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Evaluation of Antidiarrheal Activity of Aerial Parts of *Vinca major* in Experimental Animals

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Abstract: In the present study, the ethanolic extract from the aerial parts of *Vinca major* was evaluated for the antidiarrheal activity in rats. The effects of the ethanol extract from the aerial parts of *Vinca major* on castor oil induced diarrhea, castor oil and magnesium sulfate induced enteropooling, gastrointestinal motility test using charcoal meal methods were examined. This extract (250, 500 and, 1000 mg/kg, p.o.) was assayed on the latent periods, fecal frequencies in castor oil induced diarrhea. Gastrointestinal transit using charcoal meal, castor oil and magnesium sulfate induced enteropooling assays (500 mg/kg, p.o.) were conducted. These results suggested that the significant ($p<0.05$) reduction of the fecal output was observed at a dose of 500 mg/kg, p.o., in castor oil induced diarrhea, peristaltic movements in charcoal meal test, intestinal fluid secretions in castor oil and magnesium sulphate induced enteropooling, indicating its antidiarrheal activity. The results provide evidence that the ethanolic extract from *Vinca major* aerial parts possess antidiarrheal activities.

Key words: *Vinca major* % Diarrhea % Castor oil % Charcoal meal % Enteropooling

INTRODUCTION

Vinca major L. (Apocynaceae), commonly known as "Pervenche Grande" or the "Greater Periwinkle," is anevergreentrailing vine, has been mentioned frequently as a folk remedy. Phytochemical screening of Vinca major revealed the presence of alkaloids, saponins, unsaturated sterols, organic acids and phenols [1]. Vinca is used to extract its alkaloids which have multifarious medicinal properties. Vincristine sulfate and vinblastine sulfate act as antineoplastic agents and also used in treatment of hodgkin's diseases, other lymphomas and choriocarcinoma. Vinca also exhibits hypotensive, antidiabetic [2], antimicrobial [3] and antioxidant properties [4]. Though the aerial parts of *Vinca major* have been traditionally used in IndoChina for the treatment of piles, diarrhea and dysentery, no systematic studies have been carried out to confirm these activities in experimental animals [5]. Hence it was felt worthwhile to study the antidiarrheal activity of the aerial parts of *Vinca major*employing conventional animal models of diarrhea. This study aimed to investigate antidiarrheal activity of the ethanol extract from the aerial parts of *Vinca major* on castor oil induced diarrhea, castor oil induced enteropooling and magnesium sulfate induced enteropooling and gastrointestinal motility test using charcoal meal method in rats.

MATERIALS AND METHODS

Plant Materials and Chemicals: The fresh aerial parts of *Vinca major* were collected in months of May-June, 2010 from local garden of Indore, India. Authentication of plant material was done by the Botanical Survey of India, Pune, India. A voucher specimen number (MITHVM3) has been deposited in our college herbarium for future reference. The following drugs and analytical grade of chemicals were used: atropine sulfate and loperamide (standard reference antidiarrheal drugs), castor oil (laxative agents), normal saline solution (0.9 % sodium chloride), charcoal meal (10 % activated charcoal in 0.5 % w/v sodium carboxy methyl cellulose) and vehicle (0.5 % w/v sodium carboxy methyl cellulose) were used. All the other chemicals and reagent used were of analytical grade, obtained from Kasliwal Brothers, Indore, India.

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Preparation of the Extract: The shade dried aerial parts of the plant material were coarsely powdered. The powder was subjected to extraction with 95 % ethanol in soxhlet apparatus at (60-70 °C) and the marc concentrated *in vacuo* to obtain *Vinca major* ethanolic extract (VMEE). The extract was stored in amber colored bottle and refrigerated. The extract was analyzed for the presence of various phytoconstituents using standard methods [6].

Animals: Wistar rats of either sex weighing 120-200 g were used. Animals were housed under standard conditions of temperature (25 ± 2 °C), 12 h/12 h light/dark cycle and fed with standard pellet diet and water *ad libitum*. The animals were allowed to acclimatize for one week before the experiments. All experimental protocols were approved by the IAEC.

