

## ***Valeriana wallichii* DC (Maaliol Chemotype): Antinociceptive Studies on Experimental Animal Models and Possible Mechanism of Action**

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**Abstract: Background:** *Valeriana wallichii* DC (Valerianaceae), popularly known as Indian valerian and used indigenously as herbal medicine exist as three chemotypes. The aim of the study was to evaluate chemical composition and effect of extract and essential oil of *V. wallichii* (maaliol chemotype) on experimental models of nociception and to elucidate possible mechanism of action. **Materials and Methods:** Essential oil was subjected to GC-MS analysis and dichloromethane extract of the plant was analyzed using HPLC. Antinociceptive effect was evaluated using acetic acid induced writhing and tail flick model. Both the extract and essential oil were then studied for possible mechanism of analgesic action. **Results:** The main components detected in the essential oil were maaliol (36.81%) followed by  $\beta$ -gurjunene, acoradiene, guaiol and  $\alpha$ -santalene. The extract demonstrated the presence of valepotriates like isovalerohydroxydidrovaltrate, 1 $\alpha$ -acevaltrate and didrovaltrate. Both the extract and essential oil significantly inhibited the number of writhings in acetic acid induced writhing and increased the latency time after 2 h of administration in tail flick model ( $p < 0.05$ ). Subeffective dose of essential oil significantly potentiated the effect of aspirin while no potentiation was seen in case of extract. Similarly in tail flick test naloxone completely antagonised the analgesic action of essential oil while no reversal of analgesic action was seen with DCM extract. **Conclusion:** The results demonstrated both weak central and a strong peripheral antinociceptive effect of *V. wallichii* (maaliol chemotype). The data suggested that essential oil exerted peripheral antinociceptive effect via inhibition of prostaglandin synthesis and central analgesic action via opioidergic pathway.

**Key words:** Antinociceptive, essential oil, valepotriates, *Valeriana wallichii*, maaliol chemotype

### INTRODUCTION

*Valeriana* (Valerianaceae) is distributed in the temperate and sub-tropical areas globally and is among the important herbal traditional drug in various pharmacopeias (Anonymous, 1988, 1995). Valerian is the common name given to the crude drug consisting of the underground parts of species of *Valeriana*. The Valerian derived phytomedicines have been used for curing nervous unrest, emotional troubles (as tranquillizer/ sedative), epilepsy, insanity, snake-poisoning, eye-trouble, skin-diseases, relaxant, carminative and for complexion improvement (Anonymous, 1976, 1988, 1995; Houghton, 1999). Valerian continues to be a safe sedative/hypnotic choice for patients with mild to moderate insomnia (Hadley and Petry, 2003).

*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 medicine. The root/ rhizome parts are highly aromatic and contain valepotriates and essential oils. The volatile composition of *V. wallichii* oil has been the subject of previous studies which established the existence of three chemically distinct species within *V. wallichii* (Mathela *et al.*, 2005a, b; Mathela *et al.*, 2009). The chemotype-I possess maaliol (Mathela *et al.*, 2005a,b). The periodic collections showed that maaliol content vary between 48.0-67.0% in the samples collected from different micro-climatic and at different stages of growth. In contrast, chemotype-II contain patchouli alcohol (40%) (Mathela *et al.*, 2005b), while the chemotype-III is characterized by kanokonyl acetate (Mathela *et al.*, 2009). Notably, the marker constituents of one chemotype of *V. wallichii* were completely absent in other chemotypes.

There are various studies reporting isolation and bioactivities of valepotriates from *Valeriana wallichii* (Becker *et al.*, 1984; Lin *et al.*, 2009; Wang *et al.*, 2008; Cao and Hong, 1994). A study depicts, that mild

myorelaxant action of *Valeriana* is attributed to the valepotriates component of the herb (Dunaev *et al.*, 1987). There are preliminary reports demonstrating antinociceptive effect of dried leaves, roots and rhizomes of *V. wallichii* (Schultz and Eckstein, 1962; Shrivastava and Sisodia, 1970). *Valeriana wallichii* has been used in different gastrointestinal disorders, such as diarrhoea and abdominal spasm (Gilani *et al.*, 2005). In the present study antinociceptive effect of maaliol chemotype of *V. wallichii*, which is commercially used as substitute for the official drug has been evaluated to validate its ethnomedicinal use.

