

**ANTIOXIDANT AND ANALGESIC ACTIVITIES OF
VALERIAN OFFICINALIS LINN. ROOT EXTRACT**

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Summary

Methanolic root extract of *Valerian officinalis* was screened for analgesic effect using hot plate and tail immersion at various doses (100, 200 and 500 mg/kg). The results were also compared with standard drug diclofenac sodium. It showed significant activity at 500 mg/kg. The extract was also screened to evaluate its reducing power and free radical scavenging effect on DPPH free radical, superoxide radical and nitric oxide production at different concentrations (100–500 µg/ml). All these antioxidant activities were concentration dependent which were compared with standard antioxidants such as BHA and ascorbic acid. The highest antioxidant activity of *V. officinalis* root extract was observed at a concentration of 500 µg/ml.

Keywords: *Valerian officinalis*, Tail immersion, Hot plate, Antioxidant, Reducing power, Nitric oxide

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Introduction

Pain is a sensorial modality and primarily protective in nature, but often causes discomfort. It is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause (1). Cyclooxygenase products are well known to play an important role in pain, and it has been reported that extracellular calcium is involved in the formation of prostaglandins (2). Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. Moreover, a number of pathologies are known to be ultimately associated with the imbalance of pro and antioxidant factors in living systems. Exogenous antioxidant compounds may therefore exert beneficial actions upon systems which have been deprived from sufficient amounts of endogenous antioxidants as in some cardiovascular diseases, tumors, inflammation, ulcer and aging (3).

Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, and also by acting as oxygen scavengers (4). Currently available synthetic antioxidants have been suspected to cause negative effects. Therefore, these are substituted with naturally occurring antioxidants. Plant products including phenolics, flavonoids, tannins, proanthocyanidins, are reported to be radical scavengers and inhibitors of lipid peroxidation (5). Currently, the possible toxicity of synthetic antioxidants has been criticized. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity (6, 7). Thus interest in natural antioxidant, especially of plant origin, has greatly increased in recent years (8).

Valerian officinalis is a perennial herb belonging to family Valerianaceae. Traditionally it is used in hysteria, antispasmodic, hypotensive and neurosis. It also reduces irritation and pain. *Valerian* root extracts have been used for centuries in popular medicine for the treatment of anxiety, epilepsy, and sleep disorders (9, 10). It contains alkaloids actinidine, valerine, chatinin and naphtyridyl methyl ktone, terpenes, tannins, gums and resins.(9, 11) Considering its traditional uses and presence of phenolic compounds in the plant, the present study has been designed to examine the analgesic and antioxidant activity of methanolic root extract.

Materials and Methods

Chemicals

Chemicals used in this study were 1,1-diphenyl-2-picrylhydrazyl (DPPH) obtained from Sigma-Aldrich, India, nicotinamide adenine dinucleotide hydrogen (NADH) and sulfanilamide obtained from Himedia, Laboratories Pvt. Ltd., India, potassium ferricyanide and sodium nitroprusside obtained from Qualigens Fine Chemicals, Glaxo Smithkline Pharmaceutical Ltd., India, naphthylethylenediamine dihydrochloride, N-1-naphthylethylenediamine dihydrochloride, sodium nitrite, trichloroacetic acid, butylated hydroxy anisole (BHA), ascorbic acid, nitro blue tetrazolium, phenazine methosulfate, ferrous ammonium sulfate and dimethylsulfoxide (DMSO) are obtained from sd Fine Chemicals Ltd, India.. All reagents used in the study were of analytical grade.

Plant material

Valerian officinalis (VO) plant material was purchased from yucca Enterpizes, Mubmai, India. and was identified by Dr. B.D. Vashishta, Department of Botany, Kurukshetra University, Kurukshetra, India. A voucher specimen of the plant is preserved in the herbarium of the Faculty of Pharmaceutical Sciences, Kurukshetra University (No. IPS/KUK/V-1/2009).

Extraction

The roots of *V. officinalis* were cleaned and dried in the shade, then powdered to 40 mesh and stored in an airtight container at 25°C. Dried roots were coarsely powdered and 500 g of this powdered material was soaked in 1 L methanol for 48 h. All the extracts were dried below 45 °C in rotary evaporator and stored in airtight containers in refrigerator below 10°C (yield - 3.6% w/w).

Analgesic Screening

Tail immersion test

The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice (12). The test animals were treated with VO root extract at 250 mg/kg and 500mg/kg and control group was treated with solvent.

1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 s was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined before and 0, 30, 60 and 90 min after the administration of drugs.

