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Antidiarrhoeal activity of *Geranium incanum* Burm. f. (Geraniaceae) leaf aqueous extract in mice

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ABSTRACT

Ethnopharmacological relevance: Geranium incanum Burm. f. (Geraniaceae) is used in South Africa especially in rural communities by traditional medicine practitioners to treat diarrhoea. However, scientific evidence does not exist in any literature to corroborate the claim of therapeutic success of the plant species in diarrhoea.
Aim of study: The study intended to investigate the antidiarrhoeal activity of the leaf aqueous extract of *Geranium incanum* in mice.
Materials and Methods: Castor oil induced diarrhoeal test was used to assess the antidiarrhoeal activity of Geranium incanum. Gastrointestinal tract transit of charcoal meal test was used to assess the antipropulsive activity of the plant extract while the acute toxicity study and phytochemical analysis were carried out using well established protocols and methods.
Results: The antidiarrhoeal activity of *Geranium incanum* was investigated by studying the effect of leaf aqueous extract of the plant species on castor oil-induced diarrhoea in mice. The leaf aqueous extract of *Geranium incanum* significantly reduced faecal output in castor oil -induced diarrhoea and also significantly reduced the number of diarrhoeal episodes. *Geranium incanum* significantly delayed the onset of diarrhoea induced by castor oil and significantly reduced the number of animals exhibiting diarrhoea. Loperamide, a standard antidiarrhoeal drug, produced similar effects to the leaf aqueous extract of *Geranium incanum* on castor oil-induced diarrhoea. Both *Geranium incanum* and loperamide significantly reduced the intestinal propulsion of charcoal meal in mice. The phytochemical analysis of the leaves revealed the presence of tannins, saponins particularly steroidal saponin, and flavonoids. The LD_{50} of the plant species obtained was greater than 4000 mg/kg (p.o.).
Conclusion: The data obtained indicate that the leaf aqueous extract of *Geranium incanum* has both antidiarrhoeal and antipropulsive activities The data also show that the plant material given orally may be safe and/or non toxic in mice. However, further investigation on the acute toxicity and on the mechanism of the antidiarrhoeal effect of the plant species needs to be carried out.

1. Introduction

Diarrhoea is a killer disease worldwide (Farthing, 2000) and unfortunately, it happens to be amongst the symptoms of many other diseases. In most rural communities of developing countries including South Africa, diarrhoea poses serious problems particularly for children due to amongst other reasons, lack of adequate sanitation and pipe borne water. Traditional medicine practitioners in South Africa have been known to treat diarrhoea with a variety of medicinal plants one of which being *Geranium incanum* Burm. f. (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997). The plant belongs to the family, Geraniaceae, which also includes other *Geranium* species such as *Geranium canescens* and *Geranium robertianum* (Robert herb). All of these plant species have been used to treat various ailments including diarrhoea. *Geranium robertianum*, in particular, has been used in America and Europe to treat diarrhoea. The leaves of these *Geranium* species have been shown to contain tannins, notably geranin, which have been implicated in their antidiarrhoeal activity. The leaves have also been shown to contain several flavonoids but nothing much is known about the chemistry of *Geranium incanum* (Watt and Breyer-Brandwijk, 1962; Dictionary of Natural Products, 1996; Bruneton, 1999). *Geranium incanum* is a sprawling perennial shrublet with silvery leaves. *Geranium incanum* Burm. f. can grow up to 0.2–1 m in height and grows at an altitude of 10–610 m above see level. The flowers can be white, pale pink, violet or magenta in colour. The plant species is widely distributed in the Western and Eastern Cape Provinces of South Africa. It is locally known as "vrouebossie" in Afrikaans and "tlako" in Sotho (Hilliard and Burtt, 1985; Van Wyk et al., 1997; Germishuizen and Meyer, 2003). The plant has been used to treat a

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variety of ailments. Infusions of the leaves have been used to treat bladder infection, venereal diseases, menstruation problems, colic, diarrhoea, bronchitis and fever (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997).

However, scientific evidence does not exist in literatures to corroborate the claims by traditional medicine practitioners of the therapeutic successes of the plant species. The main aim of the present study was, therefore, to investigate the antidiarrhoeal activity of the leaf aqueous extract of *Geranium incanum* to justify its folklore use in diarrhoea. The acute toxicity, the phytochemical analysis of various components and the effect of the plant species on the intestinal propulsion of charcoal meal were also investigated in mice.

