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Full Length Research Paper

In vivo antidiarrheal activity of the ethanolic leaf extract of *Catharanthus roseus* Linn. (Apocyanaceae) in Wistar rats

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***Catharanthus roseus* is a medicinal plant with various pharmacological properties. In this study, we investigated the *in vivo* antidiarrheal activity of *C. roseus* ethanolic leaf extract in Wistar rats. Castor oil was used to induce experimental diarrhea in rats pretreated with extracts administered at 200 and 500 mg/kg to determine antidiarrheal effect. Loperamide and atropine sulphate were used as standard drugs in the two experiments. Preliminary phytochemical screening of the extract revealed the presence of alkaloids, tannins, triterpenes, flavonoids and saponins. The antidiarrheal effect of ethanolic extract of *C. roseus* revealed a dose dependant inhibition of castor oil induced diarrhea at doses of 200 and 500 mg/kg. On comparison of the extract with the negative control, there was a significant difference at 200 mg/kg ($P < 0.05$) and a more significant difference at 500 mg/kg ($P < 0.001$). Test extract's effect was further evaluated on intestinal transit and it exhibited dose dependant inhibition of transit motility of charcoal meal. The results indicated that the ethanol extract of *C. roseus* possesses significant antidiarrheal effect and corroborate the use of this herbal remedy as non-specific treatment for diarrhea in folk medicine.**

Key words: *Catharansu roseus*, antidiarrheal, ethanolic, phytochemical.

INTRODUCTION

The search for new therapeutic treatments for various disease conditions is currently in vogue. In many resource poor countries, plants have been looked at as an inexhaustible source of new lead compounds for drug discovery and development (Kong et al., 2003). In Uganda, 80% of the population lives in rural areas where plants play a key role in primary health care. Many plants are traditionally used to treat clinical ailments, but very few have been pharmacologically screened for assessing their efficacy and potency.

The use of plants and plant products in treatment of diarrhea is a common practice in Africa. Various plants have been used traditionally to treat diarrhea, and in fact experimental data shows that several of these plants are efficacious (Abdulkarim et al., 2005; Adeyemi et al., 2003; Appidi et al., 2008). However, several plants used in Uganda for traditional treatment of diarrhea have not been pharmacologically screened. There is need for such data to facilitate formulation of plant extracts into clinically useful drugs for management of diarrhea.

Catharanthus roseus L. (Apocyanaceae) also known as *Vinca rosea*, is a medicinal plant assumed to have originated from Madagascar, and it is used for the treatment of diarrhea. The plant is rich in alkaloids, some of which are clinically used to treat various forms of cancer (El-Sayeed et al., 2004). Other pharmacological uses of *C. roseus* include wound healing, anti-diabetic, analgesic, vasodilatory, hypoglycaemic and anti-

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Alzeihmer's disease (Nayak and Lexley, 2006). Extensive review of literature related to this plant reveals no data on its antidiarrheal activity. The aim of this study was to investigate the *in vivo* antidiarrheal activity of the ethanolic extract of *C. roseus* in Wistar rats.

MATERIALS AND METHODS

Plant

The leaves of *C. roseus* were collected from Kamukuzi division in Mbarara district, in Western Uganda. Identification and authentication of the plant was done by a taxonomist from Makerere University Herbarium; and a voucher specimen number was deposited at the Herbarium. The collected leaves were shade dried and crushed into powder mechanically using an electric grinder.

Experimental animals

Forty-eight (48) Wistar male albino rats weighing (150 to 200 g) were obtained from the animal house of the Faculty of Veterinary Medicine, Makerere University. The rats were given standard pellet diet and water *ad libitum*. All the animals were kept under study laboratory conditions of 12 h natural light/12 h darkness, 20 to 30°C warmth and 60% humidity. Rats were allowed an acclimatization period of 14 days before actual experiments.

Plant extraction

Extraction was done using ethanol solvent system by cold maceration method (Hassan et al., 2011). 200 g of the powdered material was suspended in 600 ml of absolute ethanol for 72 h at room temperature and stirred twice daily. The macerate was filtered using Whatman filter paper and concentrated at 55°C using a rotary evaporator (BIBBY STERLIN LTD Model RE 100) to obtain a crude extract with yield of 10%. The phytochemical constituents were identified by methods previously described in other studies (Nayak and Lexley, 2006). Twenty (20 g) of the extractive was dissolved in 200 ml of distilled water to make a concentration of 100 mg/ml for use in the experiment. The solution was kept in a refrigerator at temperature of 2 to 8°C throughout the study period.

