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## Comparative Anxiolytic Activity of Petroleum Extract of *Valeriana jatamansi* from Different Accessions in Mice.

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### Research Article

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#### ABSTRACT

*Valeriana jatamansi* Jones., (Family- valerianaceae) is a perennial herb or under shrub with a short, often strong smelling root stock. It is widely distributed in the temperate and cold regions of North West Himalaya to Bhutan at altitude of 1200-3000m. The objective of the study was to test the anxiolytic effect of *Valeriana jatamansi* Jones., grown in India and Pakistan. Petroleum ether extract of both accessions were evaluated using ambulatory activity, elevated plus maze, spontaneous locomotor activity, motor coordination activity and sodium thiopental induced sleep in mice at dose of 75, 150 and 300 mg/ kg. Both the extract produced significant and dose dependent activity in the ambulatory and elevated plus maze test. The spontaneous locomotor activity count, measured using an actophotometer, was significantly decreased in animals at 120 min after administration of extract in both accessions. None of the extracts produced skeletal muscle relaxant effect assessed by rotarod test. Both extract produced a significant and dose dependent lengthening in the time of sodium thiopental sleep with the Pakistan valerian extract being more potent than Indian valerian. Thus we may conclude that, Pakistan valerian possess high anxiolytic potentials than Indian valerian.

#### INTRODUCTION

Anxiety affects one tenth of the total population worldwide [1] which is a disorder that has a significant negative impact on one's quality of life. Anxiety is most commonly known to be treated with the Benzodiazepine (BDZ) group of compounds but unfortunately this class of compounds has a very narrow safety margin which has incited many a studies [2]. We have been blessed with a very rich wealth of flora and fauna by nature worldwide and this has regenerated interest and demand of traditional medicines [3].

Valerian genus belongs to Valerianaceae family which consists of more than 250 species, native to Europe and Asia. Around the world, valerian species have been used for their sedative properties in traditional medicine [4]. These species have found to be useful to treat hypertension, convulsions, irritable bowel syndrome and anxiety [5].

*Valeriana jatamansi* Jones., is a perennial herb distributed in Himalayas from Pakistan to Bhutan at an altitude between 1800 to 3000 m. Roots and rhizomes of this plant are rich in aromatic volatile oils and valepotriates. The roots also contain alkaloids viz: chitanine and valeranine [6]. There is substantial variation in phytoconstituents in plant from different sources and growing condition, collection and storage. Therefore we undertook the study to evaluate anti-anxiety potential of two accessions of valerian grown in India and Pakistan.

## MATERIALS AND METHODS

### Plant material

Dried roots and rhizome of both the India and Pakistan variety of *Valeriana jatamansi* Jones were procured from Natural Remedies Pvt. Ltd. Bangalore. Both the samples were authenticated by Dr. H.B Singh, Head Department of Herbarium and Raw materials, National Institute of Science Communication And Information Resources (NISCAIR), New Delhi and voucher specimens (NRVA-01/08-09) and (NRVP-01/08-09) were deposited in Department of Pharmacognosy and Phytochemistry, Karnataka Ligayat Education Society's College of Pharmacy Hubli for future reference.

### Processing of plant material

The roots and rhizome of both Indian and Pakistan accessions of *Valeriana jatamansi* were extracted with petroleum ether (40-60) below 40°C by refluxation. The extracts were filtered and concentrated under reduced pressure in rotary flash evaporator. The percentage yield was 0.35 & 1.1 % for Indian and Pakistan variety respectively.

### Animals

Albino mice 20-25 gm were purchased from CPCSEA approved registered breeder, were used for acute toxicity studies and sedative activity. Animals were kept at room temperature (27 ± 2°C) for one week to acclimatize to laboratory condition before starting the experiment; they were given free access to water and standard rat feed *ad libitum*. The experimental protocol was approved by the institutional animal ethical committee (KLESCOPH / IAEC. Clear / 2008-09 / Ph.D Dated 28/01/2009).

