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Full Length Research Paper

In-vitro pharmacological study and preliminary phytochemical profile of *Viola canescens* Wall. Ex Roxb

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The crude ethanolic extract of *Viola canescens* whole plant was evaluated for their analgesic, gastrointestinal motility and preliminary phytochemical profile. The analgesic effect was assessed in mice, using acetic acid induced writhing test at a dose of 50, 100 and 200 mg/kg; the effect on GIT motility was tested in mice using activated charcoal as marker, as due to its black color the distance traveled in intestine was easy to observe. The preliminary phytochemicals constitutes were identified using various qualitative tests. The plant showed dose dependent analgesic effect in compression with standard drug (diclofenac sodium 10 mg/kg); the extracts significantly ($p<0.05$) inhibited writhing at 100 and 200 mg/kg, while the analgesic effect was none significant at the dose of 50 mg/kg. The percentage analgesic effect of diclofenac sodium was 72.92, while the extract showed 16.92, 47.18 and 65.64% analgesia at the dose of 50, 100 and 200 mg/kg, respectively. The analgesic effect of the standard drug was higher than the extract. The ethanolic extract of the plant increased the intestinal motility at dose dependant manner and produced a significant effect at 100 and 200 mg/kg. The percent GIT motility of the extract (200 mg/kg) and standard drug was similar. Various phytochemicals were identified such as carbohydrates, phenolic compounds, flavoniod, alkaloids etc in ethanolic extract of the plant.

Key words: *Viola canscens,* analgesic, gastrointestinal motility and phytochemical.

INTRODUCTION

Viola canescens Wall. ex Roxb is a prostrate, pubescent herbaceous plant growing at high altitude areas like Swat, Buner and Chitral. It belongs to Violaceace family and locally it is known as banafsha. It is commonly used as antipyretic, analgesic and as anti-constipating agent. The antimalarial activity of the plant has been reported (Verma et al., 2011). The flower and leaves are used in cough, cold, fever and jaundice (Gilani et al., 2006). The whole plant is used as purgative, demulcent, antipyretic and anticancer (Hamayun, 2007). In addition to the aforementioned folk use of banafsh, the local people are using this medicinal plant for various therapeutic purposes (Ibrar et al., 2007). In continuation of our research work on rationalization of medicinal plants for its various pharmacological profiles (Barkatullah et al., 2011; Muhammad and Saeed, 2011; Rahman et al., 2011a; Rahman et al., 2011b; Raziq et al., 2011; Saeed et al., 2011) we tested the crude ethanolic extract of *V. canescens* for it analgesic and GIT motility effect.

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MATERIALS AND METHODS

Plant material

The fresh whole plant *V. canescens* was collected from upper Swat, Pakistan in April 2011. After the botanical authentication, the plant materials were washed under running tap water to remove adhering dust. The plant was air dried under shade and powdered into small frictions. The resulting powder material was subjected to extraction. A specimen of the plant was deposited under voucher number 1102/bot, in the herbarium of department of botany university of Peshawar.

Preparation of extracts

The plant ethanolic extract was prepared using the well recommended methods (Saeed et al., 2010 ; Barkatullah et al., 2011; Khan et al., 2011). About 100 g of powdered materials of whole plant was taken in a large, clean beaker and soaked in 500

Table 1. Analgesic activity of ethanolic extract of *V. canescens.*

Treatment	Dose (ml/kg)	No, of writhing (mean)
Control	10	65±3.46
Diclofenac	10	17.6±1.14**
	50	54±1.00
Ethanolic extract	100	34.33±1.53**
	200	22.33±1.53**

Values (mean ± SEM) present the numbers of writing after treatment with diclofenac sodium (10 mg/kg) and extract (50, 100 and 200 mg/kg). The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control; *, $p < 0.05$, **, $p<0.01$

ml of 90% ethanol. The beaker was sealed and kept for a period of one week accompanying occasional shaking and stirring. The solution was then filtered through muslin cloth. The filtrate (ethanol extract) obtained was evaporated by using rotary evaporator. The gummy black crude extract was obtained which was screened for their pharmacological and primarily phytochemicals studies.

Chemicals

Diclofenac sodium, castor oil, activated charcoal, acetic acid and sterile normal saline was used in all the experiments as control while extracts was prepared in normal saline.

Animals

Albino mice of either sex (18 to 22 g) were used. Animals were purchased from the Pharmacology Section of the Department of Pharmacy, University of Peshawar. The animals were maintained in standard laboratory conditions (25°C and light/dark cycles that is 12/12 h and were fed with standard food and water. The experimental protocol was recommended from the university ethical committee (ECUOP) following the well established procedures.

