

ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC AND AQUEOUS EXTRACTS OF *VALERIANA WALLICHII* DC RHIZOME

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Abstract

Valeriana wallichii DC (Valerianaceae) is an indigenous medicinal plant used in ethno medicine for the treatment of habitual constipation, insomnia, epilepsy, neurosis, anxiety and as a diuretic, hepatoprotective, analgesic and cytotoxic. The aqueous extract (Aq.Ext) and methanolic extract (Me.Ext) were subjected to anti-inflammatory activity using experimental animal model of carrageenan induced paw oedema in the presence of control for comparison. The results showed that both Aq and Me extracts significantly ameliorate oedema comparable to reference standard, aspirin. The results of this study explicate justification of the use of this plant in the treatment of inflammatory disease conditions.

Key words: Anti-inflammatory activity, *Valeriana wallichii*, Rhizome extracts

Introduction

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical conditions, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Pakistani traditional systems of medicine. Thus the present investigation was carried out to evaluate the anti-inflammatory potential of *Valeriana wallichii* DC.

Valeriana wallichii DC (Valerianaceae) is a small perennial herb of 14-45 cm height, with root stock, 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 major active principles of *Valeriana wallichii* are Valpotriates, dihydrovaltrate (Bounthanh *et al.*, 1981), isovalerianate (Thies, 1968), 6-methylapigenin, hesperidins (Marder *et al.*, 2003) and sesquiterpenoids (Ron, *et al.*, 2000). Its rhizome and root contains volatile oil (valerianic oil) which is composed of alkaloids, bornyl isovalerianate, chatinine, formate, glucoside, isovaleronic acid, 1-camphene, 1-pinene, resins, terpineol and valerianine (Nadkarni, 1976). From the rhizomes some important compounds, such as citric acid, malic acid, maliol, succinic acid and tartaric acid have been isolated (Kapoor, 1990).

Valeriana wallichii has considerable reputation for its traditional use in inflammatory conditions such as scorpion stings and jaundice (Nadkarni, 1976) and in pain conditions (Vohora & Dandiya, 1992) epilepsy, insomnia, neurosis, sciatica (Nadkarni, 1976; Marder, *et al.*, 2003). The plant is widely used in the treatment of anxiety and depression either alone or in combination with other herbs specifically St. John's Wort (Ron *et al.*, 2000; Panijel, 1985; Leathwood & Chauffard, 1982). The plant is also used in habitual constipation (Baquar, 1989), antispasmodic (Gilani *et al.*, 2005) and as cytotoxic (Bos, *et al.*, 1986). A herbal preparation (Dhanya Panchaka Kashaya) containing *Valeriana wallichii* has been

found to be effective in dyspeptic symptoms (Tripathi *et al.*, 1982). Its essential oil exhibited antimicrobial activity against large number of pathogenic bacteria and potent antifungal activity against different human and plant fungal pathogens (Suri & Thind, 1978). Although this herb has many useful claims, no specific scientific study has been carried out to examine the anti-inflammatory activity of this plant, that's why the current study was designed.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (200-250 g) bred in our own animal house were used to study the anti-inflammatory activity. The animals (five per cage) were maintained under standard laboratory conditions (light period of 12 h/day and temperature $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$), with access to standard laboratory chow and water *ad libitum*. Animals were fasted for 12 hours before the start of experiment. The experimental procedures were carried out in strict compliance with the Animals Scientific Procedure Act U.K 1986.

Chemicals and Drugs

Aspirin was purchased from market (Oval Pharmaceutical (PVT) Ltd Lahore). Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, supplied carrageenan generously. Methanol was purchased from Merck, Germany. All drugs and extracts were dissolved in normal saline prior to use.

Plant Material

Fresh rhizomes of *Valeriana wallichii* were collected from Northern parts of Pakistan (Galyat) in August 2002. Voucher specimen (82002pup) has been submitted to the herbarium of Department of Botany of the same University.

