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Antidiarrhoeal and spasmylytic activities of the methanolic crude extract of Alstonia scholaris L. are mediated through calcium channel blockade.

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This study was aimed to provide a pharmacological basis to the medicinal use of *Alstonia scholaris* as an antidiarrhoeal and antispasmodic by using *in vivo* and *in vitro* techniques. In the *in vivo* study the crude extract of *Alstonia scholaris* (As.Cr), which tested positive for the presence of alkaloids, provided 31–84% protection against castor oil-induced diarrhoea in mice at 100–1000 mg/kg doses, similar to loperamide. In isolated rabbit jejunum preparation, the As.Cr caused inhibition of spontaneous and high K\(^+\) (80 mM)-induced contractions, with respective EC\(_{50}\) values of 1.04 (0.73–1.48) and 1.02 mg/mL (0.56–1.84; 95% CI), thus showing spasmylic activity mediated possibly through calcium channel blockade (CCB). The CCB activity was further confirmed when pretreatment of the tissue with the As.Cr (0.3–1 mg/mL) caused a rightward shift in the Ca\(^{2+}\) concentration-response curves similar to verapamil, a standard calcium channel blocker. Loperamide also inhibited spontaneous and high K\(^+\) preconstructions as well as shifted the Ca\(^{2+}\) CRCs to the right. These results indicate that the crude extract of *Alstonia scholaris* possesses antidiarrhoeal and spasmylic effects, mediated possibly through the presence of CCB-like constituent(s) and this study provides a mechanistic base for its medicinal use in diarrhoea and colic. Copyright © 2009 John Wiley & Sons, Ltd.

**Keywords:** *Alstonia scholaris*; antidiarrhoeal; rabbit jejunum; antispasmodic; calcium channel antagonist.

### INTRODUCTION

*Alstonia scholaris* (L.) also known as *Echites scholaris* (devil’s tree) belongs to the family Apocynaceae and is found throughout tropical Eastern Asia and the Malayan Archipelago. The plant has been used in the traditional system of medicine for various gastrointestinal disorders such as diarrhoea, colic, dysentery, ulcers, dyspepsia and liver disorders (Wiert, 2002). Phytochemical studies of *Alstonia scholaris* shows that it contains mainly alkaloids (Chatterjee *et al.*, 1965) isolated from its leaves such as scholarisine A (Cai *et al.*, 2008), monoterpenoid indole alkaloids (19,20)-E-\(\alpha\)-alstoscholarine (1) and (19,20)-Z-\(\alpha\)-alstoscholarine (2) (Cai *et al.*, 2007), manilamine (1) (Macaboe *et al.*, 2005), akuammginone (1) (Salim *et al.*, 2005), ditamine and echitamine (echitenine) along with oxalates of calcium, crystallizable acid and several fatty resinous substances (Nadkarni, 1976). The plant is reported to possess neuropharmacological (Bhattacharya *et al.*, 1979), anticancer (Jagetia and Baliga, 2006), bronchodilatory (Channa *et al.*, 2005), antifertility (Khan *et al.*, 2003) and hepatoprotective (Lin *et al.*, 1996) activities. This investigation was aimed at providing pharmacological basis for some of the medicinal use of *Alstonia scholaris* in gastrointestinal disorders, such as diarrhoea and gut spasms.

### MATERIALS AND METHODS

**Plant materials and extraction.** Aerial parts of the *Alstonia scholaris* (2.5 kg) were collected from the Aga Khan University campus Karachi, and authenticated by Dr Rubina Abid, Department of Botany, University of Karachi, Pakistan. A specimen voucher (AS-AP-05-02-51) was deposited at the herbarium located at the Department of Biological and Biomedical Sciences, Aga Khan University, Karachi. The plant material was cleaned, shade dried and soaked in 70% aqueous-methanol at room temperature (23–25 °C) for 3 days thrice. The extract was filtered after each 3 days through a muslin cloth and finally through filter paper (Whatman qualitative grade 1). The combined filtrate was then concentrated in a rotary evaporator (35–40 °C), under reduced pressure (−760 mmHg) to a thick, dark brown coloured crude extract (As.Cr) with a yield of 2% (40 g) and stored at −4 °C for future use. The crude extract was dissolved in normal saline and distilled water for

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in vivo and in vitro experiments, respectively, and dilutions were made fresh on the day of experiment.