2. Materials and methods

2.1. Plant materials

The leaves of the plant species were collected fresh from Kirstenbosch National Botanical Gardens, Cape Town, South Africa. The identification of the plant was done by both the curator of the Gardens and Mr. Franz Weitz, a taxonomist in the Department of Biodiversity and Conservative Biology, University of the Western Cape, and the voucher specimen (6866) was deposited in the Herbarium, University of the Western Cape.

2.2. Preparation of leaf aqueous extract of Geranium incanum

The method previously described by Amabeoku et al. (2007) was used. 1 kg of leaves of the plant species was washed with distilled water and dried at 35 °C for 4 days. The dried leaves were ground to fine powder (850 μm) and a yield of 185.3 g was obtained. 80 g of fine powder was refluxed in 1 L of boiling water, allowed to cool and filtered. The filtrate was then frozen at −80 °C and freeze-dried for 120 h.

A yield of 10.4 g of dried leaf aqueous extract was obtained. Fresh extract solutions were prepared on each day of the experiment by dissolving weighed quantities of the extract in appropriate volumes of physiological saline. The solution was administered orally (p.o.) in a volume of 1 ml/100 g of animals using a bulbed steel needle.

2.3. Assessment of antidiarrhoeal activity

Male albino mice bred in the Animal House of the Discipline of Pharmacology, University of the Western Cape, Bellville, South Africa and weighing between 20 and 30 g were used in groups of six per dose of plant extract or loperamide, a standard antidiarrhoeal drug, throughout the experiments after fasting for 16 h. The method described by Williamson et al. (1996) was modified and used to assess the antidiarrhoeal activity of the plant extract. Castor oil, a laxative, known to cause frequent stooling within 4 h, was used to induce diarrhoea in a control group of mice pretreated with 0.3 ml of physiological saline for 15 min prior to the oral administration of 0.7 ml of castor oil. The onset of diarrhoea, the number of diarrhoeal episodes, stool mass and the number of animals exhibiting diarrhoea were obtained over a 5 h period of observation. The ability of plant extracts to reduce the number of animals exhibiting diarrhoea and the number of diarrhoeal episodes is taken as an antidiarrhoeal activity (Williamson et al., 1996). Experiments were repeated with animals pretreated for 15 min with either leaf aqueous extract (25–400 mg/kg) of the plant or the standard antidiarrhoeal drug, loperamide (20 mg/kg), both given orally in a volume of 1 ml/100 g of animals prior to the administration of castor oil. The doses and pretreatment times used were obtained from preliminary studies in our laboratory. All experiments were carried out between 08:00 and 17:00 h in a quiet laboratory with an ambient temperature of 22 ± 2 °C.

2.4. Assessment of gastrointestinal propulsion of charcoal meal

The method described by Williamson et al. (1996) and Kitano et al. (1994) were used to assess the effect of the plant extract on the gastrointestinal transit of charcoal meal. Animals were used in groups of six per dose of plant extract or loperamide, a standard drug after fasting for 16 h. Control group was pretreated with 0.3 ml of physiological saline given orally, for 20 min and then given 0.4 ml of charcoal meal (an aqueous suspension of 5% charcoal and 5% gum acacia) orally. 20 min after the charcoal meal, the animals were killed by ether inhalation and the intestine was removed from the cardia to the rectal end. The distance travelled by the charcoal meal was measured and expressed as a percentage of the total length of the intestine. Experiments were repeated with other groups of animals pretreated with either the leaf aqueous extract (25–400 mg/kg) of the plant or the standard drug, loperamide (20 mg/kg), both given orally in a volume of 1 ml/100 g of animals, prior to the administration of 0.4 ml of charcoal meal. All experiments were carried out between 08:00 and 17:00 h in a quiet laboratory with an ambient temperature of 22 ± 2 °C.

2.5. Acute toxicity testing

Modified method of Lorke (1983) was used to assess the acute toxicity of *Geranium incanum* leaf aqueous extract. Male albino mice were used in groups of eight per dose of plant extract after fasting for 16 h. *Geranium incanum* was administered orally to mice in graded doses (400, 800, 1200, 1600, 2000, 2400, 2800, 3200, 3600 and 4000 mg/kg). Another group of eight mice used as control received 0.3 ml of physiological saline orally. Both the test and control animals were then allowed access to food and water and observed over a period of 5 days for any deaths or acute toxicity symptoms. Depending on the results obtained, log dose/response (% death) curve may be plotted from which the median lethal dose (LD_{50}) of the plant extract would be obtained.