## MATERIALS AND METHODS

**Plant material and its extraction:** Roots and rhizomes of *V. wallichii* DC were collected from Kumaun region of India in October 2008 and authenticated from Botanical Survey of India, Dehradun (Voucher specimen number Chem/DST/V.01). The plant material was dried in shade and then subjected to dichloromethane extraction. The extract was concentrated and dried in rotary evaporator at 35°C to yield a brown dry mass (DE). The yield of the extract was 2% w/w. Similarly, the Essential Oil (EO) was extracted from the roots and rhizomes by steam distillation. The essential oil was stored under refrigeration for three days and then used immediately for analysis and evaluation of analgesic activity.

**Essential oil analysis:** The essential oil was analyzed on Nucon 5765 GC (30 m×0.32 mm, FID) with split ratio 1:48, N<sub>2</sub> flow of 4.0 kg cm<sup>-2</sup>. GC/MS analysis was done on thermoquest trace GC-2000 interfaced with Finnigan MAT Polaries-Q ion trap mass spectrometer fitted with RTX-5 MS (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<sup>-1</sup> using helium as carrier gas at 1.0 mL min<sup>-1</sup>. The injector temperature was 210°C, injection volume was 0.1 µL prepared in hexane, split ratio 1:40. Mass spectra were taken at 70 eV (EI) with mass scan range of m/z 40-450 amu with mass scan time 4 sec. Identification of the constituents was done on the basis of retention indices (Kovats, 1965) and their mass spectra (NIST and WILEY), which were compared with literature data (Adams, 2001).

**HPLC analysis:** The extract was analyzed for the presence of valepotriates 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.

**Animals:** LACA mice of either sex (20-40 g) bred in Central Animal House facility of Panjab University, Chandigarh housed in cages with food and water *ad libitum* and maintained on a natural light and dark cycle were used. All the experimental protocols were approved by the Panjab University Animal Ethical Committee research and adhered to the Principles of Laboratory Animal Care.

**Drugs:** Aspirin was procured from Panacea Biotech, Lalru, India. For the pharmacological assays, aqueous suspension of test drugs (DE and EO) in 2% v/w Tween 80 were used.

**Acute toxicity studies:** Acute toxicity test was performed according to the Organization for Economic Cooperation Development (OECD) guidelines No. 425 (OECD, 1996). It is based on a stepwise procedure with the use of a minimum number of animals per step. Three female nulliparous and non pregnant albino LACA mice were used for each step (each dose level). The dose level to be used as the starting dose was selected from one of three fixed dose levels of 25, 200 and 2000 mg kg<sup>-1</sup>, b.wt. (OECD, 1996). The animals were fasted before the oral administration of the extract and observed individually, after dosing, at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first four hours and daily thereafter, for a total of 14 days. The animals were observed for general behavior and any toxic symptoms produced by the test drug under investigation, such as cyanosis, tremors, convulsions, ataxia, extension of the limbs, increased muscle tone, piloerection, tail flick, salivation, drowsiness, diarrhea, ptosis, respiratory effects, arched back, loss of righting, abdominal gripping and blanching. The number of animals that die in a 14 day period after a single dose was recorded.

### Analgesic activity

**Acetic acid-induced writhing test:** Acetic acid (1% w/v, 10 mL kg<sup>-1</sup>) when injected intraperitoneally to mice produces writhing response characterized by abdominal constriction and hind limb stretching. Groups of mice (n = 6) received different doses (20, 40 and 80 mg kg<sup>-1</sup>, p.o.) of extract and essential oil, 1 h before the acetic acid injection. Vehicle (2% v/w Tween 80) treated group served as negative control. Aspirin (100 mg kg<sup>-1</sup>, p.o.), treated group served as positive control. The number of writhings in all the groups were counted for 10 min and compared with control groups (Kulkarni, 1999).