Acetic acid induced writhing test

All animal (18 to 22 g) were fasted for 2 h before starting experiment. Animals were divided into five groups. Group I was injected with normal saline (10 ml/kg i.p) as control while Group II was injected with standard drug diclofenac sodium (10 mg/ kg i.p) and the remaining three groups were injected with 50, 100 and 200 mg/kg i.p. of ethanolic extract. After 30 min of saline, diclofenac sodium and plant extract injection, the animals were treated i.p. with 1% acetic acid. The writhing was counted after 5 min of acetic acid injection. The number of abdominal constrictions (writhing) was counted for 10 min (Khan et al., 2010).

Gastrointestinal motility test

For this purpose, the mice of either sex (25 to 35 g) were fasted 18 to 24 h before the experiment start. Animals were divided in five groups each of six animals. First group were given normal saline (10 ml/kg) i.p, Groups II were treated with castor oil (0.1 ml/kg) as standard drug, remaining three groups were treated with ethanolic plant extract (50,100 and 200 mg/kg i.p), after 30 min of injecting saline, castor oil and extract 10 % charcoal suspension in 5% gum acacia was administer (5 mg/kg p.o). After 15 min of administering charcoal, the animal was kill by cervical dislocation and dissected out. The dissected animals were place on clean surface and measure the distance travelled by charcoal. Then GIT motility was calculated for all groups (Marona and Lucchesi, 2004).

Phytochemical screening

To identify the presence of chemical constituents of ethanol extracts, various qualitative tests were performed using the following chemicals and reagents: Phytochemical screening of the prepared extracts was conducted with various qualitative tests to identify the presence of chemical constituents. Carbohydrates were identified with Molisch's test, glycoside with water and sodium hydroxide solution, saponins, steroids with sulphuric acid, flavonoids with HCl, tannins with ferric chloride solution; alkaloids were identified with Mayer's reagent and Hager's reagent. The method was followed as in our previous studies (Ismail et al., 2011).

Statistical analysis

The results were presented as mean ± SEM of six animals. For statistical analysis, ANOVA was followed by post hoc Dunnetts test for multiple comparisons. Effects were considered to be significant at the $p < 0.05$ level.

RESULTS AND DISCUSSION

The inhibition of writhing induced by acetic acid is presented in Table 1, while the percent analgesic effect is shown in Figure 1. The ethanolic extract (100 and 200 mg/kg) was significant ($p < 0.05$) as compared to 50 mg dose, however the analgesic effect was less than that of standard drug (diclofenac sodium).

The effect on gastrointestinal motility is depicted in Table 2, while the percent increase in motility is shown in Figure 2. It is clear from results that the ethanolic extract of this plant is good purgative and it is used for this purpose in folk medicines. The GIT motility was significantly increased with extract at dose dependent manner, however the effect of the highest dose (200 mg) and castor oil was similar. Various phytochemical tests revealed that the plant is a good source of different phytochemical constituents' like carbohydrates, alkaloids,

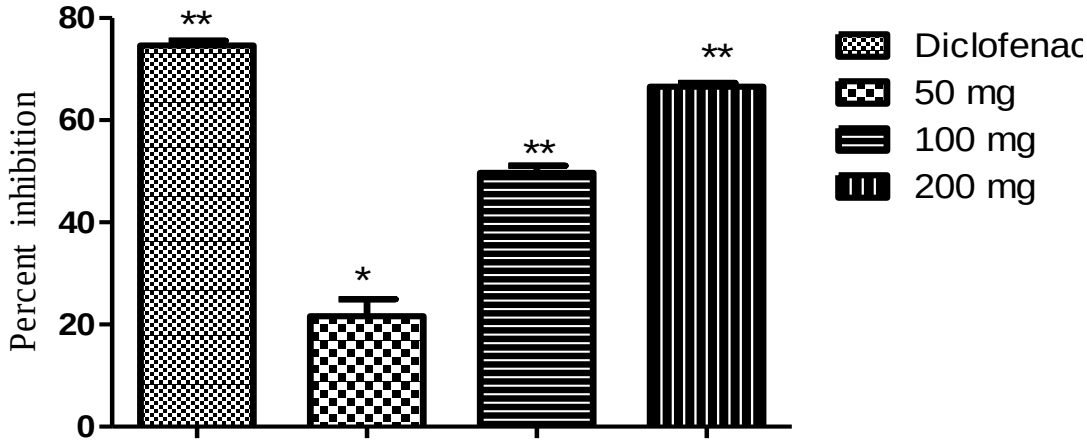

Figure 1. Analgesic effect ethanolic extract of *V. canescens* in mice. Bar presents the percent inhibition of writing after treatment with diclofenac sodium (10 mg/kg) and extracts (50, 100 and 200 mg/kg). The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control; *, $p < 0.05$, **, $p<0.01$.

Table 2. GIT mortality activities of ethanolic extract of *V. canescens*.