**Preliminary phytochemical analysis.** *Alstonia scholaris* crude extract was screened for the presence of saponins, flavonoids, flavanols, flavones, tannins, phenols, coumarins, sterols, terpenes, alkaloids and anthraquinones by using methods described by Wall et al. (1952).

**Drugs and standards.** The following reference chemicals were obtained from the sources specified: loperamide hydrochloride, acetylcholine chloride, verapamil hydrochloride, potassium chloride (Sigma Chemical Company, St Louis, MO, USA) and castor oil (Karachi Chemical Industries, Karachi, Pakistan). All chemicals used were of the highest purity grade. Stock solutions of all the chemicals were made in distilled water and the dilutions of the highest purity grade. Stock solutions of all the chemicals were made fresh on the day of experiment.

**Animals.** The experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC, 1996) and approved by the Ethical Committee of Aga Khan University Karachi. Balb c mice (20–25 g) and local rabbits (1.5–2 kg) of either sex used in the study were bred and housed in the animal house of the Aga Khan University under controlled environment (23–25 °C). Animals were given tap water ad libitum and a standard diet consisting of (g/kg): flour 380, chokar 380, molasses 12, salt 5.8, nutrivet L 2.5, potassium meta bisulphate 1.2, vegetable oil 38, fish meal 170 and powdered milk 150.

**Castor oil-induced diarrhoea.** The in vivo antidiarrhoal activity of the extract was conducted following the methods described previously (Awouters et al., 1978). In the present study Balb c albino mice were fasted for 18 h. The animals were divided in five groups, housed in five steel cages, five in each, and the bottom of each cage was covered with a blotting sheet. The first group received saline as the vehicle control (10 mL/kg, p.o.) and so acted as the negative control. The doses of the crude extract of *Alstonia scholaris* used were selected on a trial basis and were administered orally (100, 300 and 1000 mg/kg) by an intragastric feeding needle as a suspension to three groups of animals. The fifth group received loperamide (10 mg/kg) as a suspension, for comparison and served as a positive control. One hour after treatment each animal received 10 mL/kg of castor oil orally and was then observed for defeacation. Up to 6 h after the castor oil challenge, the presence of characteristic diarrhoeal droppings was noted on the blotting sheets in the individual mouse cages. The percent protection against the castor oil-induced diarrhoea was calculated based on the number of dry faeces in each cage in comparison with the wet.

**Isolated rabbit jejunum tissue preparations.** The isolated tissue experiments were carried out as previously described (Gilani et al., 2005). The animals had free access to water but were fasted for 24 h before the experiment. The animals were killed by cervical dislocation, the abdomen was cut open and the jejunal portion isolated. Preparations 2 cm long were mounted in 10 mL tissue baths containing Tyrode’s solution maintained at 37 °C and aerated with a mixture of 5% carbon dioxide in oxygen (carbogen). The composition of Tyrode’s solution in mM, was: NaCl 136.9, KCl 2.7, MgCl 1.1, NaHCO 3 11.9, NaHPO 4 0.4, CaCl 2 1.8 and glucose 5.6 (pH 7.4). A preload of 1 g was applied and the tissues kept undisturbed for an equilibrium period of 30 min after which control responses to a sub-maximal dose of acetylcholine (0.3 µM) were obtained and the tissue presumed stable only after the reproducibility of the said responses.

Under these experimental conditions, the rabbit jejunum exhibits spontaneous rhythmic contractions, allowing the relaxant (spasmolytic) activity to be tested directly without the use of an agonist (Gilani et al., 1994).

