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Anti-anxiety Activity Studies of Various Extracts of *Pulsatilla nigricans* Stoerck

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ABSTRACT
Pulsatilla nigricans Stoerck (*Ranunculaceae*) has been traditionally used in nervousness, sadness, mild restlessness and mental unrest. Yet, the plant has never been subjected to systematic biological investigation. Therefore, the present investigation has been designed to evaluate anti-anxiety activity of *P. nigricans*. In the present investigation, petroleum ether (60-80°C), chloroform, methanol, and water extracts of *P. nigricans* aerial parts were evaluated for anti-anxiety activity in mice using elevated plus-maze apparatus. Among all the extracts, only methanol extract exhibited significant anti-anxiety activity at the dose of 200 mg/kg with respect to control as well as standard (diazepam, 2 mg/kg). As phytochemical screening of methanol extract showed presence of polyphenols, i.e., flavonoids and tannins, thus, these constituents might be responsible for anxiolytic potential of *P. nigricans.*

Keywords: *Pulsatilla nigricans*, *Ranunculaceae*, Elevated plus maze, Anxiety.

INTRODUCTION

Anxiety affects most of the population nearly one-eighth of the total population world-wide. [1] Benzodiazepines, being major class of compounds used for treatment of anxiety [2], present a narrow margin of safety between the anxiolytic effect and unwanted side effects, has prompted researchers to evaluate new compounds specially plant based drugs having less undesirable effects. [3]

Pulsatilla nigricans belongs to buttercup family, Ranunculaceae and grows in Turkey, Russia, Germany, France, Denmark, Sweden, England and Asia. [4-6] *P. nigricans* has been used in nervousness, sadness, mild restlessness and mental unrest. [6] A formulation containing *P. nigricans* as a constituent has also been employed for treatment of vaginal discharge and associated weakness and mental problems. [7] Despite a long tradition of use, no work has been carried out to justify its traditional claims, specially, CNS depressant properties. Thus the present investigation were undertaken to evaluate antianxiety activity of *P. nigricans.*

MATERIALS AND METHODS

Plant Material

P. nigricans was procured from K. R. Indo German American Trading Company, Kurukshetra, India. The identity of the plant was confirmed through Dr. Avneet Singh, Department of Botany, S. D. College, Barnala. A voucher specimen of the plant has been deposited in the Herbarium-cum-museum of S. D. College of Pharmacy, Barnala.

Animals

Swiss albino mice (either sex), bred at the Central Animal House, S. D. College of Pharmacy, Barnala, were allowed a standard pellet diet (Ashirwad Industries, Chandigarh) and water *ad libitum*. The animals were housed in polyacrylic cages with not more than three animals per cage and maintained under standard laboratory conditions (temperature 25±2°C) with 12 h dark and 12 h light cycle. Groups of five mice (20-24 g) were used in all sets of experiments. The animals were fasted for 18 h before use. The approval from the Institutional Animal Ethical Committee of S. D. College of Pharmacy, Barnala was taken before carrying out biological studies.

Drugs and Chemicals

Petroleum ether (60-80°C), chloroform (Ranbaxy Laboratory Chemicals), and methanol (S. D. Fine Chemicals), all of LR grade, were employed for extraction of the plant material. Diazepam (Triko Pharmaceuticals, Rohtak, Haryana) was used as a standard drug for evaluation of anti-anxiety activity.

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Phytochemical Screening

Aerial parts of *P. nigricans* (250 g) were powdered (# 60) and Soxhlet extracted successively with petroleum ether, chloroform and methanol. The marc was air dried, and water extract was obtained by boiling the residue with distilled water for 2 hours. It was filtered, concentrated, and dried in an oven at 40-50^{o}C. Exhaustive extraction with each of the solvents was ensured. The four extracts were dried using Buchi Rotary Vacuum Evaporator (Gupta scientific store, Ambala) and the dried extracts were preserved in vacuum desiccators. All the extracts were dissolved in respective solvents and were screened for different classes of phytoconstituents. [8]

Evaluation of Anti-anxiety Activity

Preparation of doses

The extracts of *P. nigricans* were separately suspended in a vehicle comprising 1% w/w carboxymethylcellulose in distilled water. Various doses viz., 100, 200 or 400 mg/kg of each extract of *P. nigricans* were prepared by suspending the dried extracts in vehicle. The doses of extracts were so adjusted as to administer 0.25 ml of the suspension of extracts. Diazepam 2 mg/kg suspended in the vehicle was used as standard anxiolytic. The suspending vehicle (0.25 ml) was used as control.

