



RESEARCH ARTICLE

STUDY OF ANALGESIC ACTIVITY OF ROOTS OF *VALERIANA WALLICHII*

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ABSTRACT

Valeriana wallichii (Family: Valerianaceae) is being used in Indian Folk Medicine for the treatment of various diseases including management of pain. In the present study, we investigated the analgesic potential of ethanolic extract of *Valeriana wallichii* roots in different animal models viz. Hot plate method & Formalin test. The ethanolic extract (300 mg/kg) of *Valeriana wallichii* roots significantly produced analgesic activity against different pain stimuli. Thus, our present study results clearly demonstrate that ethanolic extract of *Valeriana wallichii* roots is in possession of good analgesic activity (particularly on late phase of pain) and supports the traditional uses of the plant.

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INTRODUCTION

In recent years plant derived drugs secured importance because of their unfeared efficacy as phyto-medicines. The active compounds present in these natural products serve as templates^[1]. The use of plants in herbal medicine is an age old practice and is still prevalent all over the world, while the dependence on plants as the source of medicine is still very common in developing countries, the traditional medicine plays a major role in health care delivery^[2]. The active compounds isolated from various parts of the plant such as leaves, fruits, stems, root, and seeds have been shown to possess excellent medicinal value. A wide range of plant varieties used in folklore medicine have been studied for treatment of various diseases, viz.. cancer, diabetes, arthritis, infectious disease, etc. However, still it remains as an area of interest for research for unveiling the medicinal value of certain plant species^[2].

Recently the use of natural products has enhanced enormously because of their reduced side effects when compared to synthetic drugs. People tend to rely on traditional and other forms of complementary and alternative medicine for chronic conditions which do not respond well to conventional or modern drug treatments. Among these are neurological disorders such as anxiety, pain and epilepsy. Analgesics play an essential role in relieving the unpleasant sensory experience with actual or potential tissue damage^[1]. Medicinal plants used for the therapy of analgesics in traditional medicine practice possess promising activities in animal models of anticonvulsant screening and these can be an invaluable source for search for

new analgesic compounds^[3]. In this aspects *Valeriana wallichii* is one such plant with powered medicinal properties.

Therefore the present study aims at establishing the potential of *Valeriana wallichii* as a potential analgesic agent.

MATERIALS & METHODS

Plant Material

The plant material used for this study consist of dried roots & rhizomes of *Valeriana wallichii* belonging to family Valerianaceae which were collected from Koraput, Odisha during the month of January. The plant was identified and authenticated by a scientist of M. S. Swaminathan Research Foundation, Jeypore, Koraput, Odisha by comparing with the voucher specimen present in the herbarium.

Valeriana wallichii roots were dried under shade separately powdered in mechanical grinder. The powdered plant materials were then passed through a sieve No. 22 and stored in an air tight container until the time of use.

About 500gms of coarse powder of roots of *Valeriana wallichii* was taken in the soxhlet apparatus and extracted with ethanol. The extraction was carried out for 24 to 48 hours and the temperature was maintained between 25-30°C. The extract was concentrated to dryness at 40°C under reduced pressure in a rotary vacuum evaporator.

Experimental Animals

Swiss albino mice (20-30g) of either sex were procured from Animal House of Jeypore College of Pharmacy. The animals were maintained in a well-ventilated room with 12:12 hour

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light/dark cycle in polypropylene cages. Standard pellet feed (Hindustan Lever Limited., Bangalore) and drinking water (ad libitum) was provided through out experimentation period. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments.

Drugs and Chemicals

Table No. 1 Chemicals used for the Experimental Procedure

Normal saline	Qualigens Fine Chem., Mumbai
Double Distilled water	S.D. Fine Chemicals Ltd., Mumbai
Sodium CMC	S.D. Fine Chemicals Ltd., Mumbai
Ethanol	Bengal Chemicals & Pharmaceuticals Ltd.
Formalin	Sigma Chemical Co., USA
Morphine	S.D. Fine Chemicals Ltd., Mumbai
Fentanyl	Sigma Chemical Co., USA

Acute Toxicity Study

The toxicity study was determined in mice by modified method of Lorke.^[4] Overnight fasted mice were randomly divided into groups of 4 mice per group and were administered orally with the extract in doses ranging from 500-3000 mg/kg. The results are tabulated in Table No. 2.

