Diuretic, Laxative and Toxicity Studies of *Viola odorata* Aerial Parts

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Summary

The aqueous extract of the *Viola odorata* aerial parts (200 and 400 mg/kg, p.o.) showed significant diuretic activity. Butanolic and aqueous extracts (200 and 400 mg/kg, p.o.) showed good laxative effect in rats. The acute toxicity, orally evaluated in rats, and was found to be higher than 2000 mg/kg.

Key words: *Viola odorata*; Diuretic activity; Laxative activity

Introduction

The medicinal plant *Viola odorata* Linn. (Violaceae) is a popularly known as “Banafshah” and sweet violet in Asia and Europe respectively. It is found in high altitudes of Himalyas, Europe and throughout North America. It is a long trailing Plant of less than 6 inches height. The Plant has thick and scaly underground stem, with rooting runners. It possesses a heart shaped leaves with scalloped or slightly serrated edges are dark green, smooth or sometimes downy underneath, and grow in a rosette at the base of plant. Flowers are deep purple or blue to pinkish or even yellow whitish [1-2]. *Viola odorata* is a part of the extensively medicinal plants utilized by the tradipractitionars. Indeed, the flowers are used as demulcent, diaphoretic, diuretic, laxative and root is used as emetic in larger doses.

The whole aerial part including stem, flowers and leaves are used in bronchitis, cancer, cough, fever, urinary infections, rheumatism, sneezing, kidney and liver disorders [3, 4]. Despite various ethno pharmacological data and the wide spread use and marketing of this plant studies on pharmacological activities are not available. Because of traditional uses we therefore examined the effects of four extracts of this Plant (aerial part) for diuretic, laxative and acute toxicity studies in experimental animal models.

Materials and method

Plant material

V. odorata (authenticated at K.N.K. college of horticulture, Mandsaur by Dr. S.N. Mishra) were collected by Dehradun (India) 2007 and specimen prepared and deposited at the herbarium of B.R. Nahata college of pharmacy, Mandsaur with voucher specimen no. BRNCP/V/003/2007.

Preparation of extracts

Pulverised dried aerial part of *V. odorata* were packed into the thimble of the Soxhlet extractor and refluxed continuously for 72 hours. The solvents system i.e. n-hexane, butanol, methanol and aqueous was changed at an end of every 72 hours. The solvent from n-hexane, butanol and methanol were removed by distillation on boiling water bath at atmospheric pressure. While aqueous residue was evaporated in an oven at 55°C. The following fractions were obtained. n-hexane (2.768 % w/w), butanol (1.386 % w/w), methanol (9.552 % w/w) and aqueous(16.917 % w/w). All extracts were suspended in 1% Tween 80 solution.

Animals

Experimental animals consisted of adult wistar strain rats weighing (150-200 gm) of both sexes and aged between 3 and 4 months old housed in the animal house of pharmacology department, B.R. Nahata college of pharmacy, Mandsaur in standard conditions (21°C, 60-70% humidity) under a 12 hour light:12 hour dark cycle. The animals were fed with a standard diet and water ad libitum.

Preliminary Phytochemical analysis

All the four extracts i.e. n-hexane, butanol, methanol and aqueous extracts of *V. odorata* was subjected to a preliminary phytochemical screening for the presence of steroids, flavanoids, tannins, alkaloids and saponins[5,6].

Acute toxicity studies

Test animal were divided into groups (n=5 per group) which were administered doses of the crude extracts (2000 mg/kg, p.o.), while the control group received only the vehicle (1% Tween 80 in water, p.o.). The general signs and symptoms of toxicity were observed for 5 hr, 72 hr, and 14 days and mortality was recorded for each group at the end of this period [7].

Diuretic activity

The test was performed according to *Bose A. et al*., 2007 [8] method on male rats. The animals fasted and deprived of water for 18 hr prior to the experiment and were divided into ten groups of six rats each. For screening procedure three groups of 2 animals are used for one dose of the test compounds, standard and control. The first group of animals, serving as control, received 1% Tween 80 p.o.; the second group received Furosemide (4 mg/kg, p.o.) in 1% Tween 80 as standard; the other groups received test compounds (extracts) at doses of 200 and 400 mg/kg, respectively in 1% Tween 80 solution. Immediately after dosing, the animals were placed in metabolic cages provided with a wire mesh bottom and a funnel to collect the urine in graduated measuring cylinder. Urine was collected after 5 and 24 hrs while animals deprived of food and water. Urine vol. and Na^+, K^+, concentration in the urine were determined.

Laxative activity

Laxative activity by metabolic cage method

The test was performed according to *Razina Rouf et al.,* 2007 [9] on rats of either sex, fasted for 12 hrs before experiment, but with water provided ad libitum.