**Determination of calcium antagonist activity.** To assess whether the spasmolytic activity of the test substances, was through calcium channel blockade, K+, as KCl was used to depolarize the preparations (Farre et al., 1991). K+ (80 mM) was added to the tissue bath, which produced sustained contraction. Test materials were then added in a cumulative fashion to obtain concentration-dependent inhibitory responses (Van-Rossum, 1963). The relaxation of intestinal preparations, preconstricted with K+ (80 mM), was expressed as the percent of the control response mediated by K+.

To confirm the calcium antagonist activity of test substances, the tissue was allowed to stabilize in normal Tyrode’s solution, which was then replaced with Ca²⁺-free Tyrode’s solution containing EDTA (0.1 mM) for 30 min in order to remove calcium from the tissues. This solution was further replaced with K⁺-rich and Ca²⁺-free Tyrode’s solution, having the following composition: NaCl 91.04, KCl 50, MgCl 2 1.05, NaHCO 3 11.90, NaHPO 4 0.42, glucose 5.55 and EDTA 0.1 mM. Following an incubation period of 30 min, control concentration-response curves (CRCs) of Ca²⁺ were obtained. When the control CRCs of Ca²⁺ were found to be superimposable (usually after two cycles), the tissue was pretreated with the plant extract for 60 min to test the possible calcium channel blocking effect. The CRCs of Ca²⁺ were reconstructed in the presence of different concentrations of the test material.

**Statistics.** The data expressed are mean ± standard error of the mean (SEM), and the median effective concentrations (EC₅₀ values) are given with 95% confidence intervals (CI). The statistical parameter applied is the Student’s t-test with p < 0.05 noted as significantly different (GraphPad Prism).

**RESULTS AND DISCUSSION**

Based on the medicinal use of *Alstonia scholaris* in hyperactive gut disorders such as diarrhoea and spasm (Wiatr, 2002), its crude extract was tested for a possible antidiarrhoal effect in mice. When tested against the castor oil-induced diarrhoea, As.Cr like the standard antidiarrhoal agent, loperamide, inhibited significantly (p < 0.05) the frequency of defeacation when compared with untreated mice (i.e. mice receiving neither As.Cr, nor loperamide, but castor oil only). Both substances also reduced greatly the wetness of the faecal droppings and provided around 31–84% and 94% protection,
respectively (Table 1). The induction of diarrhoea by castor oil results from the action of ricinoleic acid formed in the hydrolysis of the oil (Iwao and Terada, 1962), which produces changes in the transport of water and electrolytes resulting in a hypersecretory response and generation of giant contraction of the transverse and distal colon (Croci et al., 1997). It was studied further in the in vitro model to see if the plant extract inhibited gut motility.

Spontaneously beating isolated rabbit jejunum preparation is used routinely to test the possible inhibitory (spasmolytic) effect of test substances without use of spasmoden (Gilani et al., 1994). When tested in isolated rabbit jejunum preparations, cumulative addition of the As.Cr, loperamide and verapamil caused concentration-dependent inhibition of the spontaneous contractions (Fig. 1), with a respective EC50 value of 1.04 mg/mL (0.734–1.484), 37.16 µM (190.09–72.36) and 0.10 µM (0.06–0.15) (Fig. 2), thus showing intestinal smooth muscle relaxant (spasmolytic) activity. The contraction of smooth muscle preparations, including rabbit jejunum, is dependent upon an increase in the cytoplasmic free [Ca++], which activates the contractile elements (Karaki and Wiess, 1983). The increase in intracellular Ca++ occurs either via an influx through voltage-dependent Ca++ channels (VDCs) or its release from intracellular stores in the sarcoplasmic reticulum. Periodic depolarization and repolarization regulates the spontaneous movements of the intestine and at the height of depolarization, the action potential appears as a rapid influx of Ca++ via VDCs (Brading, 1981). Thus, the inhibitory effect of the As.Cr on spontaneous movements of rabbit jejunum may appear be due to interference either with the Ca++ release or with the Ca++ influx through VDCs.