Preparation of Valeriana aqueous extract (Vw. Aq-Ext) and methanolic extract (Vw.Me-Ext)

The plant material (rhizomes) was cleaned, shade dried and coarsely ground. The powdered material was soaked in solvents 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 rotary evaporator under reduced pressure and temperature ($\leq 50\text{C}^{\circ}$) to a semi-solid mass of dark brown color, yielding approximately 46.66 % of Aq-Ext and 25% of Me-Ext. The crude extracts were completely solubilized in normal saline (0.9% sodium chloride) for use in *in-vivo* experiments.

Preliminary Phytochemical Analysis

Preliminary Phytochemical Analysis of *Valeriana wallichii* was performed for alkaloids, tannins, saponins, terpenes, coumarins and anthraquinones according to Sofowora (1993) (Table 1)

Anti-Inflammatory Activity

Carrageenan-Induced Paw Oedema in Rats

Anti-inflammatory activity was assessed by the method described by Winter *et al.* (1962). The rats were divided into four groups of eight animals each. First group (negative control) received 0.2 ml of normal saline, second group (positive control) received aspirin (100, 150 & 200 mg/kg p.o.), third and fourth group received aqueous and methanolic extracts (100, 150 & 200 mg/kg, p.o.) of *Valeriana wallichii* respectively. After 1 h, the rats were challenged with subcutaneous injection of 0.1 ml of 1 % w/v solution of carrageenan into the plantar side of the right hind paw. Oedema was assessed for 3 hours, at 1 hour intervals after administration of the extract, in terms of an increase in circumference of the carrageenan-injected paw compared to the saline. The size of oedema was measured by wrapping a piece of cotton thread round the paw and the length of the thread corresponding to the paw circumference was determined using a meter ruler. Anti inflammatory effect was assessed as the percentage reduction in oedema level when drug was present, relative to control (Duffy *et al.*, 2001).

Activity = $100 - (100 \times \text{mean drug treated group} / \text{mean control group})$

Statistical Analysis

Results of the study were expressed as mean \pm S.E.M. ANOVA followed by Tukey-Kramer multiple comparisons test were used to determine significant differences between groups. *P-values* less than 0.05 were considered as indicative of significance.

Results and Discussion

Folkloric treatment of inflammation of various etiologies, using medicinal plants is well known to masters of the art of traditional medicine practice. Carrageenan induced paw oedema is used widely as a working model of inflammation in search for new anti inflammatory drugs (Manueli *et al.*, 1994) and appeared to be the basis of discovery of Indomethacin anti inflammatory drug (Winter *et al.*, 1963). Carrageenan-induced rat paw oedema is a suitable test for evaluating anti-inflammatory drugs, which have been frequently used to assess the anti oedematous effect of natural products. Dirosa *et al.* (1971) earlier reported that Carrageenan-induced inflammation is useful in detecting orally active anti-inflammatory agents. The development of oedema in the paw of the rat after the injection of carrageenan is due to release of histamine, serotonin and prostaglandin like substances (Vinegar *et al.*, 1969).

The aqueous and methanolic rhizomes extracts at doses (100, 150 & 200mg/kg) exhibited significant ($P < 0.05$) anti-inflammatory effect as compared to saline control in the carrageenan induced paw oedema (Figs. 2 & 3). The activity was comparable to the reference drug aspirin. At different dose range, Aq-Ext showed dose dependent activities (Fig. 2) however, the effect was not dose dependent in the case of methanolic extract (Fig. 3).

Our results revealed that administration of both Me & Aq-Exts inhibited the oedema even in the first hour. Maximum effect was observed at the end of the third hour. The significantly high anti-inflammatory activity of both methanolic and aqueous extracts may be due to inhibition of mediators of the inflammation such as histamine, serotonin released during the first phase of inflammation and prostaglandins and bradykinnins which are released during the second phase of inflammation (Vinegar *et al.*, 1969; Crunkhon & Meacock, 1971; Hernandez-Perez & Rabanal-Gallego, 2002). The qualitative phytochemical screening of *Valeriana wallichii* revealed the presence of alkaloids, flavonoids, tannins and saponins (Table 1). The anti-inflammatory activity of both the extracts of *Valeriana wallichii* rhizomes could be attributed to the high amount of flavonoids (Falodun *et al.*, 2003; Emim *et al.*, 1994) and tannins (Starec *et al.*, 1988) present in the plant. The research work also justified the traditional use of this plant in the treatment of inflammatory disease conditions such as scorpion stings and as hepato protective remedy.

