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Research Article

Screening of Antianxiety Activity of Extracts and Fractions of *Verbena officinalis* Aerial Parts

Pawan Kumar[1*], Reecha Madaan[2], Shabir Sidhu[1]

[1]*IKG Punjab Technical University, Kapurthala – 144 601, Punjab, India*
[2]*Chitkara College of Pharmacy, Chitkara University, Rajpura – 140 401, Punjab, India*

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ABSTRACT

Verbena officinalis L. (Vervain; family-Verbenaceae) has been traditionally used in the treatment of various ailments especially in mental disorders, but no systematic work has been carried out to validate traditional claims of plant. Therefore, it was planned to screen antianxiety activity of extracts and fractions of *V. officinalis* aerial parts. Crude extracts of *V. officinalis* aerial parts were prepared by extracting the plant material successively with *n*-hexane, chloroform, methanol and water. The antianxiety activity of crude extracts was performed at the doses of 200 or 400 mg/kg, *p.o.*, upon acute administration in mice using elevated plus maze model. The bioactive extract was partitioned using ethyl acetate, and ethyl acetate fraction (50 or 100 mg/kg, *p.o.*) and remaining bioactive extract (150 or 300 mg/kg, *p.o.*) were also screened for antianxiety activity. The statistical significance was checked by comparing with standard drug and control using one way analysis of variance (ANOVA), which was followed by post hoc analysis – Student-Newman-Keul's test. The methanol extract and ethyl acetate fraction exhibited significant antianxiety activity at the dose of 400 mg/kg and 100 mg/kg, respectively, with respect to control and statistically equivalent to the standard drug (Diazepam, 2 mg/kg, *p.o.*). The bioactive ethyl acetate fraction is rich in phenolic compounds and flavonoids. Finally, it is concluded that these are major anxiolytic constituents of *V. officinalis*.

Keywords: Antianxiety activity, Diazepam, *Verbena officinalis*, Vervain, Verbenaceae.

INTRODUCTION

Verbena officinalis L. (Vervain; family-Verbenaceae) has been traditionally used as nervine tonic, antidepressant, anticonvulsant; prescribed in liver and gall bladder complaints (spasm of the bladder and strangury), nervous and menstrual disorders; also for bronchitis, asthma and febrile affections[1]. The whole plant is useful in nerve complaints and amenor; fresh leaves are used as febrifuge, tonic, as rubefacient in rheumatism and diseases of the joints; roots are considered as a remedy for scrofula and snake bite[2,3]. In many parts of Europe, the herb is used in treatment of fits, convulsions and nervous disorders[3]. The aerial parts have been used traditionally for many conditions, including stimulation of lactation and treatment of dysmenorrhea, jaundice, gout, kidney stones, headache, depression, anxiety and insomnia[4-6].

V. officinalis has been reported to contain secoiridoid glycosides – verbenoside A and verbenoside B[7]; phenyl propanoid glycosides – verbascoside and eukovoside[4]; triterpenoids – oleanolic acid and ursolic acid[8]; flavonoids – apigenin-7-glucuronide and luteolin-7-glucoside[9]; volatile oil – limonene and cineole[10] and phytosterol – *β*-sitosterol and daucosterol[11]. The plant has been reported to possess a number of activities including larvicidal[12], anti-inflammatory, analgesic[4], pro-apoptotic and antioxidant[13].

A close scrutiny of the available literature revealed a startling fact that the plant has not been explored pharmacologically for validation of its traditional claims of antianxiety activity. Thus, it was considered worthwhile to screen antianxiety activity of extracts and fractions of *V. officinalis*.

MATERIALS AND METHODS

Plant Material - The dried aerial parts of *V. officinalis* were procured from Himalaya Herbs Store, Madhav Nagar, Saharanpur, (Uttar Pradesh), India in September, 2012. The plant was identified by Dr. Avneet Pal Singh, Assistant Professor, Department of Botany, Punjabi University, Patiala, India (Reference No. SPL-101/Bot, dated 15-10-2013).