It was observed previously that the spasmyloytic constituents present in different medicinal plants mediate their effect usually through a CCB effect (Gilani et al., 1999, 2005, 2006). To see whether the spasmyloytic effect of the plant extract observed in this study is also mediated through CCB, a high concentration of K+ (80 mM) was introduced to depolarize the tissue. The crude extract was then added in a cumulative fashion, where it caused a dose-dependent relaxation of the induced contractions with an EC50 value of 1.02 mg/mL (0.56–1.84) as shown in Fig. 2A, suggesting that the spasmyloytic effect is possibly mediated through CCB. Similarly, verapamil and loperamide also caused a dose-related inhibitory effect against high K+-induced contractions. When the inhibitory effect against K+ was compared with that on spontaneous contractions, the crude extract showed a similar potency, as was shown by loperamide, while verapamil was more potent against K+-induced contractions, a typical characteristic of CCB (Triggle, 1992). Loperamide is also known to exhibit the CCB effect (Reynolds et al., 1984), in addition to its spasmyloytic effect through opioid receptors (Croci et al., 1997). It is possible that the plant extract also possesses some relaxing component in addition to its CCB effect.

The contractions induced by high K+ (>30 mM) are dependent on the entry of Ca++ into the cells through voltage-dependent calcium channels (VDCs) (Bolton, 1979) and a substance which can inhibit high K+-induced contractions is therefore considered to be a CCB (Godfraind et al., 1986). Thus, the inhibition of high K+ (80 mM)-induced contractions of rabbit jejunum by As.Cr may reflect the restricted Ca++ influx by VDCs. This hypothesis was further strengthened when pretreatment of the tissues with As.Cr (0.3–1 mg/mL) caused a rightward shift in the Ca++ CRCs (Fig. 2C), similar

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Total number of faeces in 4 h</th>
<th>Total number of wet faeces in 4 h</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>10 mL/kg</td>
<td>14.80 ± 1.10</td>
<td>0.20 ± 0.31</td>
<td>98.46 ± 0.88</td>
</tr>
<tr>
<td>Castor oil</td>
<td>10 mL/kg</td>
<td>11.80 ± 0.80</td>
<td>11.6 ± 0.77</td>
<td>1.33 ± 0.82</td>
</tr>
<tr>
<td>+ As.Cr</td>
<td>100 mg/kg</td>
<td>19.80 ± 1.03</td>
<td>14.20 ± 0.96</td>
<td>31.73 ± 1.02*</td>
</tr>
<tr>
<td>+ As.Cr</td>
<td>300 mg/kg</td>
<td>20.80 ± 1.14</td>
<td>9.20 ± 0.58</td>
<td>52.85 ± 1.88*</td>
</tr>
<tr>
<td>+ As.Cr</td>
<td>1000 mg/kg</td>
<td>13.80 ± 1.15</td>
<td>2.40 ± 0.87</td>
<td>84.38 ± 1.93*</td>
</tr>
<tr>
<td>+ Loperamide</td>
<td>10 mg/kg</td>
<td>8.80 ± 1.18</td>
<td>0.4 ± 0.34</td>
<td>94.82 ± 1.39</td>
</tr>
</tbody>
</table>

*p values were calculated vs control (saline treated).*

* p < 0.05, ** p < 0.01, *** p < 0.001.

As.Cr, crude extract of Alstonia scholaris.

Table 1. Effect of the crude extract of Alstonia scholaris on castor oil-induced diarrhoea in mice

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GASTROINTESTINAL EFFECTS OF *ALSTONIA SCHOLARIS* L.

Figure 2. Concentration-response curves of (A) the crude extract of *Alstonia scholaris* (As.Cr), (B) loperamide and (C) verapamil on spontaneous and K⁺ (80 mM)-induced contractions. Figures (D), (E) and (F) show the inhibitory effect of As.Cr, loperamide and verapamil, respectively, on Ca²⁺ concentration response curves in isolated rabbit jejunum preparations. Values shown are mean ± SEM (n = 3–5).

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This study thus showed that the crude extract of *Alstonia scholaris* possesses a spasmolytic effect mediated through a CCB-like effect, which may provide a pharmacological base to its medicinal use in diarrhoea and spasms.

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