**Tail-flick model:** 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 tail. Vehicle (2% v/w Tween 80) treated group served as control. Aspirin (100 mg kg<sup>-1</sup>, p.o.) and meperidine (5 mg kg<sup>-1</sup>, i.p.) were used as the reference analgesics. The tail flick latency was recorded 15, 30, 60, 120 and 180 min after the administration of vehicle, aspirin, meperidine, EO and DE (20, 40 and 80 mg kg<sup>-1</sup>, p.o.) (Kulkarni, 1999).

**Effect of valerian extract and oil in combination with aspirin in acetic acid induced writhing:** For mechanistic study, sub-effective doses (20 mg kg<sup>-1</sup>) of DE and EO were combined with 5 mg kg<sup>-1</sup> dose of aspirin and 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<sup>-1</sup> aspirin was also studied in acetic acid writhing.

**Effect of valerian extract and oil in combination with naloxone in tail flick test:** To test the involvement of opioidergic pathway in the central analgesic action of extract and oil, effective doses (80 mg kg<sup>-1</sup>) of DE and EO were combined with 2 mg kg<sup>-1</sup>, s.c. dose of naloxone and then studied for effect on tail flick latency. Naloxone (2 mg kg<sup>-1</sup>, s.c.) was administered 30 min before the administration of EO and DE. Similarly, the opioid antagonist naloxone (2 mg kg<sup>-1</sup>, s.c.) was tested along with meperidine in tail flick test.

**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 roots and rhizomes of *Valeriana wallichii* yielded 0.16% (v/w) pale yellow colored essential oil. The results of the GC-MS analysis of the essential oil showed maaliol (36.8%) as the major constituent followed by the presence of β-gurjunene (21.3%), acoradiene (9.9%), guaiol (8.6%) and α-santalene (5.5%). The HPLC analysis of extract demonstrated the presence of valepotriates viz., isovalerohydroxydihydrovaltrate, 1α-acevaltrate and

Table 1: Chemical constituents of extract (DE) and Essential Oil (EO) of *Valeriana wallichii* (maaliol chemotype)

Sample	Major constituents
DE (valepotriates)	isovalerohydroxydihydrovaltrate 1α-acevaltrate didrovaltrate
EO (mono- and sesquiterpenoids)	maaliol β-gurjunene acoradiene guaiol α-santalene

Table 2: Effect of different doses of essential oil and extract of *Valeriana wallichii* in tail flick latency

Group (mg kg <sup>-1</sup> )	Tail flick latency in (sec)				
	Pre-drug reaction	0.5 h	1 h	2 h	3 h
Control	3.6±0.16	2.57±0.18	3.1±0.15	2.8±0.1	3±0.1
DE (20)	2.09±0.3	2.63±0.2	2.6±0.4	2.5±0.4	2.15±0.4
DE (40)	2.32±0.3	2.06±0.5	2.9±0.3	3.03±0.6	3.57±0.7
DE (80)	2.18±0.7	2.69±0.5	2.9±0.4	3.48±0.1*	2.3±0.8
EO (20)	3.5±0.17	2.88±0.17	2.5±0.5	3.29±0.5	2.97±0.2
EO (40)	2.88±0.3	5.17±1.9	3.8±0.9	3.97±0.6	3.14±0.7
EO (80)	2.57±0.2	3.32±0.52	3.4±0.95	4±0.4*	3.4±0.175
Mep (5)	3.79±0.2	4.05±0.9	8.17±0.07*	6.77±0.8*	3.88±0.19
Mep (5)+Nal (2)	3.05±0.1	4.0±0.09	3.9±0.14	3.67±0.7	3.94±0.8
DE (80)+Nal (2)	2.75±0.6	3.00±0.2	3.6±0.12	4.02±0.06*	3.1±0.4
EO (80)+Nal (2)	3.1±0.19	2.47±0.3	3.3±0.11	3.87±0.5	2.9±0.3

didrovaltrate (Table 1). The results of chemical compositional analysis thus demonstrated that out of the three reported chemotypes, it is maaliol chemotype of *V. wallichii*.