Preparation of Various Extracts and Fractions

Dried and powdered plant material (5 kg) was exhaustively (24 hrs) and successively extracted in a Soxhlet apparatus, on water bath maintained at 80°C, using solvents (25 L each) in increasing order of polarity *viz.*, *n*-hexane, chloroform and methanol (S.D. Fine Chemicals, New Delhi, India). The marc was then boiled with distilled water (25 L) for 2 h on a hot plate maintained at 100ºC. The methanol extract (500 g) of plant material was suspended uniformly in water, and partitioned successively with ethyl acetate (S.D. Fine Chemicals, New Delhi, India) as per the standard

**Author for Correspondence: pawanptukumar@gmail.com*

procedure[14]. The solvents from crude extracts and fractions were recovered under reduced pressure using rotary vacuum evaporator (BUCHI, Switzerland) to get *n*-hexane extract (HE), chloroform extract (CE), methanol extract (ME), water extract (AQE), ethyl acetate fraction (EAF) and remaining bioactive extract (RBE). Qualitative chemical tests were performed on extracts and fractions to ascertain various classes of phytoconstituents present therein[15].

Acute Toxicity Studies of Extracts of V. officinalis - Various extracts were subjected to acute oral toxicity study as per Organization for Economic Cooperation and Development (OECD) guidelines-423[16].

Antianxiety Activity Studies of Extracts and Fractions of V. officinalis

Animals - Swiss albino mice (either sex) of body weight 20-25 g purchased from the Panacea Biotec Ltd., Ambala-Chandigarh highway, Lalru, Punjab, India were used for antianxiety activity studies. 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 animals were fed with normal laboratory pellet diet (Shri Jagdamby Feed Industry, Moga) and water *ad libitum*. Groups of six animals were used in all sets of experiments.

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

Experimental Design - Two experimental protocols were designed with 16 groups of mice and each group comprised 6 mice.

Experimental protocol I, consisting of 10 groups, was designed to assess antianxiety activity of various extracts of *V. officinalis* aerial parts.

Group 1 served as control group received vehicle (0.25 ml, *p.o.*); Group 2 served as standard group received diazepam (2 mg/kg, *p.o.*); Group 3 and 4 served as test groups received 200 and 400 mg/kg doses of HE respectively; Group 5 and 6 served as test groups received 200 and 400 mg/kg doses of CE respectively; Group 7 and 8 served as test groups received 200 and 400 mg/kg doses of ME respectively and Group 9 and 10 served as test groups received 200 and 400 mg/kg doses of AQE respectively.

Experimental protocol II, consisting of 6 groups, was designed to assess antianxiety activity of various fractions obtained from the bioactive methanol extract of *V. officinalis* aerial parts.

Group 1 served as control group received vehicle (0.25 ml, *p.o.*); Group 2 served as standard group received diazepam (2 mg/kg, *p.o.*); Group 3 and 4 served as test groups received 50 and 100 mg/kg doses of EAF respectively; Group 5 and 6 served as test groups received 150 and 300 mg/kg doses of RBE respectively.

Evaluation of Antianxiety Activity Using Elevated Plus Maze Model - Antianxiety activity of various extracts and fractions was performed using elevated plus maze (EPM) model[14].

Statistics - The results are presented as mean ± standard deviation (SD). The statistical significance was checked by comparing with standard drug and control uisng one way analysis of variance (ANOVA), which was followed by post hoc analysis – Student-Newman-Keul's test[17].

RESULTS

AQE contained maximum content of extractable constituents, i.e., 6.12% w/w followed by ME (5.98% w/w), CE (1.89% w/w) and HE (1.15% w/w). General qualitative chemical tests, performed on crude extracts, showed presence of lipids in HE; steroids and triterpenoids in CE; tannins, flavonoids, coumarins, carbohydrates and proteins in ME; tannins, saponins, carbohydrates and proteins in AQE.