Activity on castor oil induced diarrhea

Twenty four (24) Wistar rats were fasted for 18 h and divided into four groups of six animals each. The four groups of rats were treated as follows; first group (control group) received the vehicle (0.5% Tween 80 in distilled water); second group received 200 mg/kg *C. roseus* extract; third groups received 500 mg/kg *C. roseus* and fourth group received loperamide (3 mg/kg orally). 30 min after drug administration, all groups received castor oil at a dose of 1 ml per animal orally to induce diarrhoa. Rats were then placed separately in transparent cages with white filter paper (A4 size) placed at the bottom, which was changed every hour to observe the consistency of fecal matter and frequency of defecation for 4 h. The severity of diarrhea was assessed each hour for 6 h. The total number of feces and diarrheal feces excreted and the total weight of feces were recorded and compared with the control group. The total number of diarrheal feces of the control group was considered 100% and the results were expressed as a percentage of inhibition of diarrhea (Dahiru et al., 2006).

Gastro intestinal motility test

Charcoal meal was used as a diet marker to determine gastrointestinal transit. The rats were divided into four groups (n = 6) and fasted for 18 h before the experiment. The first group (control group) was orally administered the vehicle (0.5% Tween 80 in distilled water). The second and third orally received 200 and 500 mg/kg of body weight of the extract, respectively. The fourth group was orally administered with the standard drug, atropine sulphate (5 mg/kg body weight). Each animal was given 1 ml of charcoal meal orally (10% activated charcoal in 5% gum acacia) 30 min after administration. Each animal was then sacrificed after 30 min and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as a percentage of the total length of the intestine. The results were compared with the control group and the distance traveled by the charcoal meal of the control group was considered 100%. The results were expressed as a percentage of inhibition of the propulsive effect (Mabeku et al., 2006).

Statistical analysis

GraphPad Prism software, version 5.0 was used in the statistical analysis of experimental data. The data for castor oil induced diarrhea test and gastro intestinal motility test was first analyzed using one-way analysis of variance followed by Turkeys least significant difference (LSD) post hoc test. P-values were calculated in comparison to the negative control groups. P-values less than 0.05 were considered significant at 95% level of confidence.

Ethical considerations

The animals used were handled in accordance to the Laboratory Biosafety Guidelines (Laboratory Biosafety Guidelines, 2004).The experimental procedures were approved by the Faculty of Veterinary Medicine, Makerere University and the protocols were approved by the Division of Pharmacology and Toxicology, Faculty of Veterinary Medicine.

RESULTS

Phytochemical composition of *C. roseus* ethanolic extracts

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, triterpenes and tannins (Table 1). Based on color intensity, the extract had higher content of alkaloids and tannins followed by flavonoids. Saponins and triterpenes were present in trace amounts.

Effect of *C. roseus* ethanolic extract on castor oil induced diarrhea

C. roseus ethanolic extract inhibited castor oil induced diarrhea in Wistar albino rats at doses of 200 and 500 mg/kg. The extracts significantly reduced the number and weight of wet fecal pellets with extract treated groups showing lower diarrheal severity than control rats (Tables 2 and 3).

Table 1. Preliminary phytochemical composition of ethanolic extract of *C. roseus*.

Test	Alkaloids	Flavonoids	Saponins	Triterpenes	Tannins
Inference	+++	++	+	+	+++

+ = Present, - = absent.

Table 2. Number of wet fecal/soft fecal pellets.

Group (n = 6)	1 h	2 h	3 h
Control	4.833 ± 0.4773	3.667 ± 0.5578	3.000 ± 0.5774
200 mg/kg *C. roseus*	3.333 ± 0.5578	2.833 ± 0.3073*	1.500 ± 0.4282*
500 mg/kg *C. roseus*	2.000 ± 0.5164***	1.333 ± 0.4944**	0.667 ± 0.2108**
Loperamide	1.167 ± 0.4773***	0.833 ± 0.3073***	0.000 ± 0.000***

Values are presented as mean ± SEM; $^{*}P < 0.05$; $^{**}P < 0.001$; $^{***}P < 0.0001$ at 95% confidence level (CL).

Table 3. Weight of wet fecal pellets.

Group (n = 6)	Mean ± SEM	95% C.I	T-value (df = 10)	P-value
Control	22.67 ± 0.882			
200 mg/kg *C. roseus*	8.67 ± 1.542	10.04 to 17.96	7.881	< 0.0001
500 mg/kg *C. roseus*	6.50 ± 1.176	12.89 to 19.44	11.00	< 0.0001
Loperamide	3.00 ± 0.516	17.39 to 21.94	19.24	< 0.0001

Table 2 shows that within 1 h after castor oil administration, rats treated with *C. roseus* extract (500 mg/kg) and loperamide had less severe diarrhea in comparison with the control rats. After 2 and 3 h, *C. roseus* extract (200 and 500 mg/kg) both inhibited castor oil induced diarrheoa. Loperamide, a standard antidiarrheal agent inhibited castor oil induced diarrhea throughout the experimental period.

Effect of *C. roseus* ethanolic extract on gastrointestinal motility in Wistar rats

C. roseus extracts exhibited inhibitory effect on gastrointestinal transit *in vivo* indicated by reduced mean distance travelled by charcoal (Table 4).