Oral administration of essential oil at doses ranging from 10-2000 mg kg<sup>-1</sup> did not produce any toxic effect or lethality. Similarly no toxic effect or mortality was detected in mice up to 2000 mg kg<sup>-1</sup>, p.o. dose of extract during 48 h of observation period suggesting that LD<sub>50</sub> values of both are greater than 2 g kg<sup>-1</sup>.

Administration of DE (20, 40 and 80 mg kg<sup>-1</sup>, p.o.) produced a dose dependent inhibition of acetic acid-induced writhes in mice and the effect being significant at 80 mg kg<sup>-1</sup> (Fig. 1). The percentage reduction in the writhing response was 37.8% with 80 mg kg<sup>-1</sup> dose. Aspirin (100 mg kg<sup>-1</sup>, p.o.) produced significant inhibition of writhing (63.5%). Similarly essential oil EO at doses 40 and 80 mg kg<sup>-1</sup> produced significant inhibition of writhings i.e., 28.2 and 47.9%, respectively (Fig. 1). The inhibition produced at 80 mg kg<sup>-1</sup> dose of EO was comparable to standard drug aspirin.

Oral administration of extract and essential oil produced a time dependent increase in tail flick latency at 80 mg kg<sup>-1</sup> and the effect was significant after 2 h of drug administration as compared to vehicle group (Table 2). However, meperidine (5 mg kg<sup>-1</sup>, i.p.) used in this study elicited a significant analgesic effect after 60, 120 and 180 min of administration.

When DE was studied for mechanism of action in acetic acid writhing, no potentiation was seen with aspirin (5 mg kg<sup>-1</sup> i.p.). However, EO (20 mg kg<sup>-1</sup>, p.o.) potentiated the antinociceptive action of aspirin in acetic

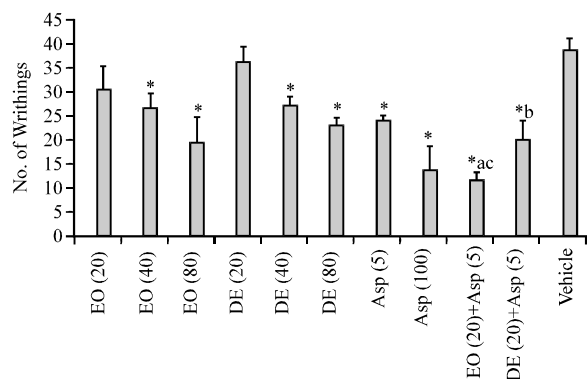


Fig. 1: Effect of oral treatment with EO and DE in acetic acid induced writhing and the effect of EO and DE in combination with aspirin (5 mg kg<sup>-1</sup>, i.p.). Aspirin (100 mg kg<sup>-1</sup> p.o.) was used as positive control. Data are mean±SEM values. p<0.05 as compared to \*vehicle, <sup>a</sup>EO (20 mg kg<sup>-1</sup>) *per se*, <sup>b</sup>DE (20 mg kg<sup>-1</sup>) *per se*, <sup>c</sup>aspirin (5 mg kg<sup>-1</sup>, i.p.) *per se* (ANOVA, Tukey's test)

acid-induced writhing (Fig. 1). Essential oil (20 mg kg<sup>-1</sup>, p.o.) *per se* did not produce any antinociceptive effect however, the inhibition was found to be 51.3% with the combination of EO and aspirin which was statistically significant as compared to aspirin (5 mg kg<sup>-1</sup>, i.p.) and EO (20 mg kg<sup>-1</sup>, p.o.) *per se*.

In tail flick model, naloxone completely antagonised the analgesic action of essential oil (80 mg kg<sup>-1</sup>) while no reversal of analgesic action was seen with 80 mg kg<sup>-1</sup> dose of DCM extract.