It was observed in acute toxicity studies that none of the crude extracts, after administration of 2 g/kg, *p.o.*, showed any lethality and mortality in mice. Therefore, 200 and 400 mg/kg, ($1/10^{th}$ and $1/5^{th}$ dose used in acute toxicity study) of crude extracts of *V. officinalis* aerial parts were selected for antianxiety activity using EPM. It is clearly evident from table 1 that ME exhibited significant antianxiety activity with respect to control, whereas HE, CE and AQE did not show antianxiety activity. The mice treated with ME (400 mg/kg) showed significant increase in number of entries and time spent in open arms with respect to control. ME exhibited antianxiety activity at the dose of 400 mg/kg in a similar manner as exhibited by the standard drug, diazepam at the dose of 2 mg/kg. These observations infer strong anxiolytic potential of ME. An attempt was made to purify crude bioactive methanol extract of the plant so that main classes of phytoconstituents which are responsible for anxiolytic potential are highlighted. The methanol extract was fractionated with ethyl acetate by adopting a standard procedure. EAF extracted about $1/4^{th}$ portion of chemical constituents, i.e., 25.77% w/w in relation to ME. The remaining methanol was also collected and concentrated to get RBE. Phytochemical screening of EAF showed presence of coumarin, flavonoids and tannins, whereas proteins and carbohydrates were observed in RBE. EAF (50 or 100 mg/kg) and RBE (150 or 300 mg/kg) were also screened for antianxiety activity in mice using EPM. The doses of EAF and RBE are selected on the basis of their respective percentage yields with respect to crude ME. EAF exhibited significant antianxiety activity at the dose of 100 mg/kg with respect to control**,** and the activity was equivalent to the standard drug. RBE was found to be devoid of antianxiety activity.

DISCUSSION

EPM was selected to assess antianxiety activity because it is simple model where no anxiogenic agent is administrated to animal, only phobia of height (acrophobia) induces anxiety in animals. In this model, two parameters are observed – (a) number of entries in open arms and (b) average time spent in open arms, in

Table 1: Antianxiety activity of various extracts of *V. officinalis* aerial parts using EPM.

Treatment	Dose (mg/kg)	Number of entries in open arms (Mean^n ± S.D.)	Time spent in open arms (sec) (Mean^n ± S.D.)
Control	Vehicle	2.50 ± 0.54^a	2.87 ± 0.22^a
Standrad drug (Diazepam)	2	7.67 ± 1.03*	14.71 ± 0.93*
HE	200	2.67 ± 0.51^a	2.90 ± 0.34^a
	400	2.83 ± 0.75^a	2.93 ± 0.22^a
CE	200	2.33 ± 0.75^a	2.91 ± 0.22^a
	400	3.00 ± 0.63^a	2.98 ± 0.27^a
ME	200	6.33 ± 1.03^{*a}	8.73 ± 0.87^{*a}
	400	7.33 ± 1.21*	13.01 ± 1.17*
WE	200	3.00 ± 0.89^a	2.95 ± 0.22^a
	400	3.17 ± 0.75^a	3.11 ± 0.20^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.

Table 2: Antianxiety activity of various fractions of *V. officinalis* aerial parts using EPM.

Treatment	Dose (mg/kg)	Number of entries in open arms (Mean^n ± S.D.)	Time spent in open arms (sec) (Mean^n ± S.D.)
Control	Vehicle	2.67 ± 0.51^a	2.87 ± 0.17^a
Standrad drug (Diazepam)	2	8.00 ± 1.09*	14.27 ± 0.79*
EAF	50	6.00 ± 0.89^{*a}	7.91 ± 0.86^{*a}
	100	8.17 ± 1.32*	13.26 ± 0.88*
RBE	150	2.83 ± 0.75^a	2.88 ± 0.19^a
	300	3.00 ± 0.63^a	3.43 ± 0.31^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.

anxious conditions, the animals prefers to stay in closed arms[14,16]. Anxiolytic drugs increase number of enters and time spent by animal in open arms of EPM. Increased number of entries and time spent in open arms after administered of ME (400 mg/kg) and EAF (100 mg/kg) in mice was observed. These observations suggest that EAF has taken main anxiolytic constituents of bioactive methanol extract. As EAF contained flavonoids and phenolic compounds as major classes of phytoconstituents, it is concluded that anxiolytic activity of *V. officinalis* is attributed to these constituents. The available literature also reveals that flavonoids such as apigenin, kaempferol and quercetin exert strong anxiolytic activity through different modes of action[14].

CONCLUSION

Phenolic compounds and flavonoids are main bioactive constituents of plant drugs. These constituents have shown varied bioactivities, especially CNS activities. In present study, EAF (rich in phenolic compounds and flavonoids) exhibited significant antianxiety activity. On the basis of these observations, it is concluded that flavonoids and phenolic compounds are responsible for antianxiety activity of *V. officinalis* aerial parts.

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