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Evaluation of Anti-anxiety Activity of *Actaea spicata* Linn.

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ABSTRACT
Actaea spicata Linn. (*Ranunculaceae*) has been traditionally used for the treatment of various ailments such as rheumatism, inflammation, nerve diseases, lumbago, scrofula and chorea. Despite a long tradition of use, no systematic phytochemical and pharmacological work has been carried out on this potential plant. Thus, *A. spicata* was subjected to preliminary anti-anxiety screening studies, with a view to ascertain the verity of its traditional use as an anxiolytic. In the present investigation, roots of the plant were extracted using solvents in order of increasing polarity viz., petroleum ether (60-80°C), chloroform, methanol and distilled water. All the crude extracts were evaluated for anti-anxiety activity in mice using elevated plus maze apparatus. Among all these extracts, only methanol extract exhibited significant anti-anxiety activity at a dose of 100 mg/kg in mice with respect to control as well as standard (diazepam, 2 mg/kg). Phytochemical screening showed presence of alkaloids and polyphenols in methanol extract of *A. spicata*. Thus, Specific methods were adopted to extract total alkaloidal and polyphenol fractions from the plant material and methanol extract of plant, respectively. Polyphenol fraction exhibited significant anxiolytic activity at the dose of 50 mg/kg, while alkaloidal fraction was found to be devoid of any activity.

Keywords: *Actaea spicata*, Alkaloids, Anti-anxiety, Polyphenols, *Ranunculaceae*.

INTRODUCTION

In present era, a sudden holocaust of mental disorders, and recognition of severe side effects and addiction liabilities associated with long term administration of widely prescribed synthetic drugs have aroused the attention of researchers towards natural resources. Plants like *Valeriana officinalis*, *Nardostachys jatamansi*, *Withania somnifera* and *Panax ginseng* have been used extensively in various traditional systems of therapy because of their adaptogenic and psychotropic properties. Inclusion of these well-established CNS affecting plants in the arsenal of modern therapeutics has revived the faith of researchers in the plants. [1] A survey of literature on plant derived anxiolytics and sedatives revealed several reports on the traditional uses of a number of plants, *Actaea spicata* being one of the important plants.

Actaea spicata Linn., commonly known as Baneberry, belongs to family *Ranunculaceae*. A survey of ethnopharmacologic records reveals that the plant has been traditionally used in the treatment of rheumatism, inflammation, rheumatic fever, lumbago, scrofula, nervous disorders, chorea, and as emetic, expectorant, laxative, stomachic and purgative. [2-4] The plant has also been used in traditional systems of medicines of various countries for the treatment of snake bite, asthma, and externally for skin complaints. In some parts of Europe the powdered leaves, stems and flowers are used as an insecticide. [5]

A. spicata has been reported to contain isoquinoline alkaloids magnoflorine, corytubrine; triterpene glycosides including actein and trans-aconitic acid. [6] An exhausted literature survey on *A. spicata* revealed that sporadic phytochemical and pharmacological reports are available on this plant. As *A. spicata* has been used traditionally for the treatment of various ailments, this plant holds great potential for in depth phytochemical and pharmacological evaluations.

Despite a long history of use of *A. spicata* as a traditional medicine for the treatment of various ailments, especially in CNS disorders, the plant has never been subjected to CNS activity studies. Thus, it was considered worthwhile to subject *A. spicata* to anti-anxiety screening studies.

MATERIALS AND METHODS

Plant material

Dried roots of *A. spicata* were procured from K. R. Indo German American Trading Company, Kurukshetra (Haryana), India in the month of November 2008. Identity of the plant was confirmed through Dr. H. B. Singh, Scientist F, Head of Raw Material Herbarium and Museum (RHMD), National and

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Animals

Laca mice (either sex) were bred at the Central Animal House, S.D. College of Pharmacy, Barnala. The animals were allowed a standard pellet diet and water *ad libitum*. Groups of five mice (20-24 g) were used in all sets of experiments. The animals were fasted for 18 hours before use. The approval from the Institutional Animal Ethical Committee of S. D. College of Pharmacy, Barnala was taken before carrying out biological studies.

Solvents

Petroleum ether (60^o–80^oC), chloroform (CHD, Mumbai) and methanol (S. D. Fine Chemicals Pvt.), all of LR grade, distilled under normal atmospheric pressure were employed for extraction of the plant material.

Recovery of solvents

Solvents from extracts were recovered under reduced pressure using Rotary vacuum evaporator (Gupta Scientific Store, Ambala), and the dried extracts were preserved in a vacuum desiccator containing fused calcium chloride (S.D. Fine Chemicals).