Conclusion

Valeriana wallichii showed anti inflammatory properties, similar to those observed for non-steroidal anti inflammatory drugs, such as aspirin. It is also suggested that the mechanism of action of *Valeriana wallichii* might be associated with the inhibition of histamine, serotonin and prostaglandins synthesis. However further studies are needed to isolate and characterize anti inflammatory chemical constituents present in both Methanolic and Aqueous extracts of the plant.

Table 1. Phytochemical screening of different extracts of *Valeriana wallichii* rhizome

Test	Observation	Inferences	
		Me-Ext	Aq-Ext
Alkaloids : Extract + Draggendorff's reagent	Turbidity/precipitation	++	++
Saponins : Extract vigorously shaken in a test tube for 2 minutes	Frothing less than 1 cm	-	++
Flavonoids : Defatted residue of Extract+Ethanol→Filter→Filtrate+AlCl ₃	Yellow colour	++	-
Tannins : Extract + Few drops of FeCl ₃	An immediate green precipitate formed	++	++
Terpenes : Decolorized Extract residue + Chloroform + acetic anhydride+conc:H ₂ SO ₄	Brown precipitate formed	-	-
Anthraquinones :Extract+1%HCl+Filter+Benzene+NH ₄ OH	No violet colour	-	-
Coumarins : Extract covered with filter paper moistened with NAOH + boiling water + UV light	No fluorescence	-	-

++ = Positive

- = Absent

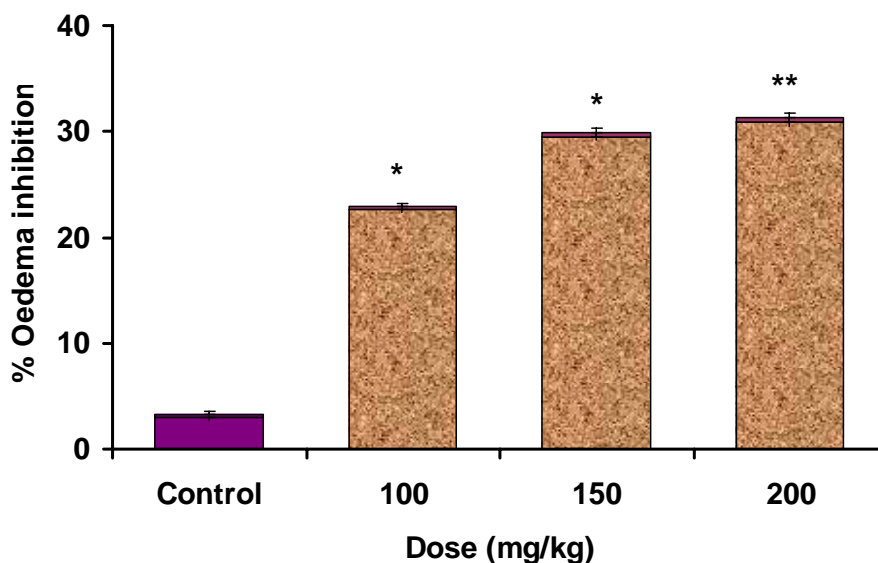


Fig 1. Effect of aspirin on Carrageenan induced hind paw oedema in rats. Values are mean \pm SEM (n=8) *P<0.05, **P<0.01 indicates significant difference between aspirin & Saline (ANOVA followed by Dunnett post hoc analysis).