Castor Oil Induced Diarrhea: The antidiarrheal activity of ethanolic extract was evaluated according to the method described by Teke *et al*. Wistar rats were fasted for 18 hours and divided into five groups of five animals each. Castor oil at a dose of 1 ml was given orally to all groups of animals for the induction of diarrhea. Thirty minutes after castor oil administration various treatments were given, Group I (control) animals were treated with 0.5 % sodium carboxymethyl cellulose, Group II (standard) animals were treated with loperamide (3 mg/kg, p.o.), a positive control. Group III-V served as treated and *Vinca major* ethanolic extract at dose of 250, 500, 1000 mg/kg were administered respectively by oral route. Animals were placed separately in individual cages lined with filter paper. The filter papers were changed every hour and the severity of diarrhea was assessed hourly for 4 hours [7].

Gastrointestinal Motility Test: Wistar rats were fasted for 18 h and divided into three groups of five animals each, group I animals served as control and were treated orally with 0.5 % w/v sodium carboxymethyl cellulose in distilled water. Group II animals served as standard and treated with atropine (5 mg/kg, i.p.) a positive control. Animals of group III received orally 500 mg/kg ethanolic extract of *Vinca major*. After 1 h, each animal was administered orally with charcoal meal 0.25 ml (10% charcoal in 0.5 % w/v Sodium carboxymethy cellulose). Thirty minutes later, the animals were sacrificed. Total small intestine from pylorus to caceum was isolated and the total length and the length traveled by the charcoal meal were measured. This distance was expressed as a percentage of the length of the small intestine [7].

$$\% \text{ Inhibition} = (Mc\text{-}Md/\ Mc) \times 100$$

Mc: mean number of defecation travelled by charcoal meal; Md: mean number of defecation travelled by drug or extract.

Castor Oil Induced Enteropooling: Wistar rats were fasted for 18 h and divided into three groups of five animals each. Group I which received normal saline (2 ml, p.o.) served as the control group. Group II served as standard and received loperamide (3 mg/kg, p.o.). Group III animals received *Vinca major* ethanolic extract of 500 mg/kg, p.o. one hour before the oral administration of castor oil (2 ml). One hour later, the rats were sacrificed and the small intestine was removed after tying the ends with threads and weighed. The intestinal content was collected into a graduated cylinder and their volume was measured. The intestine was reweighed and the difference between the full and empty was calculated [8, 9].

Magnesium Sulfate Induced Enteropooling: Wistar rats were fasted for 18 h and divided into three groups of five animals per group. Solutions of magnesium sulfate were made in the 10 % w/v aqueous solution. Group I animals which received normal saline (2 ml, p.o.) served as the control group. Group II animals served as standard and received loperamide (3 mg/kg, p.o.). Group III animals received *Vinca major* ethanolic extract of 500 mg/kg, p.o. Immediately after the treatment magnesium sulfate (10 % w/v) was administered. After 30 minutes following administration of magnesium sulfate the rats were sacrificed, the small intestine was removed after tying the ends with threads and weighed. The intestinal content was collected into a graduated cylinder and their volume was measured. The intestine was reweighed and the difference between the full and empty was calculated [9, 10].

Statistical Analysis: Data was expressed in as mean ± standard error of mean (S.E.M.) and statistical analysis was carried out employing one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test at $p<0.05$ significance level using "Graphpad Instat" version 3.00 for windows 95, graphpad software, San Diego California USA, (www.graphpad.com).

RESULTS

Phytochemical Investigation: The percentage yield of ethanolic extract was found to be 10.36 %. The ethanolic extract showed the presence of alkaloids, saponins, phenolic compounds, carbohydrates and sterols.

Castor Oil Induced Diarrhea: One hour after castor oil administration, all the rats in the control group of animals produced copious diarrhea. Pretreatment of rats with ethanolic extract of *Vinca major* (250, 500, 1000 mg/kg, p.o.) dose dependently and significantly ($p<0.05$) delayed the onset of diarrhea, reduced the frequency of defecation and the wetness of the fecal droppings (reduction in the no. of wet stool and the general diarrheal scores including the hard and copious stool (Table 1). The standard antidiarrheal drug loperamide (3 mg/kg, p.o.) produced a marked significantly greater ($p< 0.05$) inhibitory effects in all the diarrheal parameters.

