Malaysian Journal of Pharmaceutical Sciences
Vol. 11, No. 2, 31–39 (2013)

EVALUATION OF ANTIDIARRHOEAL ACTIVITY OF *ELYTRARIA ACAULIS* EXTRACTS ON MAGNESIUM SULPHATE- AND CASTOR OIL-INDUCED DIARRHOEA IN WISTAR RATS

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Elytraria acaulis is traditionally used in the treatment of diarrhoea. E. acaulis extracts were prepared in alcohol, water, hydroalcoholic solution, chloroform and ethyl acetate. Acute toxicity test was performed in albino mice and antidiarrhoeal activity was studied in Wistar rats. Castor oil- and magnesium sulphate-induced diarrhoea in Wistar rats were treated with E. acaulis extracts, standard antidiarrhoeal drug loperamide and the results were compared to control. Extracts of E. acaulis showed neither mortality nor any toxic effect in albino mice up to the dose of 5 g/kg during the period of 48 hours, which was further extended for 14 days. Water extract of E. acaulis was 60.68% ($p<0.01$) effective in reducing faeces in castor oil-induced diarrhoeal rats and 62.10% ($p<0.01$) in magnesium sulphate-induced diarrhoea model. These results indicated that E. acaulis extracts are effective for treatment of diarrhoea.

Keywords: *Elytraria acaulis,* Diarrhoea, Castor oil, Magnesium sulphate, Acute toxicity

INTRODUCTION

Diarrhoea is the frequent passing of loose, watery and unformed faeces. Loss of fluids through diarrhoea can cause dehydration and electrolyte imbalance. Herbal treatment for diarrhoea in natural and traditional medicinal practices includes the use of plants or plant extracts like *Semicarpus anacardium, Achyranthus aspera, Rhus semialata* (Alexander *et al.* 2011), *Desmostachya bipinnata* (Ahmad *et al.* 2010), *Elytraria acaulis* (Katewa and Jain 2006; Jain *et al.* 2005) etc.

E. acaulis is one of the plants belonging to Acanthaceae family, commonly known as *nela marri* (Sankaranarayanan *et al.* 2010), *ho-muli* (Kotwal and Srivastava 2013), *kala gathia*, *galobi* (Katewa and Jain 2006), *sahamuria* (Sikarwar *et al.* 2008), *patharchattaa, dasmor* and *shat-muuli* (Khare 2007). *E. acaulis* is traditionally used in treatment of abscess of mammary glands, boils, burns, colic, diarrhoea, rickets, throat compliments and tonsillitis (Jain *et al.* 2005). Leaves of *E. acaulis* are used to cure fever, venereal diseases (Khare 2007), kidney stone and urticaria (Katewa and Jain 2006). Roots of *E. acaulis* are claimed to have therapeutic benefits in treating stomach ache (Katewa and Jain 2006), tooth ache, asthma, expulsion of guinea worms (Katewa and Jain 2006; Jain *et al.* 2005), migraine (Katewa and Jain 2006), leucorrhoea, piles (Sikarwar *et al.* 2008), mammary tumours, pneumonia and infantile diarrhoea. The plant's infusion is prescribed as a remedy for cough (Khare 2007).

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Pyrazole alkaloids like withasomnine and 4'-hydroxywithasomnine have been isolated from *E. acaulis* (Ravikantha *et al.* 2001).

A literature review revealed that antidiarrhoeal activity of *E. acaulis* has not been investigated. The present study was carried out to examine traditional claims for the antidiarrhoeal activity of the plant and to determine acute toxicity test. Several extracts of *E. acaulis* were prepared and their antidiarrhoeal activity was investigated in Wistar rats using castor oil-induced diarrhoea and magnesium sulphate-induced diarrhoea models. Acute toxicity test was performed in albino mice to determine safe dose of the extracts.

METHODS

Plant Material: Collection and Authentication

Whole plants of *E. acaulis* were collected from hills in Shakumbhari Mata in Shekhawati Region of Rajasthan, India. The plant was authenticated by Dr. R. P. Pandey at Botanical Survey of India, Jodhpur, India. A voucher specimen, JNU/PH/2010/E_aE_1, was deposited in the herbarium of Jodhpur National University, Jodhpur, India.

