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 Senticorus anacardium, Achyranthes 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-muli (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).
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, 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).

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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 antidiarrhoal 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 antidiarrhoal 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 antidiarrheal effect in Wistar rats. Loperamide, the standard antidiarrheal 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 antidiarrheal 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 antidiarrhoeal effect in Wistar rats (Table 2). Water extract showed 62.10% reduction in faeces, which outperformed slightly compared to the standard antidiarrheal 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.

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

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.

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

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 gastrointestinal 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. α2-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 α2-adrenergic receptor also controls the balance of absorption and secretion in the ileum. Stimulation of these α2-adrenergic receptor in ileum results in a decline of ion fluxes, which is consistent to the α2-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 (Niemeggeers 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+-ATPase 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. 1998a). 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 flavonoids 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 anti-diarrhoeal action of various plant extracts, *viz.* *C. gabunensis* (Mimosaceae), *Zizyphys spinacristi* (Rhamnaceae), *Xanthium indicum* (Compositae), *Emmilia cocinea* (Asteraceae), *Sphaeranthus senegalensis* (Asteraceae), *Ficus hispida* (Moraceae), *Clome 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 anti-diarrhoeal 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 anti-diarrhoeal 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 anti-diarrhoeal properties. Phytoconstituent differences among these extracts may be responsible for their differences in anti-diarrhoeal potencies. Further studies may be directed to investigate the actual phytoconstituents responsible for anti-diarrhoeal activity of these extracts.

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