Gastrointestinal Motility Test: Compared with the control group, *Vinca major* ethanolic extract 500 mg/kg, p.o. significantly ($p<0.05$) decrease the propulsive movement and transit of charcoal meal to the gastrointestinal tract. The standard antidiarrheal drug atropine (5 mg/kg, p.o.) produced greater antimotility effect then the higher dose of *Vinca major* ethanolic extract 500 mg/kg, p.o. (Table 2).

Castor Oil Induced Enteropooling: Oral administration of castor oil (2 ml, p.o.) produced a marked and significant ($p<0.05$) increase in the intestinal fluid volume of castor oil treated groups of rats compared to control group of animals treated with normal saline (2 ml, p.o.) only. Compared with the control group of rats, pretreatment of the test group of rats with *Vinca major* ethanolic extract (500 mg/kg, p.o.) dose dependently and significantly ($p<0.05$) inhibited castor oil induced enteropooling in rats (Table 3). The standard drug, loperamide produced a marked and significant greater ($p<0.05$) inhibitory effects

Table 1: Effect of *Vinca major* aerial part extract on castor oil induced diarrhea

Treatment	Dose (mg/kg)	Total no. of feces	Total no. of diarrheal feces	Wt. of dry feces	Wt. of wet feces
Control	-	0.90 ± 0.09^{b}	3.70 ± 0.40^{b}	$0.06 \pm 0.02^{a,b}$	$0.71 \pm 0.04^{a,b}$
LOP	3	0.19 ± 0.05^{a}	0.00 ± 0.00^{a}	0.23 ± 0.06^{a}	0.00 ± 0.00^{a}
VMEE	250	0.70 ± 0.02^{b}	$2.07 \pm 0.50^{a,b}$	0.81 ± 0.01^{a}	0.07 ± 0.04^{a}
VMEE	500	0.44 ± 0.08^{a}	$0.92 \pm 0.03^{a,b}$	$0.60 \pm 0.03^{a,b}$	0.02 ± 0.02^{a}
VMEE	1000	0.45 ± 0.08^{a}	$0.85 \pm 0.00^{a,b}$	$0.60 \pm 0.03^{a,b}$	0.02 ± 0.02^{a}

Results are expressed as mean ± SEM; n=5 in each group. Data was analyzed by one way ANOVA followed by Tukey-Kramer multiple comparisons test. $^{a}p<0.05$ when compared to control group, $^{b}p<0.05$ when compared to standard group (loperamide 3 mg/kg).

VMEE = *Vinca major* ethanolic extract; LOP = loperamide

Table 2: Effect of *Vinca major* aerial part extract on gastro intestinal motility test

Treatment	Dose (mg/kg)	% movement by charcoal
Control	-	86.54 ± 7.91^{b}
Atropine sulfate	3	39.46 ± 2.59^{a}
VMEE	500	58.06 ± 6.12^{a}

Results are expressed as mean ± SEM; n=5 in each group. Data was analyzed by one way ANOVA followed by Tukey-Kramer multiple comparisons test. $^{a}p<0.05$ when compared to control group, $^{b}p<0.05$ when compared to standard group (atropine sulfate, 3 mg/kg).

VMEE = *Vinca major* ethanolic extract

Table 3: Effect of *Vinca major* aerial part extract on castor oil induced enteropooling

Treatment (mg/kg)	Dose	Volume of fluid (ml)	Weight of intestinal content (gm)	Percentage of inhibition
Control	-	1.95 ± 0.21^{b}	2.86 ± 0.55b	-
LOP	3	0.74 ± 0.50^{a}	0.81 ± 0.38^{a}	71.67
VMEE	500	$1.49 \pm 0.02^{a,b}$	$1.07 \pm 0.45^{a,b}$	62.58

Results are expressed as mean ± SEM; n=5 in each group. Data was analyzed by one way ANOVA followed by Tukey-Kramer multiple comparisons test. $^{a}p<0.05$ when compared to control group, $^{b}p<0.05$ when compared to standard group (loperamide 3 mg/kg).