Elevated plus maze (EPM) model of anxiety

The plus-maze apparatus consisting of two open arms (16 × 5 cm) and two closed arms (16 × 5 × 12 cm) having an open roof, with the plus-maze elevated (25 cm) from the floor was used to observe anxiolytic behaviour in animals. [7-8] Each mouse was placed at the centre of the elevated plus maze with its head facing the open arms. During this 5 minutes experiment, the behavior of the mouse was recorded as: (a) the number of entries into the open arms, (b) average time spent by the mouse in the open arms (average time = total time spent in open arms/number of entries in arms). Extracts of *A. spicata* were administered orally using a tuberculin syringe fitted with oral canula. The dose administration schedule was so adjusted that each mouse was having its turn on the elevated plus-maze apparatus 45 minutes after the administration of the dose. During the entire experiment, the animals were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of plus-maze could invoke anxiety in the animals.

Vehicle and standard

Distilled water + Tween 80 (5%) was used as vehicle for preparing the suspension of various test doses of different extracts. Diazepam (2 mg/kg) (Triko Pharmaceuticals) was used as standard drug.

Statistics

The results have been expressed as mean ± standard deviation (S.D.). The test doses were compared among themselves, and also with diazepam and control by analysis of variance (ANOVA) followed by Student Neumann Keuls test. [9] Control group was also compared with the standard group.

Preparation of extracts, and their phytochemical screening

Dried, coarsely powdered roots of *A. spicata* (500 g) were successively extracted with petroleum ether, chloroform and methanol using a Soxhlet apparatus. The marc was air dried, and water extract was obtained by boiling with distilled water for 2 h, filtering, concentrating and drying in an oven at 40-50°C. All the four extracts were dissolved in respective solvents, and were screened for different classes of phytoconstituents. [10]

Preparation of polyphenol and alkaloidal fractions of *A. spicata* roots

The alkaloidal fraction was isolated from roots of *A. spicata* by following method: Roots (2 kg) of *A. spicata* were treated with lime, and then soxhlet extracted with chloroform. The chloroform extract was concentrated to $1/4^{th}$ of its original volume under reduced pressure. It was then partitioned in a separator using 5×50 ml of 2% acidulated water (HCl-water). The aqueous fraction was basified using NaOH solution to pH 8-9 followed by partitioning with chloroform (5×50 ml). The chloroform fraction was rich in alkaloids (0.089% w/w). The bioactive methanol extract (25 g) of *A. spicata* roots was suspended uniformly in water, placed in three-necked round bottom flask connected with teflon stirrer, and partitioned with ethyl acetate by heating for 30 min at 50^oC with continuous stirring. This procedure was repeated five more times. All the shakings of ethyl acetate were concentrated under reduced pressure (6.982 g).

Table 1: Yield of various extracts of *A. spicata* roots

Extract	Yield (% w/w)
Petroleum ether	1.90
Chloroform	2.35
Methanol	13.38
Water	4.87

Table 2: Results of phytochemical screening of various extracts of *A. spicata* roots

Class of phytoconstituents	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract
Alkaloids	-	+	+	-
Anthraquinone glycosides	-	-	-	-
Cyanogenic glycosides	-	-	-	-
Cardiac glycosides	-	-	-	-
Steroids/Triterpenoids	+/-	-/-	-/-	+/-
Saponins	-	-	-	-
Flavonoids	-	-	+	-
Coumarins	-	-	-	-
Tannins	-	-	+	-
Carbohydrates	-	-	+	+
Proteins	-	-	+	+

+ : present, - : absent

RESULTS

Table 1 shows the yield of various extracts and Table 2 shows results of phytochemical screening of various extracts of *A. spicata* roots. The mean time spent by the mice in open arms after oral administration of 100, 200 or 400 mg/kg of the extracts of *A. spicata* roots, diazepam (2 mg/kg) and the control (vehicle) has been shown in Table 3. Among the extracts tested, maximum anxiolytic activity was observed in the methanol at the dose of 100 mg/kg, p.o. Phytochemical screening showed presence of alkaloids and polyphenols in methanol extract of *A. spicata*. Thus, Specific methods were adopted to extract total alkaloidal fraction and polyphenol fraction from the plant material and methanol extract, respectively. Alkaloidal fraction and polyphenol fraction (25, 50 or 100 mg/kg, p.o.) were subjected to biological evaluation for anti-anxiety activity in mice using EPM apparatus. The mean time spent by the mice in open arms after oral administration of 25, 50 or 100 mg/kg of the alkaloidal and polyphenol fractions of *A. spicata* roots, diazepam (2 mg/kg) and the control (vehicle) has been shown

in Table 4. Polyphenol fraction exhibited significant anxiolytic activity at the dose of 50 mg/kg, while alkaloidal fraction was found to be devoid of any activity.

