pm 0.8	4.5 \pm 0.5	7.1 \pm 0.07*	5.77 \pm 0.8*	3.88 \pm 0.19

P values: <* 0.05 as compared to pre drug reaction

produce any adverse effect or lethality in mice. Similarly no adverse effect or mortality was detected in albino mice up to 2000 mg/kg, p.o dose of extract (DCME) during 48 h observation period.

Administration of DCME (20, 40 and 80 mg/kg, p.o) produced a dose dependent inhibition of acetic acid-induced writhes in mice and the effect being significant at 40 and 80 mg/kg (Fig. 1). The percentage reduction in the writhing response was 29.8% and 54.3% respectively at doses 40 and 80 mg/kg. Aspirin (100 mg/kg, p.o) also produced significant inhibition of writhing (64.9%). The inhibition produced by 80 mg/kg of DCME is comparable to aspirin. Similarly essential oil VP AEO at doses 80 and 40 mg/kg produced significant inhibition of writhings i.e. 50% and 31.1% respectively (Fig. 1) and the effect being comparable to standard drug aspirin at 80 mg/kg dose.

Oral administration of extract and essential oil (20, 40 and 80 mg/kg, p.o.) failed to prolong the latency time in tail flick model when compared to predrug reaction (Table 2). However aspirin (100 mg/kg, p.o.) used in this study elicited a significant analgesic effect.

When DCME was studied for mechanism of action in acetic acid writhing, no potentiation was seen with aspirin (5 mg/kg i.p.). However VP AEO (20 mg/kg, p.o.) potentiated the antinociceptive action of aspirin in acetic acid writhing (Fig. 1). 20 mg/kg p.o. dose of essential oil *per se* did not produce any analgesic effect. The inhibition was found to be 61.7% with the combination of VP AEO and aspirin which is statistically significant when compared to aspirin (5 mg/mg, i.p) and VP AEO (20 mg/kg, p.o) *per se*.

Discussion

Analgesic activity of valerian extract and oil were investigated in both acetic acid-induced writhing and

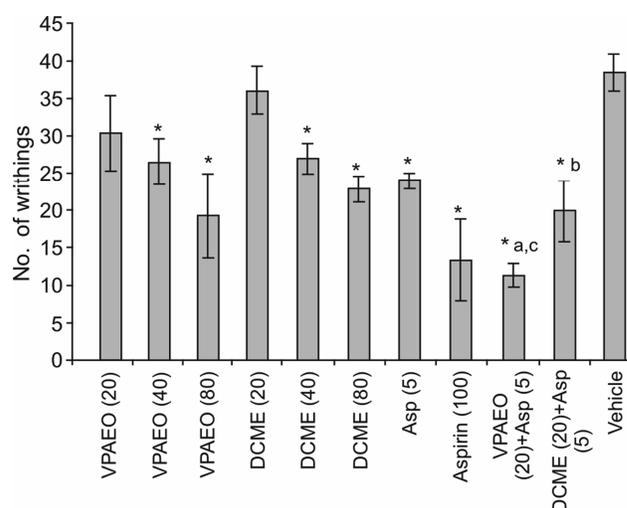


Fig. 1—Effect of different doses of VP AEO and DCME in acetic acid induced writhing and effect of aspirin (5 mg/kg, i.p) in combination with VP AEO and DCME. Aspirin (100 mg/kg p.o) was used as standard. *P* < 0.05 as compared to *vehicle, ^aVP AEO (20 mg/kg) *per se*, ^bDCME (20 mg/kg) *per se*, ^caspirin (5 mg/kg, i.p) *per se*. [Values are expressed Mean \pm SE of 6 independent observations in each group].

tail flick models in mice. The acetic acid-induced writhing in mice, a typical model for inflammatory pain, has long been used as a screening tool for the assessment of analgesic or anti-inflammatory properties of new agents¹⁸. This method presents a good sensitivity; however, it shows poor specificity, leaving scope for the misinterpretation of results which can be avoided by complementing the test with other models of nociception. Acetic acid causes inflammatory pain by inducing capillary permeability¹⁹ and liberating endogenous substances that excite pain nerve endings. Since the writhing test usually lasts for less than an hour, it is too short to cover central prostaglandin-dependent sensitization, which requires spinal COX-2 induction. However, it primarily involves COX-1 derived prostanoids and

thus non-selective COX inhibitor offers a better antinociceptive effect as compared to selective COX-2 inhibitors²⁰.

There are few reports on analgesic activity of *Valeriana wallichii*^{14,15,16} and our study also revealed that the essential oil (VP AEO) and extract (DCME) of *V. wallichii* chemotype (patchouli alcohol) produced significant inhibition in acetic acid writhing at doses 40 and 80 mg/kg, p.o. suggesting peripheral analgesic action.

Tail flick is an acute spinally mediated reflex to noxious thermal stimuli and it is known to be selective for centrally acting analgesics. In tail flick model, none of the doses of essential oil and extract were found to be effective, indicating that higher center is not involved in antinociceptive effect of the patchouli alcohol chemotype at the tested doses.

When studied for mechanism of action in acetic acid writhing model, essential oil potentiated the action of aspirin. So the mechanism of analgesic effect of essential oil could probably be due to blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of indomethacin and other NSAIDs. This is because NSAIDs can inhibit COX in peripheral tissues and, therefore, interfere with the mechanism of transduction of primary afferent nociceptors.

GC-MS screening of the plant showed that its essential oil is dominated by sesquiterpenes. Many studies report analgesic activity of essential oils and sesquiterpenes²¹⁻²⁴. Few studies suggest that terpenoids inhibit *in vitro* formation of PGE₁²⁵ and have suppressive effect on iNOS and COX-2 activity^{26,27}. Moreover natural terpenoids are reported to be natural inhibitors of NF-κB signaling²⁸ just like aspirin and other NSAIDs²⁹. All these evidences reveal that the peripheral analgesic activity of essential oil is due to the presence of terpenes and sesquiterpenes and the mechanism of action is similar to non steroidal anti-inflammatory drugs.

The results of the study shows that DCME extract of *V. wallichii* majorly contains valepotriates (iridoids), and it did not potentiate the effect of aspirin in acetic acid writhing. So the effect of extract may be attributed to mechanism other than COX inhibition, possibly to K_{ATP} channel activation which can then lead to antispasmodic effect³⁰ in acetic acid writhing.

The results reveal that both extract and essential oil of *V. wallichii* possess peripheral analgesic action and the effect being comparable to aspirin. The presence

of terpenes and valepotriates may be contributory to this activity of *Valeriana wallichii*. Preliminary acute toxicity assessment of both oil and extract performed show that lethal dose is much greater than the minimal analgesic dose which makes the plant worthy for further studies and phytochemical investigation is also proposed in order to isolate the active fraction and eventually the pure compound responsible for analgesic action.

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