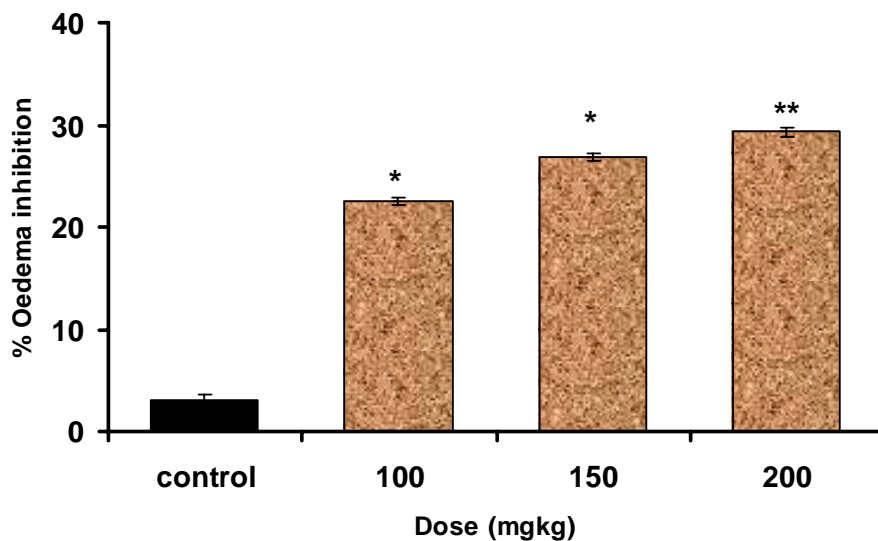


Fig 2. Effect of Aq-Ext of *Valeriana wallichii* on Carrageenan induced hind paw oedema in rats. Values are mean \pm SEM (n=8) *P<0.05, **P<0.01 indicates significant difference between Aq-Ext & Saline (ANOVA followed by Dunnett post hoc analysis)

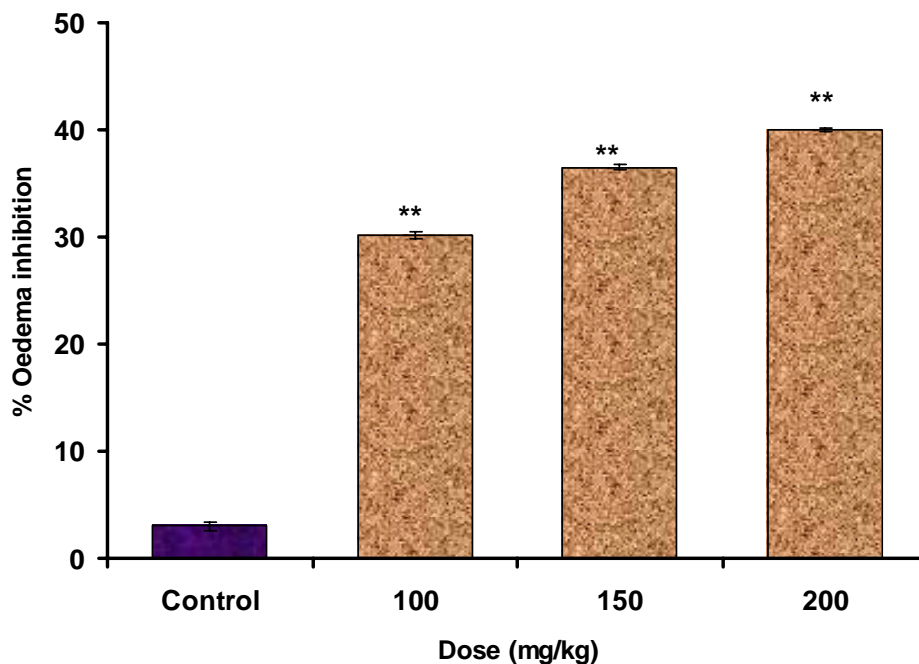


Fig 3. Effect of Me-Ext of *Valeriana wallichii* on Carrageenan induced hind paw oedema in rats. Values are mean \pm SEM (n=8) **P<0.01 indicates significant difference between Me-Ext & Saline (ANOVA followed by Dunnett post hoc analysis)

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