Elevated plus maze 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. [9]

Animals were fasted 18 h prior to the experiment. Extracts of *P. nigricans* 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 min after the administration of the dose. Each mouse was placed at the center of the elevated plus-maze with its head facing the open arms. During this 5 min experiment, the behavior of the mouse was recorded as (a) preference of the mouse for its first entry into the open arms, (b) the number of entries into the open or closed arms, (c) average time spent by the mouse in open arms (average time=total duration in the arms/number of entries). During the entire experiment, the animals were allowed to socialize. Every precaution was taken to ensure that no external stimuli could invoke anxiety in the animals. Similar observations were recorded for the standard group (Diazepam 2 mg/kg) as well as the control group (vehicle, 0.25 ml).

Statistics

The results have been expressed as mean ± standard error of mean (S.E.M.). The test doses were compared with standard and control by analysis of variance (ANOVA) followed by Student Newmann Kuel's post hoc analysis.

RESULTS AND DISCUSSION

The percentage yields (w/w) of various extracts viz. petroleum ether, chloroform, methanol and water extracts were 2.36, 1.08, 19.22 and 9.75 respectively. Table 1 shows results of phytochemical screening of various extracts of *P. nigricans* aerial parts.

Anti-anxiety activity of *Pulsatilla nigricans* was evaluated by employing a widely used model, i.e. elevated plus-maze. The mean number of entries and time spent by mice in open arms after oral administration of three doses viz. 100, 200 and 400 mg/kg of all extracts are given in Table 2.

Table 1: Phytochemical screening of various extracts of *P. nigricans* aerial parts

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

Table 2: Anti-anxiety activity of various extracts of *P. nigricans* aerial parts

S. No.	Treatment	Dose (mg/kg)	Mean[n] number of entries in open arms ± S.E.M.	Mean[n] time[b] (sec) spent in open arms ± S.E.M.
1.	Control	Vehicle	2.4 ± 0.45[a]	2.91 ± 0.28[a]
2.	Diazepam	2.0	7.2 ± 0.42*	14.53 ± 0.39*
3.	Petroleum ether extract	100	3.4 ± 0.38[a]	3.73 ± 0.47[a]
		200	3.7 ± 0.68[a]	3.21 ± 0.71[a]
		400	3.1 ± 0.51[a]	3.77 ± 0.11[a]
4.	Chloroform extract	100	2.9 ± 0.49[a]	3.03 ± 0.65[a]
		200	3.7 ± 0.36[a]	3.19 ± 0.43[a]
		400	3.4 ± 0.22[a]	3.57 ± 0.51[a]
5.	Methanol extract	100	2.7 ± 0.71[a]	3.05 ± 0.48*[a]
		200	6.8 ± 0.83*	13.46 ± 1.04*
		400	3.3± 0.11[a]	3.98 ± 0.27[a]
6.	Water extract	100	3.1 ± 0.40[a]	3.49 ± 0.46[a]
		200	2.9 ± 0.65[a]	4.03 ± 0.28[a]
		400	3.2 ± 0.31[a]	4.87 ± 0.35[a]

n=5; *P<0.05 vs control; [a]p<0.05 vs standard (Diazepam); ANOVA followed by Student Newmann Kuel's post hoc analysis

Amongst various extracts methanolic extract of *P. nigricans* significantly increased mean number of entries and mean time spent by mice in open arms of elevated plus maze apparatus at the dose of 200 mg/kg with respect to control, thereby producing anti-anxiety activity. The higher dose (400 mg/kg) did not exhibit significant anxiolytic effect, may be due its sedative activity. None of other extracts exhibited anti-anxiety activity at any dose tested.

Phytochemical screening of methanolic extract showed presence of flavonoids, tannins and carbohydrates. Flavonoids have shown anti-anxiety activity in various studies. Further, the anxiolytic effect of flavonoids has been attributed to its effect on central nervous system [10] and benzodiazepine receptors. [11-12] Therefore, flavonoids of methanolic extract of *P. nigricans* may be responsible for the anti-anxiety activity.

Finally, it is concluded from the present study that mathanolic extract of *P. nigricans* exhibits anti-anxiety activity at the dose of 200 mg/kg in mice using elevated plus maze model of anxiety. The studies are under progress to isolate bioactive constituent(s)/ fraction from *P. nigricans* responsible for anti-anxiety activity.

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