

Screening of Antianxiety Activity of *Habenaria Intermedia* D. Don Fruits

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Abstract *Habenaria intermedia* D. Don (Vrididhi; Orchidaceae) has been traditionally used in the treatment of nervous disorders, skin disorders and asthma. The available pharmacological reports on *H. intermedia* reveal that the plant has not been screened for antianxiety activity. Thus, it was envisaged to subject *H. intermedia* for screening of antianxiety activity using elevated plus maze model. The crude extracts (*n*-hexane, chloroform, methanol and water extracts) of plant material were prepared successively in increasing order of polarity. The anxiolytic activity was assessed by comparing number of entries and average time spent by mice treated with test extracts (200 or 400 mg/kg, *p.o.*) in open arms of EPM with respect to control and standard drug, diazepam (2 mg/kg, *p.o.*). Significant antianxiety activity was observed in methanol extract with respect to control, whereas *n*-hexane, chloroform and water extracts did not exhibit antianxiety activity. It is further observed that antianxiety activity exhibited by the methanol extract was statistically not equivalent to the standard drug. Based on these observations, it is concluded that the methanol extract of *H. intermedia* exhibits mild anxiolytic activity.

Keywords: Antianxiety activity, *Habenaria intermedia*, Orchidaceae, Vrididhi.

1. INTRODUCTION

The demand for anxiolytic agents has been enormously increased to deal with stressful situations in life and modernization borne disorders. Antianxiety medications help in reducing the symptoms of anxiety, panic attacks and extreme fear. For treatment of generalized anxiety disorders benzodiazepines are first choice. Antidepressants are preferred in case of panic disorder or

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social anxiety disorder (NIMH, 2016). Long term use of benzodiazepines causes depression, addiction and physical dependence (Baldessarini, 2001). Search for effective solutions is needed to treat anxiety disorders. Exploration of plant drugs, based on their traditional uses, seems to be a viable approach.

Habenaria intermedia D. Don (Vriddhi; Orchidaceae) traditionally, used in the treatment of nervous disorders, skin disorders, asthma, cold and fever (Khare, 2007). The plant is mainly distributed in India, Pakistan, Nepal and Bhutan. In India, it is found in Himachal Pradesh, Kashmir, Uttarakhand and Sikkim (Balkrishna *et al.*, 2012). *H. intermedia* has been reported to contain coumarin – scopoletin and phenol – gallic acid. The plant has been reported to possess hepatoprotective (Goudar *et al.*, 2015), immunomodulatory (Habbu *et al.*, 2012) and antistress activities (Sahu *et al.*, 2013). *H. intermedia* has not been scientifically validated for its traditional claims of anxiolytic potential. Thus, the present investigations were undertaken with a view to screen antianxiety activity of various extracts of *H. intermedia*.

2. MATERIALS AND METHODS

2.1 Plant material

The procurement of *Habenaria intermedia* fruits was made from Himalaya Herbs Store, Madhav Nagar, Saharanpur, UP in September, 2013. Identification of the plant was confirmed by Dr. Avneet Pal Singh, Assistant Professor, Department of Botany, Punjabi University, Patiala, India (Reference No. SPL-101/Bot, dated 15-10-2013).

2.2 Preparation of various extracts

Two kg coarsely powdered plant material was taken in thimble and placed in a Soxhlet apparatus. It was then extracted exhaustively using *n*-hexane (10 L) on a water bath maintained at a temperature of 100°C. The marc was dried, again packed in Soxhlet and extracted exhaustively with chloroform (10 L) and then with methanol (10 L) (E Merck, New Delhi, India). The marc of plant was dried and again extracted with distilled water (10 L) using decoction process for 2 h on a hot plate maintained at 100°C. The rotary vacuum evaporator (BUCHI, Switzerland) was used to concentrate crude extracts. All extracts *viz.*, *n*-hexane extract (HE), chloroform extract (CE), methanol extract (ME) and water extract (AQE) were tested to detect various group of phytochemicals by applying general chemical tests (Farnsworth, 1966).

2.3 Animals

Albino mice (Swiss strain; either sex; body weight 20-25 g) were procured from the Panacea Biotech Ltd., Ambala-Chandigarh highway, Lalru, Punjab, India. The animals studies were approved from Institutional Animal Ethics Committee of CT Institute of Pharmacy, Jalandhar (IAEC-CTIPS/2014/V/0029 (PCT - D), dated 15/12/2014). The laboratory pellet diet (Shri Jagdamby Feed Industry, Moga) and water *ad libitum* were given to mice.