The animals were divided into different groups of six animals each. For screening Procedure 2 groups of 3 animals each were used for one dose of test compound, standard and control. The first group of animals, serving as control, received 1% Tween 80 p.o.; the second group received Bisacodyl (2.5 mg/kg, p.o.) in 1% Tween 80 as standard; the other groups received test compounds (extracts) at doses of 200 and 400 mg/kg, respectively in 1% Tween 80 solution. Immediately after dosing, the animals were placed in metabolic cages suitable for collection of faeces. After 24 hrs of drug administration, the faeces were collected and weighed.

Laxative activity by gastrointestinal motility method

The method, described by *A.J. Akindele et al*, 2006 [10, 11] was adopted to evaluate the effect of the crude extracts on the gastrointestinal transit in rats. The test animals were starved for 24 h prior to the experiment but were allowed free access to water. The animals were divided into control and test groups containing five rats in each group. Control group received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg body weight orally. Positive control group received Bisacodyl at the dose of 2.5 mg/kg p/o, and test groups received the extracts at the doses of 200 and 400 mg/kg body weight orally. After 30 min, rats of each group were fed with 1ml of charcoal meal (3% suspension of deactivated charcoal in 0.5% aqueous methyl cellulose). After 30 min of the administration of charcoal meal, the animals of each group were sacrificed and the length of the intestine (pyloric sphincter to caceum) as well as the distance travelled by charcoal as a fraction of that length was measured. Peristaltic index (%) for each rat was expressed as percentage of distance traveled by the charcoal meal relative to the total length of small intestine.

Statistical analysis

All data were expressed as mean ± S.E.M. One way ANOVA followed by Dunnett's multiple comparison tests were used to analyse the data obtained from invivo experiments. All statistical analysis was performed with Prism 4.0 (Graph Pad software Inc., San Diego, CA). $P<0.05$ was considered to be significant.

Results

Preliminary Phytochemical analysis and acute toxicity studies

Results of Preliminary Phytochemical analysis carried out on the crude of all extracts i.e. n-hexane, butanol, methanol and aqueous extracts of *V. odorata* indicates the presence of steroids, flavanoids, tannins, alkaloids and saponins. No lethal effects were observed within 24 hrs after the administration of the extract at a dose level of 2000 mg/kg. No deaths occurred among animals treated with the extracts. Dose levels of 200 and 400 mg/kg body weight were chosen for the Pharmacological screening.

Diuretic activity

n-hexane, butanolic, methanolic and aqueous extract were subjected for diuretic study at dose level of 200 and 400 mg/kg body weight and it was found that all the extracts at a dose level of 400 mg/kg during first 5 hours showed good results and after 24 hours n-hexane and Methanolic extracts showed best results. The Preliminary studies showed the presence of flavanoids in different extracts. It is reported previously that the flavanoid glycosides are endowed with diuretic activity. It may be therefore be presumed here that the diuretic activity is due to presence of flavanoids in test extracts [8]. The data in table 1 and 2 allowed with conclusion that the extract acts as diuretic because of urinary electrolyte concentration with significant increase in the urinary output. The increase in the ratio of concentration of excreted sodium and potassium ion for the test extracts, compared to control, indicates that the extracts increases potassium ion excretion to a greater extent than sodium ion, which is very essential quality of a good diuretic.

Laxative activity by metabolic cage method

n-hexane, butanolic, methanolic and aqueous extract were subjected for laxative activity at dose level of 200 and 400 mg/kg body weight and it was found that the butanolic and methanolic extract at a dose level of 200 mg/kg showed good results. Aqueous extract at a dose level of 400 mg/kg showed good laxative activity.(Table no. 3)

Laxative activity by Intestinal transit method

n-hexane, butanolic, methanolic and aqueous extract were subjected for laxative activity at dose level of 200 and 400 mg/kg body weight and it was found that the n-hexane extract at a dose level of 200 mg/kg showed good results of laxative activity. Butanolic and aqueous extracts at dose level of 200 and 400 mg/kg body weight shows significant effect on gastro intestinal motility. (Table no. 4)

Conclusion

The qualitative chemical examination showed the presence of alkaloid, glycoside, tannins, flavonoids, saponins etc. that can be responsible for pharmacological activities of different extracts.

Toxicity study of all the extracts, performed on rats using extracts at dose of 2000 mg /kg body weight by fixed dose method showed the extract to be safe up to this dose level.

Diuretic activity of different extracts has been studied and it was found that urine output and Na^+ and K^+ level was more in case of aqueous extract at a dose level of 400 mg/kg as compared to control animals.