3. Phytochemical analysis of *Geranium incanum*

The methods of Harborne (1984) and Ikhiri et al. (1992) were used to determine the groups of chemical compounds present in the dried powder of the leaves of the plant extract.

4. Statistical analysis

The data obtained from the antidiarrhoeal and antipropulsive activity experiments were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison. However, the data on the number of animals exhibiting diarrhoea were analysed using the Chi square test. In the above cases, p values of less than 5% ($p < 0.05$), were considered to be significant.

5. Ethics clearance

The Ethics Committee of the University of the Western Cape approved the experimental protocol used in the present study and this conforms to the "Guide to the care and use of animals in research and teaching" of the University.

Table 1
Effect of leaf aqueous extract of *Geranium incanum* on castor oil-induced diarrhoea and gastrointestinal transit of charcoal meal in mice.

Treatment groups	Mass of stool (g) [Mean ± SEM]	Faecal output (%)	Onset of diarrhoea (min) [Mean ± SEM]	No. of animals exhibiting diarrhoea	No. of diarrhoeal episodes [Mean ± SEM]	Length of intestine travelled (%) [Mean ± SEM]
PS						
0.3 ml	1.65 ± 0.18	100	22.33 ± 2.87	6/6	6.40 ± 0.55	75.00 ± 3.19
Geranium incanum						
25 mg/kg	1.35* ± 0.12	81.8	30.83 ± 2.14	6/6	4.14* ± 0.98	49.82** ± 1.98
50 mg/kg	0.93** ± 0.12	56.4	40.50* ± 3.73	6/6	3.75** ± 0.67	43.52** ± 1.60
100 mg/kg	0.80** ± 0.14	48.5	45.67* ± 7.14	6/6	2.76** ± 0.73	37.83** ± 1.41
200 mg/kg	0.92** ± 0.07	55.8	65.67** ± 5.74	3/6	2.41** ± 0.09	30.22** ± 0.86
400 mg/kg	0.65** ± 0.14	39.4	70.50** ± 9.45	2/6+	2.02** ± 0.11	25.06** ± 1.24
Loperamide						
20 mg/kg	0.10** ± 0.10	6.1	210.00** ± 0	1/6++	1.00** ± 0	16.82** ± 0.54

*$p < 0.05$, **$p < 0.001$ vs castor oil (0.7 ml, p.o.) control, ANOVA ($n = 6$).
+$p < 0.01$, ++$p < 0.005$ vs castor oil (0.7 ml, p.o.) control, Chi squared test ($n = 6$).
PS: physiological saline.

6. Results and discussion

6.1. Effect of leaf aqueous extract of Geranium incanum on castor oil-induced diarrhoea and gastrointestinal transit of charcoal meal

In antidiarrhoeal experiment, castor oil (0.7 ml, p.o.) induced diarrhoea promptly within 4 h in all the animals and also produced a considerable amount of stool. *Geranium incanum* (25–400 mg/kg, p.o.) significantly ($p < 0.05$–0.001), reduced the faecal output produced by castor oil. At doses of 50–400 mg/kg (p.o.), the plant extract significantly ($p < 0.05$–0.001), and dose dependently delayed the onset of diarrhoea induced by castor oil. *G. inacnum* (200 mg/kg, p.o.) protected 50% of mice against the diarrhoea while the dose of 400 mg/kg (p.o.) significantly ($p < 0.01$), reduced the number of animals suffering from diarrhoea by protecting 66.7% of them. *Geranium incanum* (25–400 mg/kg, p.o.) significantly ($p < 0.05$–0.001) and dose dependently reduced the number of diarrhoeal episodes in the animals. Loperamide (20 mg/kg, p.o.) profoundly ($p < 0.001$), reduced the faecal output produced by castor oil. The onset of castor oil-induced diarrhoea and the number of diarrhoeal episodes were also profoundly prolonged ($p < 0.001$), and reduced ($p < 0.001$), respectively by loperamide. The number of animals suffering from diarrhoea was also significantly ($p < 0.005$), reduced by loperamide by protecting 83.3% of them (Table 1).

With the gastrointestinal transit experiment, it was observed that in the control group of animals pretreated with physiological saline, the mean length of intestine travelled by the charcoal meal was 75 ± 3.19%. *Geranium incanum* (25–400 mg/kg, p.o.) significantly ($p < 0.001$), reduced the mean length travelled by the charcoal meal in a dose-dependent manner. The propulsion of the charcoal meal was inhibited by 33.6–66.6% at the doses of 25–400 mg/kg (p.o.) of *Geranium incanum*. Loperamide (20 mg/kg, p.o.) significantly ($p < 0.001$), reduced the mean length travelled by the charcoal meal. Loperamide inhibited the propulsion of the charcoal meal by 77.6%.