2.4 Vehicle and standard drug

The test doses of crude extracts were prepared using vehicle {Distilled water + Tween 80 (2%)}. Diazepam (Triko Pharmaceuticals, Rohtak, Haryana) at the dose of 2 mg/kg, *p.o.* was used as standard anxiolytic drug.

2.5 Experimental design

Experimental protocol, comprises ten groups (I to X), and each group contained 6 mice.

Group I – Mice received vehicle (0.25 ml, *p.o.*) served as Control group;

Group II – Mice received diazepam (2 mg/kg, *p.o.*) served as Standard group;

Group III – Mice received HE (200 mg/kg);

Group IV – Mice received HE (400 mg/kg);

Group V – Mice received CE (200 mg/kg);

Group VI – Mice received CE (400 mg/kg);

Group VII – Mice received ME (200 mg/kg);

Group VIII – Mice received ME (400 mg/kg);

Group IX – Mice received AQE (200 mg/kg);

Group X – Mice received AQE (400 mg/kg);.

2.6 Evaluation of antianxiety activity

Elevated plus maze model was used to assess antianxiety activity. The parameters assessed were (a) number of open arms entries and (b) time spent (average) in open arms (Prakash *et al.*, 2015).

2.7 Statistics

The results of antianxiety activity studies were presented as mean \pm standard deviation (SD). The anxiolytic activity of extracts were compared with diazepam and vehicle using one way ANOVA followed by Student Newman Keul's test (Scheffer, 1980).

3. RESULTS AND DISCUSSION

The percentage yields of crude extracts *viz.*, HE, CE, ME and AQE were found to be 0.52, 0.48, 3.90 and 2.29% w/w respectively. Qualitative chemical tests showed presence of lipids in HE; alkaloids in CE; coumarins, flavonoids and tannins in ME; and carbohydrates and proteins in AQE. All crude extracts of *H. intermedia* fruits were screened for antianxiety activity using elevated plus maze model (EPM). It is clearly evident from table 1 that ME (200 or 400 mg/kg) produced significant antianxiety activity in mice using EPM when compared with control, but the anxiolytic activity exhibited by ME was statistically not similar as shown by the standard drug. HE, CE and AQE could not increase number of entries and time spent by mice in open arms of EPM with respect to control at any tested doses. These observations finally suggest that only ME exhibits mild anxiolytic activity whereas HE, CE and WE are devoid of anxiolytic activity.

The EPM model of anxiety was employed for the assessment of antianxiety activity of test drugs because this is easy to handle, less labour oriented, and does not involve any training to the mice (Kaur *et al.*, 2014). In this model the animals are exposed to approach–avoidance conflict (height), which induces anxiety (acrophobia) in animals (Kumar & Kumar, 2014).

Table 1: Antianxiety activity of various extracts of *H. intermedia* fruits using EPM.

Treatment	Dose (mg/kg)	Number of entries in open arms (Meann \pm S.D.)	Time spent in open arms (sec) (Meann \pm S.D.)
Control	Vehicle	2.00 \pm 0.63 ^a	3.13 \pm 0.28 ^a
Diazepam	2	7.67 \pm 1.03*	12.76 \pm 0.47*
HE	200	2.33 \pm 0.52 ^a	2.93 \pm 0.34 ^a
	400	2.50 \pm 0.55 ^a	3.08 \pm 0.23 ^a
CE	200	2.50 \pm 0.84 ^a	2.81 \pm 0.29 ^a
	400	2.67 \pm 0.52 ^a	2.92 \pm 0.42 ^a
ME	200	5.00 \pm 0.89 ^{*a}	8.88 \pm 1.25 ^{*a}
	400	5.50 \pm 1.05 ^{*a}	9.00 \pm 0.90 ^{*a}
WE	200	2.50 \pm 0.84 ^a	2.86 \pm 0.21 ^a
	400	2.67 \pm 0.82 ^a	2.85 \pm 0.22 ^a

n=6; * P <0.05 vs Control; ^a P <0.05 vs Diazepam (Standard drug); one way ANOVA followed by Student Newman Keul's test.

As coumarins, flavonoids and tannins are major classes of phytoconstituents of bioactive ME, it is finally concluded that phenolic compounds may be therapeutically active constituents of *H. intermedia*.

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