DISCUSSION

Anti-anxiety activity of various extracts of *A. spicata* roots was evaluated employing a widely used model, elevated plus-maze. The model was chosen as it is effective, cheap, simple, less time consuming, requires no preliminary training to the mice and does not cause much discomfort to the animals while handling. The model is principally based on the observations that the exposure of animals to an elevated and open maze results in approach–avoidance conflict which is manifested as an exploratory-cum-fear drive. The fear due to height (acrophobia) induces anxiety in the animals when placed on the elevated plus-maze. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in motor activity, which is measured by the time spent by the animal in the open arms.

Table 3: Anti-anxiety activity of various extracts of *Actaea spicata*

Treatment	Dose	Number of entries in open arms Mean[n] ± S.D.	Average time spent in open arms Mean[n] ± S.D.
Control	Vehicle	3.20 ± 0.45[a]	3.45 ± 0.39[a]
Standard	2 mg/kg	8.20 ± 0.84*	14.73 ± 0.92*
Petroleum ether extract	100 mg/kg	2.80 ± 0.45[a]	2.97 ± 0.34[a]
	200 mg/kg	3.00 ± 0.71[a]	3.16 ± 0.33[a]
	400 mg/kg	3.40 ± 0.55[a]	3.32 ± 0.24[a]
Chloroform extract	100 mg/kg	2.60 ± 0.55[a]	3.40 ± 0.44[a]
	200 mg/kg	2.40 ± 0.55[a]	3.47 ± 0.36[a]
	400 mg/kg	2.80 ± 0.84[a]	3.65 ± 0.49[a]
Methanol extract	100 mg/kg	8.00 ± 0.71*	14.92 ± 1.21*
	200 mg/kg	5.80 ± 0.84[a]*	9.24 ± 1.73[a]*
	400 mg/kg	3.40 ± 0.55[a]	3.50 ± 0.38[a]
Water extract	100 mg/kg	2.80 ± 0.45[a]	3.3 ± 0.45[a]
	200 mg/kg	2.80 ± 0.45[a]	3.49 ± 0.51[a]
	400 mg/kg	3.00 ± 0.71[a]	3.33 ± 0.62[a]

n=5; The data is expressed as Mean ± S.D.; *P<0.05 vs Control; [a]P<0.05 vs Standard; ANOVA followed by Student Newmann Keul's test.

Table 4: Anti-anxiety activity of alkaloidal and polyphenol fractions of *Actaea spicata*

Treatment	Dose	Number of entries in open arms Mean[n] ± S.D.	Average time spent in open arms Mean[n] ± S.D.
Control	Vehicle	2.40 ± 0.55[a]	3.23 ± 0.48[a]
Standard	2 mg/kg	7.80 ± 0.84*	15.24 ± 1.29*
Alkaloidal fraction	25 mg/kg	2.60 ± 0.55[a]	3.23 ± 0.48[a]
	50 mg/kg	2.40 ± 0.55[a]	3.53 ± 0.36[a]
	100 mg/kg	3.00 ± 0.71[a]	3.47 ± 0.45[a]
Polyphenol fraction	25 mg/kg	5.20 ± 0.84[a]*	9.20 ± 0.63 [a]*
	50 mg/kg	7.60 ± 0.89*	14.86 ± 1.31*
	100 mg/kg	3.40 ± 0.54[a]	3.80 ± 0.55[a]

n=5; The data is expressed as Mean ± S.D.; *P<0.05 vs Control; [a]P<0.05 vs Standard; ANOVA followed by Student Newmann Keul's test.

Dried petroleum ether, chloroform, methanol and water extracts of *A. spicata* roots, separately suspended in a suitable vehicle, were administered orally to mice, and the activity was compared with that observed in the control group as well as with the group treated with the standard anxiolytic drug diazepam. Complete manifestation of anxiety in mice of the control group is evident from the minimum mean time spent in the open arms of elevated plus-maze by these animals. Among the extracts tested, maximum anxiolytic activity was observed in the methanol at the dose of 100 mg/kg which was at par with that of diazepam as is evident from statistical equivalence between the results of this dose and that manifested by diazepam. However, the activity decreased at higher doses, which might be due to sedation.

Phytochemical screening showed presence of alkaloids and polyphenols in methanol extract of *A. spicata*. Thus, Specific methods were adopted to extract total alkaloidal fraction and polyphenol fraction from the plant material and methanol extract of plant, respectively. The alkaloidal fraction was isolated from roots of *A. spicata* by adopting standard procedures. Alkaloidal and polyphenol fractions (25, 50 or 100 mg/kg, p.o.) were subjected to biological evaluation for anti-anxiety activity in mice using EPM apparatus. Polyphenol fraction exhibited significant anxiolytic activity at the dose of 50 mg/kg, while alkaloidal fraction was found to be devoid of any activity.

The authors are involved in bioactivity directed fractionation of polyphenol fraction with a view to isolate bioactive fraction/constituent(s).

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