Extraction

Different underground parts, *viz* roots, rhizome, etc., were dried in shade for one month, ground using electric mixer-grinder and screened using British standard sieve (BSS) no. 22 (average aperture size 710 µm). The powdered crude drug (10 g) was extracted in soxhelet extractor with petroleum ether, ethyl acetate, chloroform, ethanol, ethanol (50%) and water, separately, to extract non-polar and polar compounds. The obtained extracts were filtered through Whatman filter paper, concentrated and dried by evaporating the solvent on water bath. The residual moisture in the extract was removed by drying in an oven followed by storage of powdered extracts in a desiccator.

Animals

The antidiarrhoeal studies were conducted on healthy female Wistar rats weighing 150–200 g and albino mice of either sex weighing 25–30 g were used for acute toxicity studies. Approval by the institutional animal ethical committee (registration number 1258/ac/09/CPCSEA) was obtained for conduct of animal experiments. The animals were kept in colony cages at standard husbandry conditions. All animals had free access to feed and water ad libitum.

Preliminary Acute Toxicity Test

E. acaulis extracts were administered orally in doses of 250, 500, 1000, 2000 and 5000 mg/kg body weight to albino mice (one dose per group; five animals in a group). Simultaneously, the control animals received normal saline (5 mL/kg). The general signs and symptoms of toxicity, intake of food and water, and mortality were recorded for a period of 48 hours and then for a period of 14 days as per Organisation For Economic Co-Operation and Development (OECD) guideline 423 (OECD 2001).

Experimental Procedure for Antidiarrhoeal Activity

Healthy Wistar rats were distributed into 7 groups, each group consisting of 5 animals, which received the treatments in following manner:

group I: normal control [1% carboxymethylcellulose (CMC) 10 mL/kg, body weight]
group II: standard drug (loperamide 3 mg/kg body weight)
group III: water extract of *E. acaulis*
group IV: hydroalcoholic extract of *E. acaulis*
group V: alcoholic extract of *E. acaulis*
group VI: chloroform extract of *E. acaulis*
group VII: ethyl acetate extract of *E. acaulis*.

All animals were initially screened for induction of diarrhoea by administering 1 mL of castor oil or 2 g/kg body weight dose of magnesium sulphate. Only animals which developed diarrhoea were selected for antidiarrhoeal studies.

Castor Oil-induced and Magnesium Sulphate-induced Diarrhoea in Rats

Wistar rats weighing 150–200 g were selected and kept for overnight fasting. The potential antidiarrhoeal agents (loperamide, Yashica Pharmaceuticals Ltd., Thane, Maharashtra, India) and test samples (*E. acaulis* extracts, 500 mg/kg body weight) to be tested were administered orally by gavage. For castor oil-induced diarrhoea, 1 mL of castor oil was administered orally to each animal after one hour after administration of drug/extract. For magnesium sulphate-induced diarrhoea, magnesium sulphate was administered at a dose of 2 g/kg orally to each animal, 30 minutes after administration of drug/extract. All animals were placed in cages, where floor was lined with non-wetting paper sheets of uniform weight. Non-wetting paper sheets were changed every hour up to 6 hours. Characteristic diarrhoeal droppings of every hour up to the 6th hour were recorded after draining the urine by gravity. A numerical score based on stool consistency was assigned. Normal stool was assigned as 1, semi-solid stool as 2 and watery stool as 3. Mean of diarrhoeal droppings passed by treatment groups was compared to control group (Akter *et al*. 2009, Shilpi *et al*. 2006, Vogel and Vogel 1998).

Statistical Analysis

The data obtained in the studies was subjected to one way analysis of variance (ANOVA) for comparing different groups with control by Dunnett's 't' test. p-value <0.01 was considered significant and results were expressed as mean ± SD.

RESULTS

Acute toxicity studies for *E. acaulis* extracts were performed for extracts of underground plant parts. Extracts of *E. acaulis* showed neither mortality nor any toxic effect up to the dose of 5 g/kg in 48 hours to 14 days. Behaviour, breathing and cutaneous effects were normal. These results showed that in single dose, there is no acute toxicity of *E. acaulis* extracts. Therefore, the studied extracts are considered to be safe in acute toxicity studies,

since general toxicity dose for rodents is limited up to 2 g/kg/day for rodents and 1 g/kg/day for non-rodents [International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) 2008].

In castor oil-induced diarrhoea model, *E. acaulis* extracts showed antidiarrhoeal effect in Wistar rats. Loperamide, the standard antidiarrhoeal drug, was superior in reducing the number of faeces by 70.94%, while among studied extracts, water extract was found to be most effective, reducing diarrhoeal droppings by 60.68%. The least potent antidiarrhoeal effect was observed in ethyl acetate extract, which reduced diarrhoea by 16.24% (Table 1). All the tested extracts significantly (p<0.01) reduced the wet faeces (stool consistency 3) and total number of faeces, when compared to control group using one way ANOVA followed by Dunnett's 't' test (Table 1).