### Acute toxicity studies

Acute toxicity studies were carried out as per OECD guidelines by employing the up and down method prior to evaluating each extract for sedative activity.

### Pharmacological evaluations

#### Treatment schedule

The anxiolytic activity was examined by using behavioral study, ambulatory activity, elevated plus-maze, spontaneous locomotor activity, motor coordination activity and sodium thiopental induced sleep test. Animals were divided in to eight groups each group consisting of six albino mice. Group-1 received vehicle (Tween-80); group 2 received standard drug, group 3-5 received petroleum ether extract of Indian valerian at 75, 150 and 300 mg/kg BW; group 6-8 received petroleum ether extract of Pakistan valerian at 75, 150 and 300 mg/kg BW.

#### Behavioral effect

Behavioral effect was assessed by the method described by Achliya, et al 2005. The animals were observed after 30 min of administration of extract, upto 2 h for behavioral changes. The observation parameters consisted of body position, locomotion, rearing, respiration, righting reflex and lacrimation [7].

#### Ambulatory activity

The ambulatory activity was studied according to the method published by Ganzalez-trujano M.E et al 2007. Sixty minutes after the administration of valerian extracts, each mouse was placed into a cage divided in 12 squares (4 × 4 cm). The number of squares explored by each mouse in a 2 min interval was registered as ambulatory activity [8].

#### Elevated plus-maze activity

The elevated plus-maze apparatus used in this study consisted of four arms elevated 40 cm above the floor. Two open arms (25 X 5 cm), facing each other and two closed arms (25 X 5 X 15 cm) with open roof and walls. Each mouse was individually placed on central platform facing towards open arm. The number of entries in to any of the arms and total time spent in each of the two arms types was observed for 5 min. The experiment was performed between 0900 and 1600 hours [9].

### Spontaneous locomotor activity

In spontaneous locomotor activity, thirty min after drug administration the spontaneous locomotor activity was recorded using an activity cage (INCO, Medcraft Mumbai) with automatic counting of animal movements on the cage floor. The locomotor count for each animal was recorded for 5 minutes at 30 minute intervals for 2 h. The results were compared with the Standard Diazepam (5 mg/kg p.o) [10].

### Motor coordination activity

Rota rod apparatus consisted of a base platform and an iron rod of 3 cm diameter and 30 cm length. This rod was divided into three equal parts by two disks. This facilitates three mice to walk on the rod same time. The animals were trained to remain for 5 min on the rod rotating at a speed of 30 rpm. On the next day either vehicle, standard or valerian extracts were administered orally and their ability to remain on the rotating rod was assessed before and 30 min after the treatment. The fall-off time from the rod was noted for each animal [11].

### Effect on the sodium thiopental-induced sleep

The method described by Rakotonirina et al 2001 was used. Sleep inducing or potentiating effects of the valerian extracts have been studied in mice treated by 100 mg/kg of sodium thiopental. The different extracts of the test groups and the Twen-80 (5% w/v) of the control group were administered 30 min before the injection of the sodium thiopental. The number of mice that slept with the dose of sodium thiopental was counted. The sleeping time was measured by observing the recovery of the straightening reflex [12].

### Statistical analysis

All values are expressed as mean  $\pm$  SEM. The values attained for the above parameters in case of the extracts were compared with standard drug and control group by using One-Way analysis of variance (ANOVA) followed by Dunnett's test. The values of  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$  were considered to indicate a significant difference between groups.

## RESULTS

### Acute toxicity studies

The valerian Petroleum ether extract prepared from both Indian and Pakistan accessions were found to be safe after oral administration up to the dose of 2000 mg/kg body weight. No mortality was observed at this dose up to 24 h.

### Behavioral assessment

The animals were observed for 2 h after oral administration of valerian extracts. Both the extracts have shown reduced alertness, locomotion and reactivity to touch and stimuli. Normal respiration was reported in the treated group. The animals treated with vehicle showed normal body position, locomotion, respiration and righting reflex.