Treatment	Dose (ml/kg)	Total length of intestine	Distance covered by charcoal
Control	10	59.33 ±0.07	15.17 ±0.27
Castor oil	10	55.75 ±0.10	33.25 ±0.17**
	50	53.6±2.07	42.67±0.58*
Ethanolic extract	100	53.8±4.55	34.67±1.53**
	200	50.6±1.34	22.33±1.53**

Values (mean ± SEM) present the movement of charcoal in GIT after treatment with castor oil (10 ml/kg) and extract (50, 100 and 200 mg/kg). The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control; *$p < 0.05$; **$p < 0.01$.

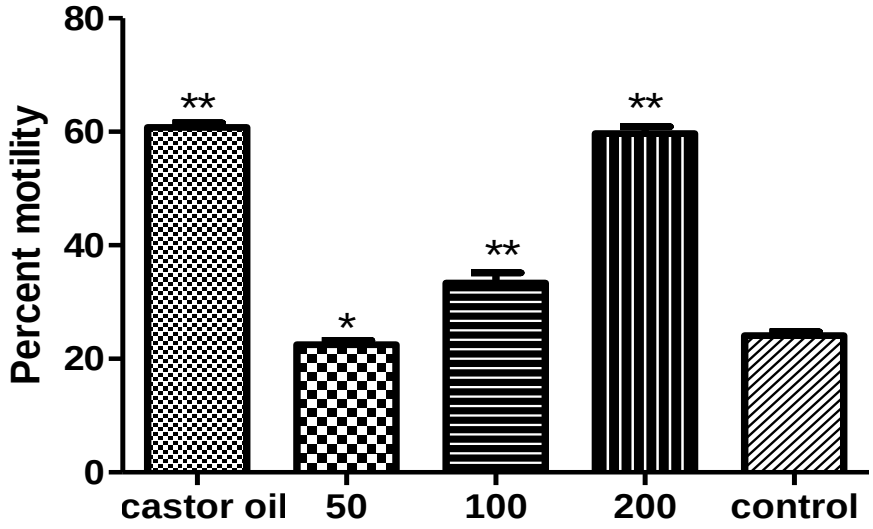

Figure 2. GIT motility effect of ethanolic extract of *V. canescens* in mice. Bar presents the percent movement of charcoal in GIT after treatment with castor oils (10 ml/kg) and extracts (50, 100 and 150 mg/kg). The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control; *, $p < 0.05$, **, $p<0.01$.

Table 3. Phytochemical evaluation of *V. canescens* crude extract.

S/N	Constituents	Test name	Ethanolic extract
1	Carbohydrates	Fehling's test	+
		Molish's test	+
		Benedict's test	+
2	Protein	Ninhydrine test	-
		Biuret's test	-
3	Alkaloids	Wagner's test	+
		Mayer's test	+
		Hager's test	-
4	Phytosterol and tr+iterpenoids	Salkoskii's test	+
		Liebermann test	-
5	Phenol	Ferric chloride test	+
6	Flavonoids	Lead acetate test	+
		Alkali test	-
7	Tannins	Copper sulphate test	
		Ferric chloride test	+
		Alkali test	-
8	Saponins	Forthing test	+
9	Anthocynanins	Hcl test	+
10	Glycosides	Killaer killani test	-
11	Fixed oil and fats	Spot test	-
12	Volatile oil	-	-

phenolic compounds, flavonoids and saponins as shown in Table 3. The analgesic effects of the crude extract of the leaves of *V. canescens* were investigated in this study using acetic acid induced writhing test, because of its sensitivity that could give different grades of injurious stimuli in chemically induced tissue damage (Owoyele et al., 2004). Similarly, the acetic acid induced writhing has been used to evaluate analgesic effects of drugs and the response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathways (Nuhu et al., 2010). The acetic acid induced writhing which allows the acid to act via peripheral mechanism. Therefore, the crude leaves extract of *V. canescens* has a significant inhibition in writhing in each mouse. The analgesic effect of this plant can be potentiated by providing the literature survey. The analgesic effect of *Viola odorant* showed good analgesic effect at dose of 400 mg/kg (Antil et al., 2011).

Constipation is one of GIT disorder and its major complication is pills/hemorrhoid, which is chronic disorder and need surgery. Although there is number of constipation reliving drugs in market but having a lot of side effects including contraindication in pregnancy. It is common perception among the public's that natural things are safe with fewer side effects; therefore the search for natural anti-constipating agents is carried out in various research institutes. In this research work, *V. canescens* is tested for their anti-constipating effects on mice, using activated charcoal as marker. This method is simple and non expensive for testing the chemicals or plant extracts for GIT motility study. It is shown that the traveling of charcoal in intestine was mostly up to 20 cm while the movement of charcoal was significant with ethanolic extract of *V. canescens.* In conclusion, the crude extract of this plant can be used as analgesic and laxative in traditional medicine.

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