In Table 4, *C. roseus* inhibited propulsion of charcoal through the intestinal tract reducing the mean percent distance travelled to 48.82 ± 0.913 (P = 0.0006) at a dose of 200 mg/kg. Increase of the dose to 500 mg/kg resulted in greater inhibition of gastrointestinal transit (P = 0.001) which was comparable to that caused by loperamide (standard drug).

DISCUSSION

In this study, we demonstrate that the ethanolic extract of *C. roseus* has antidiarrheal activity against experimentally induced diarrhea in Wistar rats. At doses of 200 and 500 mg/kg, the extract inhibited castor oil induced diarrhea as well as inhibited gastrointestinal propulsion of charcoal meal. This data corroborates the traditional usage of *C. roseus* in the treatment and management of diarrhea. The inhibition of diarrhea observed was comparable to the standard drug loperamide (3 mg/kg) with regard to severity of diarrhea.

Preliminary phytochemical screening of *C. roseus* revealed the presence of tannins, triterpenes, alkaloids, flavonoids and saponins. Identification of these compounds is in agreement with the phytochemical compounds in the same plant (Nayak and Lexley, 2007). The antidiarrheal activity of the ethanolic extract may be attributed to the phytochemicals present in this plant as supported by literature (Dahiru et al., 2006; Boominathan et al., 2005). Previous studies have shown that antidysenteric and antidiarrheal properties of plants are due to tannins, alkaloids, saponins, flavonoids, sterol, triterpenes and reducing sugars (Longanga et al., 2000). Hence, tannins, alkaloids, flavonoids, triterpenes and saponins may be responsible for the mechanism of action of *C. roseus* antidiarrheal activity.

In addition, we may hypothesize that the antidiarrheal action may also be due to the presence of tannins. It has been previously demonstrated that protein tannins make the intestinal mucosa more resistant and hence, reduce secretion and peristaltic movement (Abdulkarim et al., 2005).

Table 4. Effect of *C. roseus* ethanolic extract on gastrointestinal motility in Wistar rats.

Group (n = 6)	Mean ± SEM	95% C.I of difference	P-value
Control	77.5 ± 2.328		
200 mg/kg *C. roseus*	65.08 ± 1.207	6.797 to 18.4	0.0006
500 mg/kg *C. roseus*	48.82 ± 0.913	23.16 to 34.30	0.0001
Loperamide	37.07 ± 4.171	29.84 to 51.13	0.0001

C.I; confidence interval.

The extract also significantly reduced intestinal transit as observed by the decrease in transit motility of the charcoal meal. Comparable to atropine sulphate, the extract produced a dose dependant antimotility effect at doses of 200 and 500 mg/kg. Many plant compounds including flavonoids are known to have antispasmodic effects, delay gastrointestinal transit, suppress gut motility, stimulate water absorption or reduce electrolyte secretion (Palombo, 2006).

Conclusion

In conclusion, ethanolic leaf extract of *C. roseus* has antidiarrheal activity in Wistar rats. We therefore recommend further investigation of the fractions of the ethanolic extract responsible for the observed antidiarrheal effect of *C. roseus*.

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REFERENCES

Abdulkarim A, Sadiq Y, Gabriel OA, Abdulkadir UZ, Ezzeldin MA (2005). Evaluation of five medicinal plants used in diarrhea treatment in Nigeria. J. Ethno. Pharmacol., 101: 27-30.

Adeyemi O, Okpo S, Adesanya A (2003). Gastrointestinal activity of the aqueous extract of a Nigerian herbal preparation. West Afr. J. Pharmacol., 19: 22-27.

Appidi JR, Grierson DS, Afolayan (2008). Ethno botanical study of plants used for the treatment of diarrhea in the Eastern Cape, South Africa. *Pakistan* J. Bio. Sci., 11 (15): 1961-1963.

Boominathan R, Devi BP, Dewanjee S, Mandal SC (2005). Studies on antidiarrheal activity of *Ionodium suffruticosam* ging. (Violaceae) extract in rats. Phytotherapeutics. 10: 375-380.

Dahiru D, Sini JM, John Africa L (2006). Antidiarrheal activity of *Ziziphus mauritiana* root extracts in rodents. Afr. J. Biotechnol., 5 (10): 941-945.

El-Sayed M, Choi YH, Frédérich M, Roytrakul S, Verpoorte R (2004). Alkaloid accumulation in *Catharanthus roseus* cell suspension cultures fed with stemmadenine. Biotechnol. Lett., 26: 793–798

Kong JM, Goh NK, Chia LS, Chia TF (2003). Recent Advances in traditional plant drugs and orchids. Acta Pharmacol. Sin., 24:7-21.

Nayak BS, Lexley MPP (2006). *Catharanthus roseus* flower has wound healing activity in Sprague Dawley rats. BMC Compl. Altern. Med., 6:41.

Palombo EA (2006). Phytochemicals from traditional medicinal plants used in treatment of diarrhea: Modes of action and effects on intestinal function. Phytother. Res., 20: 717-724.