Hot Plate

The analgesic activity was determined using the hot-plate test in mice according to the method described by Eddy and Leimbach, with minor modifications (13). The paws of mice are very sensitive to heat at temperature, which are not damaging the skin. The response is in the form of jumping, withdrawal of the paws or the licking of the paws. The animals were placed on Eddy's hot plate kept at a temperature of 55±0.5⁰ C. A cut off period of 15 sec, was observed to avoid damage of the paw. Reaction time and the type of response were noted using a stopwatch. Control mice were treated with vehicle (2% Tween 80, 1 ml/kg). Diclofenac sodium was used as positive control (50 mg/kg) and extract of VO (250 and 500 mg/kg, orally) were administered. The latency was recorded before and after 15, 30, 60 and 120 min. Average reaction times were then calculated and the percentage variation calculated using following relation.

$$\% \text{ inhibition} = \left[\frac{\text{After treatment}}{\text{Before treatment}} - 1 \right] \times 100$$

Acetic acid-induced abdominal writhing test

The test was performed as described by Collier et al. (14). Nociception was induced by an intraperitoneal (i.p.) injection of acetic acid 1.0%, 0.1 ml/10g body weight. Mice were treated with the extracts of VO (100,250 and 500 mg/kg, orally) 30 min before acetic acid injection. A group of mice were treated with diclofenac sodium (50 mg/kg i.p.).

Antioxidant Screening

DPPH free radical scavenging activity

The free-radical scavenging activity of VO root extract was measured by decrease in the absorbance of methanol solution of DPPH (15). A stock solution of DPPH (33 mg in 1 L) was prepared in methanol, which gave initial absorbance of 0.493, and 5 ml of this stock solution was added to 1 ml of VO root extract solution at different concentrations (100-500µg/ml). After 30 min, absorbance was measured at 517 nm and compared with standards (10-50 µg/ml). Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\% \text{ Anti-radical activity} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}}$$

Reducing power assay

The reducing power of VO root extract was determined as per the reported method (16). Different concentrations of root extract (100-500µg/ml) in 1 ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm and compared with standards. Increased absorbance of the reaction mixture indicated increased reducing power.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically (17). Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with different concentrations of the extract (100-500 µg/ml) dissolved in methanol and incubated at 25°C for 30 min. A control without the test compound but with an equivalent amount of methanol was taken. After 30 min, 1.5 ml of the incubated solution was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% N-1-naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamine dihydrochloride was measured at 546 nm. The percentage scavenging activity was measured with reference to standard.

Superoxide radical scavenging assay

The reaction mixture consisting of 1 ml of nitro blue tetrazolium (NBT) solution (156 mM NBT in phosphate buffer, pH 7.4), 1 ml NADH solution (468 mM NADH in phosphate buffer, pH 7.4), and 1 ml of sample solution of VO root extract was mixed. The reaction was started by adding 100 μ l of phenazine methosulfate (PMS) solution (60 mM PMS in phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min and the absorbance was measured at 560 nm against blank sample and compared with standards. Decreased absorbance of reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula (18, 19).

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance of *V. officinalis* or standard compounds.

Statistical Analysis

The measurements were done in triplicate and data was expressed as mean \pm standard deviation (SD). The Student's t-test was used to test the significance of differences between results obtained for different samples and between results for samples and controls. A probability value of less than 0.05 was considered as significant.

Results

Analgesic Activity

The methanolic root extract was also screened for analgesic effect by tail immersion, writhing syndrome and hot plate at various doses (100, 250 and 500 mg/kg). The results were also compared with standard drug diclofenac sodium. The extract showed significant activity at 500 mg/kg. The tail withdrawal reflex time following administration of the extract was found to increase with increasing dose of the sample. The result was statistically significant ($p < 0.01$) and was comparable to the reference drug diclofenac sodium (Table 1). Results of hot plate test are shown in Table 2. The extract produced a dose dependent increase in latency time when compared with the vehicle. The result was found to be statistically significant ($p < 0.01$).

Table 1: Analgesic effects of VO root extract using tail immersion method

S.No.	Drug treatment	Dose /kg	No. of animals	Mean \pm SEM	
				Reduced analgesia (min)	Percentage inhibition
1	Control	Saline	6	3.5 \pm 0.47	-
2.	Standard	Diclofenac	6	13 \pm 1.12*	80 \pm 0.5
3.	VO	100 mg/kg	6	3.25 \pm 0.32	32 \pm 1.6
4.	VO	250 mg/kg	6	4.32 \pm 0.61	45 \pm 0.5
5.	VO	500 mg/kg	6	4.67 \pm 0.34*	51 \pm 1.9

Values are mean \pm SEM. The data were analyzed by one way ANOVA $p < 0.01$ compared to control group.