## DISCUSSION

The essential oil and dichloromethane extract of *V. wallichii* (maaliol chemotype) did not show any toxicity and behavioral changes in mice up to 2 g kg<sup>-1</sup>, hence doses of (20, 40 and 80 mg kg<sup>-1</sup>, p.o.) were selected for the present study. The effect of *V. wallichii* was found to be more prominent in inhibiting peripheral pain mechanism than central. Acetic acid-induced abdominal constriction (writhing) is a sensitive method for screening peripheral analgesic effect of drug. It causes an increase in concentration of PGE<sub>2</sub> and PGF<sub>2α</sub> in the peritoneal fluid (Collier *et al.*, 1968). 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 inhibitors (Kulkarni and Jain, 2005).

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, both the essential oil and extract were effective at 80 mg kg<sup>-1</sup> dose, indicating that at higher doses, higher center is involved in antinociceptive effect of maaliol chemotype.

Essential oil at doses 40 and 80 mg kg<sup>-1</sup> and extract at 80 mg kg<sup>-1</sup> dose were found to be effective in acetic acid-induced abdominal constriction method, which was used to evaluate peripherally acting analgesics. The 80 mg kg<sup>-1</sup> dose of essential oil inhibited the acetic acid-induced pain with potency comparable to aspirin.

When compared with the previous results obtained in our lab with patchouli alcohol chemotype of *V. wallichii*, we found that DCM extract of patchouli alcohol was more effective than DCM extract of maaliol chemotype in exerting peripheral analgesic effect, while oils of both the chemotypes exhibited comparable effect (Sah *et al.*, 2010). Maaliol chemotype (essential oil and DCM extract) inhibited central pain mechanism also but at higher dose while patchouli alcohol chemotype was devoid of central analgesic effect at the tested doses.

When studied for mechanism of action in acetic acid writhing model, essential oil potentiated the action of aspirin. Thus, the mechanism of peripheral 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 aspirin 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. The results correlate well with the study done on patchouli alcohol chemotype of *V. wallichii* in our laboratory (Sah *et al.*, 2010). Naloxone completely antagonized the central analgesic effect of essential oil, while was ineffective in case of extract. The results thus demonstrated that opioidergic pathway is responsible for central analgesic action of essential oil.

GC-MS screening of the essential oil of the plant revealed terpenes like maaliol, santalene, acoradiene, β-gurjune and guaiol which together may be responsible for antinociceptive effect. Many studies report antinociceptive activity of essential oils and sesquiterpenes (Golshani *et al.*, 2004; Sayyah *et al.*, 2003; Ahmed *et al.*, 1997; Santos and Rao, 2000). The studies also suggest that terpenoids inhibit *in vitro* formation of PGE<sub>1</sub> and have suppressive effect on iNOS and COX-2 activity (Burstein *et al.*, 1975; Lee *et al.*, 2002; Yoon *et al.*, 2008). Moreover, natural terpenoids are reported to be natural inhibitors of NF-κB signaling just like aspirin and other NSAIDs (Muller *et al.*, 2001; Salminen *et al.*, 2008). Central analgesic action of essential oil from

*Cymbopogon citratus* was blocked by naloxone in one study (Viana *et al.*, 2000). All these evidences reveal that the peripheral and central analgesic activity of essential oil is attributed to the presence of terpenes and sesquiterpenes.

Valepotriates (iridoids) are the major class of compounds in the extract of *V. wallichii*. The extract did not potentiate the effect of aspirin in acetic acid writhing and neither its central analgesic effect was blocked by opioid antagonist, naloxone so the effect may be attributed to mechanism other than COX inhibition and opioidergic pathway.

### CONCLUSIONS

The results of the present study suggested that both extract and essential oil of *V. wallichii* chemotype maaliol possess peripheral and central analgesic action. The peripheral antinociceptive action of essential oil being comparable to aspirin but weaker in case of central analgesic action. The extract, however possess weak peripheral and central analgesic action. The presence of terpenoids and valepotriates may be contributory to this activity of *Valeriana wallichii*. LD<sub>50</sub> values of both the extract and oil are much greater than the minimal analgesic dose which makes the plant relatively safe and nontoxic to the animals. This makes the plant worthy for further studies to see whether it is the synergic effect or a particular compound responsible for antinociceptive action.

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