In magnesium sulphate induced diarrhoea model, the extracts of *E. acaulis* showed antidiarrhoel effect in Wistar rats (Table 2). Water extract showed 62.10% reduction in faeces, which outperformed slightly compared to the standard antidiarrhoeal drug loperamide which had 71.77% reduction (Table 2). The least antidiarrhoeal effect was observed in chloroform extract with faeces reduction of 17.74% (Table 2). All extracts showed significant (p<0.01) antidiarrhoeal effect in reducing the wet faeces (stool consistency 3) and total number of faeces, when compared to control using one way ANOVA followed by Dunnett's 't' test.

In both castor oil-induced diarrhoea model and magnesium sulphate-induced diarrhoea model, the order of antidiarrhoeal effect was water extract > hydroalcoholic extract > alcohol extract > chloroform extract > ethyl acetate extract. The difference in activity of these extracts in reducing diarrhoea may be due to the nature and quantity of phytoconstituents present in these extracts.

Table 1: Effect of *E. acaulis* underground parts extracts on castor oil-induced diarrhoea in Wistar rats.

Treatment	Dose (mg/kg, p.o.)	Mean of wet faeces in 6 hours (n)	Mean of total number of faeces in 6 hours (n)	Feaces reduction (%)
Water	500	5.22± 0.78**	9.2±0.84**	60.68
Hydro-alcoholic	500	8.58±1.13**	10.6±1.34**	54.7
Alcohol	500	8.08±0.73**	10.8±0.84**	53.85
Chloroform	500	10.38±0.88**	14.0±1.87**	40.17
Ethyl Acetate	500	14.98±1.55*	19.6±2.70*	16.24
Loperamide	3	5.62±0.90**	6.8±0.84**	70.94
Control	10#	19.62±1.02	23.4±2.07	–

Notes: **Significant difference at p<0.01 vs. control and p<0.001 vs. control; one-way ANOVA followed by Dunnett's 't' test
*Significant difference at p<0.01 vs control; no significant difference at p<0.001 vs. control; one-way ANOVA followed by Dunnett's 't' test
#In mL/kg

Table 2: Effect of *E. acaulis* underground parts extracts on magnesium sulphate induced diarrhoea in Wistar rats.

Treatment	Dose (mg/kg, p.o.)	Mean of wet faeces in 6 hours (n)	Mean of total number of faeces in 6 hours (n)	Feaces reduction (%)
Water	500	4.62 ± 0.47**	9.4±1.34**	62.1
Hydroalcholic	500	7.64 ±0.84 **	10.4±2.51**	58.06
Alcohol	500	7.96 ±0.55 **	10.2±1.48**	58.87
Chloroform	500	9.84 ±0.65 **	13.2±1.92**	46.77
Ethyl Acetate	500	14.84 ±0.77*	20.4±2.07*	17.74
Loperamide	3	4.46 ±0.51**	7.0±0.71**	71.77
Control	10#	21.18 ±0.94	24.8±1.92	–

Notes: **Significant difference at $p<0.01$ vs. control and $p<0.001$ vs. control; one-way ANOVA followed by Dunnett's 't' test
*Significant difference at $p<0.01$ vs control; no significant difference at $p<0.001$ vs. control; one-way ANOVA followed by Dunnett's 't' test
#In mL/kg

DISCUSSION

Diarrhoea arises due to an imbalance between the absorptive and secretory mechanisms in the intestinal tract resulting in an excessive loss of fluid in the faeces. In some of the cases of diarrhoea the secretory component predominates, while in others diarrhoea is characterised by hypermotility (Choudhary 2012). Peristaltic activity is inhibited and tone is reduced by activation of sympathetic innervations of the intestines. α_2-adrenergic receptor on the parasympathetic terminals may also play role in inhibition of sympathetic nerve resulting in stimulation of gastrointestinal motility by inhibiting release of acetylcholine. Activation of the mucosal α_2-adrenergic receptor also controls the balance of absorption and secretion in the ileum. Stimulation of these α_2-adrenergic receptor in ileum results in a decline of ion fluxes, which is consistent to the of α_2-adrenergic receptor agonist to inhibit intestinal fluid secretion (Kagbo and Eyaru 2011).