Table 2: Effect of methanolic extract of fruits of VO in the hot plate test

S.N.	Drug treatment	Dose /kg	No. of animals	Mean \pm SEM	
				Reduced analgesia (min)	Percentage inhibition
1	Control	Saline	6	2.5 \pm 0.28	-
2.	Standard	Diclofenac	6	13 \pm 1.12*	80 \pm 1.1
3.	VO	100 mg/kg	6	3.41 \pm 0.58	42 \pm 0.7
4.	VO	250 mg/kg	6	4.15 \pm 0.42*	51 \pm 1.2
5.	VO	500 mg/kg	6	3.61 \pm 0.36	38 \pm 0.8

Values are mean \pm SEM. The data were analyzed by one way ANOVA. $p < 0.01$ compared to control group.

The effects of the extract of on acetic acid-induced writhing in mice are shown in Table 3. The oral administration of extract significantly ($p < 0.01$) inhibited writhing response induced by acetic acid in a dose dependent manner.

Table 3: Effect of methanolic extract of fruits of VO in the writhing test

S.N.	Drug treatment	Dose /kg	No. of animals	Mean \pm SEM Change in No. of wriths
1	Control	1.0 % acetic acid	6	16 \pm 2.2
2.	Standard	10 mg/kg	6	6 \pm 0.45*
3.	VO	100 mg/kg	6	4 \pm 0.34
4.	VO	250 mg/kg	6	7 \pm 0.67*
5.	VO	500 mg/kg	6	5 \pm 0.54*

Values are mean \pm SEM. The data were analyzed by one way ANOVA. $p < 0.01$ compared to control group.

Antioxidant Effect

Antioxidant activity of *Valerian officinalis* (VO) was evaluated by various in vitro assays. DPPH radical was used as a substrate to evaluate free radical scavenging activities of root extract. It involves reaction of specific antioxidant with a stable free radical 2, 2-diphenyl-1-picryl-hydrazyl DPPH. As a result, there is reduction of DPPH concentration by antioxidant, which decreases the optical absorbance of DPPH; this is detected by spectrophotometer at 517 nm. BHA and ascorbic acid were used as standards. The scavenging effect of root extract of VO on the DPPH radical was 86.23%, at a concentration of 500 μ g/ml. These results indicated that extract has a noticeable effect on scavenging the free radicals. The reductive capabilities of extract were compared with ascorbic acid and BHA. The root extract showed dose dependent reducing power.

In the Nitric oxide scavenging study, crude extract of the root was checked for its inhibitory effect on nitric oxide production. Ascorbic acid was used as a reference compound. It also showed scavenging of superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The percentage inhibition of superoxide generation by 500 μ g/ml concentration of root extract was measured as 56.93%. The antioxidant activity of VO root extract and standard compounds were compared by using specific in vitro methods (Table 4).

Table 4: Antioxidant profile of *Valerian officinalis* root extract

S. No.	Sample	Sample conc. ($\mu\text{g/ml}$)	DPPH radical scavenging activity (%inhibition)	Superoxide anion scavenging activity (%inhibition)	Percentage scavenging of nitric oxide	Reducing power activity ^a (absorbance)
1	VO	500	86.23 \pm 0.9*	56.93 \pm 2.7*	48.5 \pm 3.7*	0.237
2	Ascorbic acid	500	90.43 \pm 1.1*	85.57 \pm 2.5	53.2 \pm 3.2*	0.248
3	BHA	500	88.05 \pm 1.3*	68.08 \pm 4.6*	52.05 \pm 2.8	0.165

^aIncreased absorbance indicates increased reducing power All values represent Mean \pm SEM * $p < 0.01$

The exact constituents of VO which show free radical scavenging action are unclear. However, the phytoconstituents like polyphenol, flavonoids and triterpenoids present in the plant extract may be responsible for antioxidant and free radical scavenging activities. The antioxidant activity of fruit may be attributed to its phenolic and flavonoids content.

Discussion

The hotplate method and tail immersion test are two selective methods used to examine the analgesic effect of compounds; the extract increased mean basal latency which indicates that it may act via centrally mediated analgesic mechanism. In acetic acid-induced writhing model, the pain sensation is triggered by localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipid (20). It is a sensitive procedure to evaluate peripherally acting analgesics. Moreover, recent studies suggest that the inflammatory tissue damage is due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites (21). Plants containing polyphenolic compounds, like flavonoids, tannins and phenolic acids, have been reported to have multiple biological effects, including antioxidant activity (22-25).

Cyclooxygenase (COX) inhibitory activity together with antioxidant activity may reduce the production of free arachidonic acid from phospholipid or these may inhibit the enzyme system responsible for the synthesis of prostaglandins and ultimately relieve pain-sensation (26). Phenolic compounds present in the plant extract, as evident from phytochemical screening, may be responsible for the antioxidant action. Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals.

Conclusion

The methanolic root extract possesses good antioxidant and analgesic potential. However, further studies are needed to isolate active constituent (s) responsible for such activity.

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