Laxative activity of different extracts has been studied and it was found that alcoholic extracts at a dose level of 200 mg/kg and aqueous extract at a dose level of 400 mg/kg showed significant effect as laxative. But in case of gastrointestinal motility, butanolic and aqueous extracts at dose level of 200 mg/kg and 400 mg/kg showed the good results.

Table 1: Diuretic activity of different extracts after 5 hours

S.No.	Treatment	Dose (mg/kg)	Urine volume (ml)	Conc. (ppm)		Na^+ / K^+
				Na^+	K^+	
1.	Control	-	3.50±0.76	468.30±22.45	642.00±23.44	0.72
2.	Standard (Furosemide)	4	8.00±1.15**	635.00±29.30**	1237.00±49.48**	0.51
3.	n-hexane	200	3.16±0.72	455.00±20.21	654.70±52.84	0.69
		400	5.00±0.57	495.30±15.06	1213.00±14.72**	0.40
4.	Butanol	200	3.83±0.44	444.30±18.32	905.30±17.03**	0.49
		400	6.10±0.66	562.00±15.04	1339.00±48.89**	0.41
5.	Methanol	200	2.90±0.90	495.70±15.72	705.00±37.75	0.70
		400	4.16±0.44	493.00±23.29	1457.00±36.01**	0.33
6.	Aqueous	200	4.90±0.49	605.00±42.72*	1322.00±57.42**	0.45
		400	6.63±0.63*	748.70±49.50**	2377.00±55.93**	0.31

Values are expressed in mean ± S.E.M., n = 6 ** Significant at $p < 0.01$ Vs control, *Significant at $p < 0.05$ Vs control Dunnet's test, dose of extracts = 200,400 mg/kg , Standard (Furosemide) = 4 mg/kg

Table 2: Diuretic activity of different extracts after 24 hours

S.No.	Treatment	Dose (mg/kg)	Urine volume (ml)	Conc. (ppm)		Na^+ / K^+
				Na^+	K^+	
1.	Control	-	5.50±0.86	551.00±19.00	1036.00±41.82	0.53
2.	Standard (Furosemide)	4	13.67±1.20**	719.30±22.41**	1433.00±25.74**	0.50
3.	n-hexane	200	6.30±0.85	530.00±39.69	728.30±28.85**	0.72
		400	9.66±1.20	538.00±26.50	1100.00±34.15	0.48
4.	Butanol	200	8.00±1.15	579.30±15.45	958.30±13.02	0.60
		400	11.16±1.13**	539.00±35.57	1274.00±27.64**	0.42
5.	Methanol	200	6.60±1.60	661.00±35.84	958.30±13.02	0.68
		400	10.50±1.04*	651.70±19.65	1439.00±22.51**	0.45
6.	Aqueous	200	10.23±0.90	676.00±41.88	1341.00±26.74**	0.50
		400	11.20±1.20*	798.00±51.38**	1598.00±58.85**	0.49

Values are expressed in mean ± S.E.M., n = 6 ** Significant at $p < 0.01$ Vs control, *Significant at $p < 0.05$ Vs control Dunnet's test, dose of extracts = 200,400 mg/kg , Standard (Furosemide) = 4 mg/kg

Table 3: Laxative activity of different extracts

S.No.	Treatment	Dose (mg/kg)	Weight of faeces after 24 hrs.
1.	Control	-	1.838±0.055
2.	Standard (Bisacodyl)	0.25	2.330±0.032**
3.	n-hexane	200	2.175±0.034
		400	2.172±0.052
4.	Butanol	200	2.521±0.090**
		400	1.899±0.025
5.	Methanol	200	2.462±0.149**
		400	2.073±0.05
6.	Aqueous	200	2.175±0.009
		400	2.679±0.153**

Values are expressed in mean ± S.E.M., n=5, ** Significant at $p < 0.01$ Vs control, Dunnet's test, Standard (Bisacodyl) = 0.25 mg/ kg

Table 4: Laxative activity of different extracts

S.No.	Treatment	Dose (mg/kg)	Peristaltic index (%)
1.	Control	-	62.46±2.11
2.	Standard (Bisacodyl)	0.25	62.90±1.86
3.	n-hexane	200	84.07±4.3**
		400	35.07±0.95
4.	Butanol	200	86.01±1.75**
		400	91.84±2.28**
5.	Methanol	200	68.08±2.60
		400	70.89±3.00
6.	Aqueous	200	84.35±1.77**
		400	85.70±2.36**

Values are expressed in mean ± S.E.M., n=5, ** Significant at $p < 0.01$ Vs control, Dunnet's test, Standard (Bisacodyl) = 0.25 mg/ kg

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