VMEE = *Vinca major* ethanolic extract; LOP = loperamide

Table 4: Effect of *Vinca major* aerial part extract on magnesium sulfate induced enteropooling

Treatment	Dose (mg/kg)	Volume of fluid (ml)	Weight of intestinal content (gm)	Percentage of inhibition
Control	-	6.83 ± 0.27^{b}	11.13 ± 0.36^{b}	-
LOP	3	4.91 ± 0.25^{a}	7.27 ± 0.26^{a}	34.69
VMEE	500	$5.87 \pm 0.26^{a,b}$	$9.47 \pm 0.41^{a,b}$	14.91

Results are expressed as mean ± SEM; n=5 in each group. Data was analyzed by one way ANOVA followed by Tukey-Kramer multiple comparisons test. $^{a}p<0.05$ when compared to control group, $^{b}p<0.05$ when compared to standard group (loperamide 3 mg/kg).

VMEE = *Vinca major* ethanolic extract; LOP = loperamide

on castor oil induced fluid accumulation than the higher dose of *Vinca major* ethanolic extract (500 mg/kg, p.o.) used.

Magnesium Sulfate Induced Enteropooling: The extract reduced the intestinal fluid secretion induced by magnesium sulfate, in a dose dependent fashion. The standard antidiarrheal drug, loperamide (3 mg/kg, p.o.), produced a more marked and significantly greater ($p<0.05$) inhibitory effects on magnesium sulfate induced fluid accumulation. The reduction in the intestinal fluid secretion at 500 mg/kg of plant extract treatment was found to be almost comparable with that of treatment by 3 mg/kg dose of loperamide (Table 4).

DISCUSSION

Diarrhea is a very common ailment and national problem in many tropical countries and the cause of 4-5 million deaths throughout the world annually [11, 12]. Apart from modern medical therapy, the use of herbal drugs in the treatment of diarrheal diseases is a common practice in many countries of Asia including India and Bangladesh. A number of medicinal plants have been reported [13] to be effective against diarrhea and dysentery, as they are used in traditional herbal practice. Indigenous plants used for this purpose are: *Byrsocarpus coccineus* [14], *Cylicodiscus gabaunensis* [15], *Calotropis procera* [16], *Rumex maritimus* [17] and others. The present study was undertaken to substantiate out the scientific rationale behind the local use of *Vinca major* ariel parts in diarrhea.

The antidiarheal activity of the alcoholic extract of the aerial parts of *Vinca major* was evaluated by employing castor oil induced diarrhea, gastrointestinal motility test, castor oil and magnesium sulfate induced enteropooling methods. The results of the present study showed that the ethanolic extract of *Vinca major* in castor oil induce diarrhea at 250, 500 and 1000 mg/kg body weight doses, significantly lowered several typical parameters of diarrhea produced a statistically significant reduction in the severity and frequency of diarrhea produced by castor oil. It is known that the active component of castor oil is the ricinoleic acid which is liberated from the action of lipases on castor oil.

In the enteropooling study, the *Vinca major* ethanolic extract (500 mg/kg) significantly reduced the intestinal content of rat. The intra luminal fluid accumulation was blocked by significantly the ethanolic extract of *Vinca major*. Further, the experiments carried out on the gastrointestinal tract motility after charcoal meal administration also showed a reduction in the propulsive movement of small intestine after pre treatement with the extract of *Vinca major*. Intestinal fluid secretion has been analyzed by enteropooling assay in rat, evoked by magnesium sulfate (a standard laxative agent).

In the evaluation of intestinal transit, atropine sulfate was used as standard drug. Atropine is known to inhibit intestinal transit probably due to its anticholinergic effect [18]. The ethanolic extract of *Vinca major* aerial parts also appeared to act on all parts of the intestine. Thus, they reduced the intestinal propulsive movement in the charcoal meal treated model at a dose level of 500 mg/kg of body weight and a transit period for sixty minutes; though this was not comparable to atropine sulfate.

The results indicated that ethanolic extract of *Vinca major* possess the significant antidiarrheal activity. They inhibited the frequency of defecation and reduced greatly the wetness of fecal excretion. Moreover, the intestinal fluid secretion and gastrointestinal propulsion were inhibited. The *Vinca major* extract also showed antimicrobial activities on some gastrointestinal microorganisms [3]. These findings provided a scientific support for the utility of this plant in the treatment of diarrheal diseases. Further research is to be carried out to fraction and purify the extract, in order to find out the fractions and molecules responsible for the anti-diarrheal activity observed.

CONCLUSION

On the basis of beneficial effects of Vinca major in the literature and our own results of the experiments in the extract of same herb *Vinca major* possess significant antidiarrheal activity.

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