### Ambulatory and elevated plus-maze activity

A dose dependent decreased ambulatory activity was observed in the group treated with extracts. A significant decrease ( $P > 0.001$ ) in the number of squares travelled by the animals was recorded in both Indian and Pakistan valerian extracts at 300 mg/kg. Reduction in the ambulatory activity was pronounced as that produced by the standard dug diazepam (1 mg/kg).

In the elevated plus maze test, a significant increase in time spent ( $201.19 \pm 11.58$  sec) in the open arms by the petroleum ether extract of Pakistan valerian at 300 mg/kg was observed. The animal treated with same dose by Indian variety has spent  $181.37 \pm 11.9$  sec in open sided arm. The group treated with standard diazepam at 1 mg/kg has shown significant increased time spent in the open arm as expected. The results were depicted in table 1.

### Spontaneous locomotor activity

Petroleum ether extract of both Indian and Pakistan variety of valerian at 300 mg/kg exhibited significant decrease in Spontaneous locomotor activity ( $P > 0.001$ ) as compared to the control. The diazepam treated animals also shown significant decrease in locomotion ( $P > 0.001$ ). The observations are given in table 2.

**Table 1: Effect of petroleum extract of different accessions of Valerian on the ambulatory activity and anti-anxiety response in the plus maze test in mice.**

Group	Ambulatory activity (Counts/ 2 min)	Time spent in open sided arm (sec)
Vehicle	68.35 ± 3.12	45.6 ± 4.36
Diazepam (1 mg/kg)	46.84 ± 2.81***	248.42 ± 1.43***
Indian valerian (75 mg/kg)	58.59 ± 6.33*	78.54 ± 4.3*
Indian valerian (150 mg/kg)	47.3 ± 5.17**	102.1 ± 9.89**
Indian valerian (300 mg/kg)	37.46 ± 4.49***	181.37 ± 11.9***
Pakistan valerian (75 mg/kg)	55.61 ± 8.44*	89.54 ± 5.4*
Pakistan valerian (150 mg/kg)	43.18 ± 7.2**	132.6 ± 8.72**
Pakistan valerian (300 mg/kg)	30.11 ± 6.7***	201.19 ± 11.58***

(Data represent in Mean ± SEM; n=6; \*P< 0.05 ; \*\*P< 0.01 ; \*\*\*P< 0.001 compared with control group (Student 't' test)

**Table 2: Spontaneous locomotor activity of petroleum extract of different accessions of Valerian.**

Group	Number of movements			
	30 min	60 min	90 min	120 min
Control	236.3 ± 1.72	248.0 ± 1.88	252.2 ± 1.19	253.0 ± 1.93
Diazepam	103 ± 2.3***	46 ± 1.9 ***	30 ± 2.8 ***	12 ± 1.1 ***
Indian valerian (75 mg/kg)	218 ± 2.9	207 ± 1.65	184 ± 1.59	176 ± 3.1***
Indian valerian (150 mg/kg)	187 ± 1.35	159 ± 2.23	106 ± 2.47	83 ± 1.9***
Indian valerian (300 mg/kg)	134 ± 1.98 ***	91.0 ± 1.4 ***	68.33 ± 1.80 ***	40.33 ± 1.75 ***
Pakistan valerian (75 mg/kg)	217 ± 3.18	197 ± 2.74	145 ± 2.2	120 ± 1.3 ***
Pakistan valerian (150 mg/kg)	153 ± 2.12	112 ± 1.66	73 ± 2.41 ***	55 ± 2.69 ***
Pakistan valerian (300 mg/kg)	118.7 ± 1.5 ***	72.0 ± 1.3 ***	45.3 ± 1.53 ***	28.17 ± 1.13 ***

(Data represent in Mean ± SEM; n= 6; a = P< 0.001, compared with control; statistics ANOVA one-way followed by Dunnet 't' test)

### Motor co-ordination

Extracts of the both accessions of *Valeriana jatamansi* at the dose level of 300 mg/kg does not induce any motor incoordination in the animals. The standard Diazepam (5mg/kg p.o) exhibited significant reduction in motor coordination compared to control animals. (Data not shown).