Evaluation of Analgesic activity

Method- 1 : Hot Plate method

Procedure: All male Swiss Albino mice weighing between (20-30 g) were selected for the study and the mice were randomly divided in to 4 groups. They are control, standard, test-1, test-2 and each group contains 6 mice. Group-I (Control group) mice were administered 0.2 ml of normal saline. Group-II (Standard group) mice were administered 25 mg/kg of Morphine in single oral dose. Group-III (Test-1 group) mice were administered 150mg/kg BW of ethanolic extract of *Valeriana wallichii* in single oral dose before the half an hour of the experiment. Group-IV (Test-2 group) mice were administered 300mg/kg BW of ethanolic extract of *Valeriana wallichii* in single oral dose before the half an hour of the experiment. The specificity and sensitivity of the test is increased by measuring the reaction time of the first evoked behaviour regardless of whether it is paw licking or jumping, or by lowering the temperature. The behaviour is relatively stereotyped in the mice but is more complex in rats, which sniffs, licks its forepaw, licks its hind paw, straightening up, stamps its feet, starts and stops washing himself, among other things. These behaviour is labelled as “chaotic defensive movements”.

Group I Animals received 0.2 ml of saline

Group II Animals received **Morphine** (25mg/kg)

Group III Animals received **EEVW** (150mg/kg/p.o)

Group IV Animals received **EEVW** (300mg/kg/p.o)

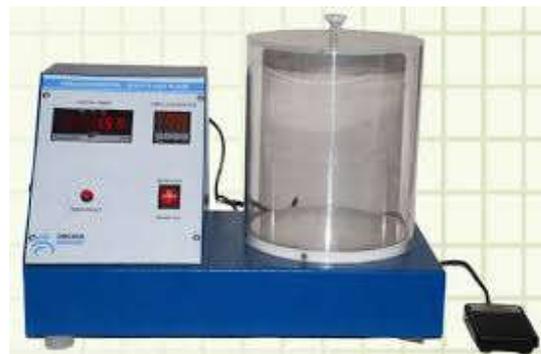


Fig 1 [Hot Plate method]

Method -2 Formalin Test

Procedure: All male Swiss Albino mice weighing between (20-30 g) were selected for the study and the mice were randomly divided in to 4 groups. They are control, standard, test-1 , test-2 and each group contains 6 mice. Group-I (Control group) mice were administered 0.2 ml of normal saline. Group-II (Standard group) mice were administered 25 mg/kg Fentanyl in single oral dose. Group-III (Test-1 group) mice were administered 150mg/kg BW of ethanolic extract of *Valeriana wallichii* in single oral dose before the half an hour of the experiment. Group-IV (Test-2 group) mice were administered 300mg/kg BW of ethanolic extract of *Valeriana wallichii* in single oral dose before the half an hour of the experiment. The test in rats has been proposed as a chronic pain model, which is sensitive to centrally active analgesic agents. Formalin is injected in to the front paw and reaction is recorded as excessive licking and biting of the paw. Analgesics response or protection is indicated, if both paws rest on the floor. The term formalin usually means a 37% solution of formaldehyde. 0.5 to 15 % solution of formalin, when injected in to the dorsal surface of the rat forepaw provokes a painful behaviour that can be assessed on a four-level scale related to posture: 0 denotes normal posture; 1 denotes the injected paw remains on the ground but not supporting the animal; 2 denotes the injected paw clearly raised and 3 denotes the injected paw being licked, nibbled or shaken.

Group I Animals received 0.2 ml of saline

Group II Animals received **Fentanyl** (25mg/kg)

Group III Animals received **EEVW** (150mg/kg/p.o)

Group IV Animals received **EEVW**, (300mg/kg/p.o)

Statistical analysis

The data were expressed as mean \pm standard error of mean (S.E.M). The Significance of differences among the group was

assessed using one way analysis of variance (ANOVA). The test followed by Dunnet's T-test, p value < 0.01 were considered as significant.

RESULTS

Acute toxicity study

There was no mortality amongst the graded dose groups of mice up to a dose of 3000 mg/kg for duration of 72 h. The animals were observed for further 14 days period for all toxicity signs. There was no considerable change in body weight before and after treatment and no sign of toxicity were observed.