6.2. Acute toxicity studies

Leaf aqueous extract of *Geranium incanum* (400, 800, 1200, 1600, 2000, 2400, 2800, 3200, 3600, 4000 mg/kg) given orally did not cause any death in the different dose groups. However, at the doses of 3600 and 4000 mg/kg (p.o.) all the animals showed the following symptom, hypoactivity. The LD_{50} value for oral administration of the plant extract was found to be greater than 4000 mg/kg.

6.3. Phytochemical analysis

The phytochemical analysis of the leaves of *Geranium incanum* revealed the presence of tannins, saponins particularly steroidal saponin, and flavonoids.

In the present study, the data obtained show that castor oil induced diarrhoea under 25 min. The ability of *Geranium incanum* to reduce the number of animals exhibiting diarrhoea and the number of diarrhoeal episodes is taken as an antidiarrhoeal activity (Williamson et al., 1996).Castor oil, an irritant or stimulant laxative, is hydrolysed in the upper small intestine to ricinoleic acid, a local irritant, that irritates the mucosa of the gastrointestinal tract resulting in increase in intestinal motility (Altman, 2001). Nitric acid mechanism has also been shown to be involved in castor oil-induced diarrhoea (Capasso et al., 1994; Mascolo et al., 1994). The data obtained in the present study show that loperamide, a standard antidiarrhoeal agent, profoundly inhibited castor oil-induced diarrhoea and also inhibited the gastrointestinal transit of charcoal meal in mice. Loperamide, an opioid derivative, has been shown to slow intestinal motility by its action on mu receptors on neurons in the submucosal neural plexus of the intestinal wall and its antimuscarinic activity in the gastrointestinal tract (Altman, 2001; Camillen et al., 2002; Waller et al., 2005). It is not surprising, therefore, that loperamide protected mice against castor oil-induced diarrhoea and also inhibited the intestinal propulsion of charcoal meal in the study. *Geranium incanum* significantly reduced the number of animals exhibiting diarrhoea and the number of diarrhoeal episodes and also inhibited the intestinal propulsion of charcoal meal in the present study. It is probable that the plant extract may be exerting its antidiarrhoeal and antipropulsive activities by slowing intestinal motility.

Furthermore, the standard chemical tests carried out in this study showed that the leaves of the plant species contain tannins, saponins particularly steroidal saponin, and flavonoids. It is pertinent to note that tannins have been reported in several studies to have antidiarrhoeal effect. Farthing (2000) reported that astringents such as tannins have been known since the last century to have antisecretory activity in the gastrointestinal tract and have been used to treat diarrhoea. Powell and Field (1980) in their publication, listed gallic acid (tannins) as one of the potential antidiarrhoeal drugs. Frei et al. (1998) and Bruneton (1999) reported that tannin containing drugs are widely used for the treatment of diarrhoea and related disorders. In view of the above publications, it is, therefore, not surprising that the standard chemical tests in this study, showed the presence of tannins in *Geranium incanum* which also may probably contribute to its antidiarrhoeal activity. This is in agreement with the report that tannins found in the leaves of the other species of *Geranium* contribute to their antidiarrhoeal activity (Dictionary of Natural Products, 1996; Bruneton, 1999). Furthermore, Ofuji et al. (1998) in their study where they compared the effect of geranium herb on its binding activity to rabbit haemoglobin and the short-circuit current across rat jejunal mucosa with those of the antidiarrhoeicas, such as tannic acid and so on, reported that geranium herb has astringent action.

They suggested that this astringent action may be involved in the antidiarrhoeal effect of both geranium herb and Phelloberin-A. The suggestion by Ofuji et al. (1998) is in agreement with the suggestion made in the present study that tannin, an astringent, found in *Geranium incanum* may be involved in its antidiarrhoeal activity. In conclusion, the data obtained in this study suggest that the leaf aqueous extract of *Geranium incanum* has antidiarrhoeal activity thus justifying its folklore use in diarrhoea. The LD_{50} obtained in this study may be greater than 4000 mg/kg (p.o.) of plant extract. With the relatively high LD_{50}, it is possible that the plant extract may be safe and/or non-toxic in mice. However, further investigation on the acute toxicity and the mechanism of the antidiarrhoeal activity of *Geranium incanum* needs to be carried out.

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