### Effect on the sodium thiopental-induced sleep

As shown in the table 3, the pretreatment with extract of Pakistan variety (300 mg/ kg) lengthened the duration of the sodium thiopental induced sleep in mice. Indian valerian extract at 300 mg/ kg and Pakistan variety at 150 mg/ kg has shown moderate activity while other groups have shown less activity.

**Table 3: Effect of petroleum extract of different accessions of Valerian on Sodium thiopental –induced sleeping time in mice.**

Group	Mean sleeping time± S.E.M (min)
Control (sodium thiopental 50 mg/kg i.p)	110 ± 3.92
Indian valerian (75 mg/kg)	119 ± 4.65
Indian valerian (150 mg/kg)	142 ± 3.98
Indian valerian (300 mg/kg)	185 s± 3.69*
Pakistan valerian (75 mg/kg)	132 ± 2.89
Pakistan valerian (150 mg/kg)	167 ± 3.1*
Pakistan valerian (300 mg/kg)	202 ± 3.21**

(Data represent in Mean ± SEM ; n=6; \*P< 0.05,\*\*P< 0.01 compared with control group (Student 't' test)

## DISCUSSION

The cause of the most of the anxiety disorder is yet not understood. The picture has become a little clear in recent years. Amongst the drugs used to treat psychosis the Benzodiazepine classes of compounds are most common in spite of their undesired adverse effects. The role of gamma amino butyric acid (GABA) in anxiety is well established [13].

GABAergic effects of herbal anxiolytics have been reported in the literature [14]. Monoterpenes present in volatile oil have shown activity with several subtypes of GABA<sub>A</sub> receptor [15]. (+) Borneol, a bicyclic monoterpene has been reported to have positive modulation against GABA receptor [16].

In our study, petroleum ether extract of both valerian accessions exhibit low toxicity which is reflected in high LD<sub>50</sub> values. A significant CNS dependent action such as reduced alertness, locomotion and diminished response to external stimuli was observed in mice treated with petroleum ether extract of both accessions as soon as 30 min after administration.

The reduction in ambulatory activity observed in mice for both accessions of valerian which supports the presence of sedative components in both the extracts. Petroleum ether extract of Pakistan valerian at 150 mg/ kg has shown better ambulatory activity than standard diazepam (1 mg/ kg).

The time spent in open arm of the plus maze is representative indices of anxiolytic activity. The results suggest both the extract have shown significant and dose dependent activity in elevated plus maze test. Extract of Pakistan valerian has shown better activity than Indian valerian in all tested doses.

The extracts of both the accessions were able to induce a motor depressant effect which was accessed through spontaneous locomotor activity. Pakistan valerian at 150 mg/ kg has shown significant reduction in locomotor at 90 min after administration. A dose dependent locomotor activity was recorded in both the accessions at 120 min after administration.

The impaired motor functions produced by drugs acting on the CNS are predicted using the Rota rod test. Both extracts fails to produce skeletal muscle relaxation in animals.

Gas chromatography mass spectroscopy (GCMS) was carried out for the volatile oil of Indian and Pakistan valerian. Higher content of borneol was observed in the volatile oil of Pakistan valerian than Indian variety. (Data not shown).

Ecological, environmental and genetic factors are seen to play a major role in determining the essence of phytoconstituents present in the plant. A comparison between the two accessions shows that, petroleum ether extract of Pakistan valerian induces a more potent anxiolytic response than the Indian valerian in the tested models. The results indicate that the anxiolytic effect produced by valerian extracts may be attributed due to presence of (+) borneol. Further investigation of the exact mechanism of action of the valerian extract as well as active constituent(s) responsible for anxiolytic activity is needed.

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