Castor oil hydrolysis produces ricinoleic acid, which induces diarrhoea as a hypersecretory response due to changes in the transport of water and electrolytes (Niemegeers *et al.* 1984; Fioramonti *et al.* 1983). Ricinoleic acid causes irritation and inflammation of gastric mucosa resulting in release of prostaglandins causing stimulation of secretion (Galvez *et al.* 1993b; Gaginella *et al.* 1975). Furthermore, ricinoleic acid also sensitizes intramural neurons of the gut. Adenylate cyclase activation, cyclic adenosine mononucleotide phosphate (cAMP) mediated active secretion (Capasso *et al.* 1994) and inhibition of Na+, K+-ATPAase activity (Gaginella and Bass 1978) have been postulated as other mechanisms to explain the diarrhoeal effect of castor oil. Water extract successfully inhibited the castor oil-induced diarrhoea, which may be due to reduction in secretion as it was evident from the reduction of total number of faeces in the test groups.

Diarrhoea in rats is also induced by administration of oral magnesium sulphate, which increases the accumulation of fluid in the intestinal lumen and enhances flow from the proximal to distal intestine. This mechanism also involves release of nitric oxide (NO), probably through stimulation of the constitutive form of NO synthase (Izzo *et al.* 1994).

Magnesium sulphate has also been reported to liberate cholecystokinin from duodenal mucosa resulting in increase of small intestine secretions and motility and thereby preventing the reabsorption of water and sodium chloride (Zavala *et al.* 1998; Galvez *et al.* 1993a). Water extract was effective in reducing diarrhoea when tests were conducted in magnesium sulphate model. This improvement is expected due to increase in water and electrolyte reabsorption from the gastrointestinal tract.

Tannins and flavanoids have been identified in preliminary phytochemical screening and chromatographic profiles of extracts prepared from underground parts of *E. acaulis* (Singh *et al.* forthcoming). Sometimes, phytoconstituents like tannins present in extracts may denature proteins resulting in reduction in intestinal secretion and make it more resistant (Tripathi 2003). Tannins act locally on the gut wall, inhibit intestinal motility and thus possess antisecretory effects (Kumar *et al.* 2010; Tripathi 2003). Tannins, studied in extracts of *Eremomastax speciosa* (Acanthaceae) and *Cylicodiscus gabunensis* (Mimosaceae), stimulate the normalisation of the deranged water transport across the mucosal cells and thus reduce the intestinal transit (Kumar *et al.* 2010). Tannins present in extracts reduce secretion and make the intestinal mucus resistant by forming protein tannate and this mechanism of action has been postulated for antidiarrhoeal action of various plant extracts, *viz. C. gabunensis* (Mimosaceae), *Zizyphys spinachristi* (Rhamnaceae), *Xanthium indicum* (Compositae), *Emmilia cocinea* (Asteraceae), *Sphaeranthus senegalensis* (Asteraceae), *Ficus hispida* (Moraceae), *Cleome viscosa* (Capparidaceae) (Kumar *et al.* 2010).

Flavonoids have been reported to inhibit prostaglandins and autacoids release resulting in reduction of motility and secretion induced by castor oil (Veiga *et al.* 2001). Methanolic extract derived from the stem bark and diet with fruit pulp of *Hymenaea stigonocarpa* displayed antidiarrhoeal effect due to presence of condensed tannins and flavonoids (Rodrigues *et al.* 2012). Therefore, it is proposed that tannins and flavonoids present in extracts of *E. acaulis* are responsible for the antidiarrhoeal action. The mechanism involved seems to be associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport, decreasing Na^+ and K^+ absorption across the intestinal mucosa. The extracts of *E. acaulis*, like loperamide reduced diarrhoea by reducing gastrointestinal motility or by increasing reabsorption of electrolytes and water or by inhibiting induced intestinal accumulation of fluid.

CONCLUSION

Various extracts of *E.* acaulis were found effective in reducing diarrhoea in Wistar rats in both castor oil- and magnesium sulphate-induced diarrhoea models. It shows that *E.* acaulis may contain pharmacologically active substances having antidiarrhoeal properties. Phytoconstituent differences among these extracts may be responsible for their differences in antidiarrhoeal potencies. Further studies may be directed to investigate the actual phytoconstitutents responsible for antidiarrhoeal activity of these extracts.

ACKNOWLEDGEMENT

The authors express their thanks to Dr. R. P. Pandey from Botanical Survey of India, Jodhpur, India, for authentication of plant material.

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