Table No. 2

Group	Dose (mg/kg)	Dead Total
I	500	0
II	1000	0
III	2000	0
IV	3000	0

Method -1

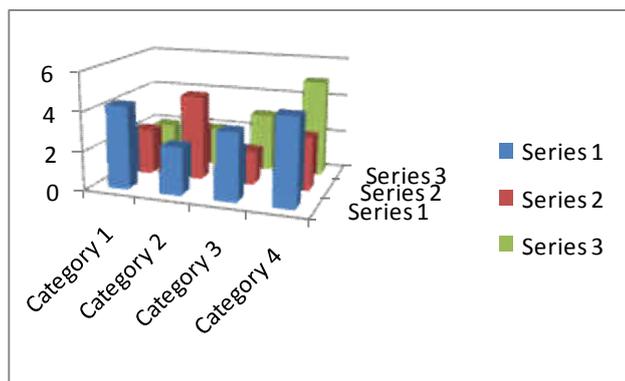
In this model the reaction time in EEVW treated group increased significantly (p<0.01) in comparison to the control group. The maximum effect was observed at the highest dose viz. 300 mg/kg at 60 min which showed a reaction time of 16.5 sec, where as the standard drug ibuprofen (10mg/kg) showed a reaction time of 17.2 sec. The extract also showed dose and time dependent activity table.

Table No. 3 Effect of EEVW in Hot plate Method

Group s	Drugs	Dose mg/kg	Reaction time in second			
			0min	30min	60min	90min
1	Control	-	3.89±0.15	4.10±0.07	4.60±0.17	4.30±0.25
2	Ibuprofen	10	12.6±0.42	14.6±0.81	17.2±0.64	14.2±0.47
3	EEVW	150	8.80±0.12	11.7±0.65	13.7±0.55	14.0±0.47
4	EEVW	300	10.6±0.52	13.6±0.67	16.5±0.33*	13.5±0.38*

Values are expressed in terms of mean ± SEM, n=6 in each group, *p<0.01 statistically highly significant as compared with control group.

EEVW = Ethanolic Extract of *Valeriana Wallichii*



Method -2

Administration of EEVW at 150 and 300 mg/kg caused reduction in duration of paw licking (43.8 and 35.11) sec, as compared to the control group (57.2 Sec). High doses of EEVW at 300 mg/kg showed complete abolishment of the early phase indicated by absence of paw licking after the

formalin injection. However the standard drug ibuprofen (10mg/kg) exhibited a reduction of paw licking time of 25.4 sec only in the early phase.

In later phase the administration of EEVW decreased the duration of paw licking dose dependently. On the other hand, ibuprofen (10mg/kg) exhibited a reduction of paw licking time of 33.84 sec in the later phase.

Table No. 4 Effect of EEVW in Formalin Test

Groups	Drugs	Dose mg/kg	Early Phase	Later Phase
1	Control	----	57.2±1.45	135.20±1.10
2	Ibuprofen	10	25.4±1.45	41.85±0.34
3	EEVW	150	39.00±0.53	61.31±1.11
4	EEVW	300	0	49.84±0.45*

Values are expressed in terms of mean + SEM, n=6 in each group, *p<0.01 statistically highly significant as compared with control group.
EEVW = Ethanolic Extract of *Valeriana Wallichii*

DISCUSSION

The word pain is applied to a wide variety of subjective phenomenon ranging from the perception of an experimental noxious stimulant to the most severe and excruciating pain in humans suffering from cancer, trigeminal neuralgia etc.

Animal models have been used extensively in basic pain research based on the premise that animal models can serve as surrogate assay that can reliably predict the potency and efficacy of the pharmacological action of, and, in some cases the molecular response to agents that work in human pain state. But in contrast to the polymorphic nature of pain in humans pain in animals can be estimated only in examining their reactions to various chemicals, thermal, and mechanical stimuli, with the latency or nature of response altered in the pain state.

Most commonly used methods for evaluation of analgesic drugs are hot plate method and tail flick method. However Formalin test also been widely used because it accesses the response of the animal to moderate, continuous pain.

Although different pain models based on use of nociceptive stimuli (electrical, thermal, mechanical, or chemical) have been used, none is ideal.

CONCLUSION

The present study on pharmacological evaluation and analgesic study of dried roots and rhizomes of *Valeriana Wallichii* will provide useful information for its identification and pharmacological property. The ethanolic extract of *Valeriana Wallichii* roots and rhizomes parts with 300mg/kg dose prove to have an analgesic activity particularly on late / second phase of pain.

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