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Assessment of the laxative activity of an ethanolic extract of *Bambusa arundinacea* (Retz.) Willd. shoot

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ABSTRACT

Ethnobotanical relevance: *Bambusa arundinacea* (Retz.) Willd., commonly known as Kanta Bans, plays an important ethnobotanical role, especially in Asia. In traditional medicine it has reportedly been used for the treatment of constipation, blood-diseases, leucodema, inflammation and urinary discharges. A number of ethnomedicinal records exist regarding the use of *B. arundinaceae* shoots as a laxative, however, there are no scientific studies reported on its laxative activity. Therefore, the aim of this present study was to evaluate the laxative activity of an ethanolic *B. arundinaceae* shoot extract in mice.

Materials and methods: *B. arundinacea* shoots were collected from Dhaka, Bangladesh in July 2015. An ethanolic shoot extract was obtained and its laxative activity was evaluated by faecal consistency, gastrointestinal transit and entero-pooling assays in a mouse model. Furthermore, a phytochemical investigation of the extract was conducted by UHPLC-ESI-QqQ MS and UHPLC-ESI-Orbitrap MS analysis.

Results: The ethanolic shoot extract of *B. arundinacea* showed significant laxative activity in our mouse model, with significant increases in (i) the amount of wet faeces, with the maximum effect at 2 h for 500 mg/kg (47.92%), (ii) gastrointestinal transit (67.18% and 60.03% for doses of 250 and 500 mg/kg, respectively), and (iii) small intestine content at the test doses of 250 and 500 mg/kg p.o. Phytochemical investigation identified a total of thirty compounds in the ethanolic shoot extract of *B. arundinacea* using UHPLC-ESI-QqQ MS and UHPLC-ESI-Orbitrap MS analysis.

Conclusions: The results of this study provide support for the traditional use of *B. arundinacea* shoot as a laxative.

1. Introduction

Constipation is a functional bowel disorder that causes discomfort in daily life, affecting about 14% of the adult population worldwide (Cirillo and Capasso, 2015; Longstreth et al., 2006; Saito et al., 2002). The use of botanicals such as senna, cascara, frangula, aloe, and rhubarb have long been utilised in the management of constipation (Cirillo and Capasso, 2015).

Bambusa arundinacea (Retz.) Willd., belonging to the family Poaceae, is a thorny plant native to Asia, where it predominantly occurs throughout India, Bangladesh, China, Sri Lanka, Myanmar and Malaya (Ghani, 2003; Ohrnberger, 1999). Since the era between 12000 and 2000 BCE, the plant has played an important role in human civilization as a structural tool and in the folk medicine in numerous tribes and locations (Inc, 2014).

The stems and leaves of the plant have been used in folk medicines to treat different inflammatory conditions (Yusuf et al., 2009). In Ayurveda the leaves, stems and roots are used as an astringent, diuretic and laxative (Nadkarni, 2000). The juice of *B. arundinacea* leaf has been used as a drink to strengthen the cartilage in sufferers of osteoarthritis and osteoporosis. It reportedly increases the integrity of the bones, arterial walls, skin, teeth, the gum, hair and nails, along with alleviating eczema and psoriasis (Vanithakumari et al., 1989). In the treatment of dysmenorrhoea and amenorrhea a decoction of leaves of *B. arundinacea* has been used to stimulate menstruation and reportedly reduces menstrual pain (Kirtikar and Basu, 1999). The shoots and spouts of *B. arundinacea* have been used in traditional medicinal systems of Bangladesh for the treatment of constipation and urinary discharges (Ghani,

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2003; Yusuf et al., 2009). The plant is reported to possess antibacterial, antifungal, anti-hyperglycemic, antifertility, anti-inflammatory and antiulcer activities (Kumar et al., 2012; Muniappan and Sundararaj, 2003; Thamizharasan et al., 2015; Vanithakumari et al., 1989; Zubair et al., 2013). Phytochemical studies have indicated that the plant contains 3′,3,6,7-tetramethoxy-4′,5,8-trihydroxy flavones; 4-methoxy benzoic acid; 4′-hydroxy flavan-3-ol and hemicelluloses (Guha and Pant, 1967; Kumar et al., 2016). However, there are no reports to-date regarding laxative activity in an animal model. Therefore, this study was carried out to evaluate the laxative activity of the ethanolic extract of *B. arundinacea* shoot in mice.

2. Materials and methods

2.1. Plant material and extraction

Shoots of *Bambusa arundinacea* (Retz.) Willd. were collected July 2015 from Dhaka, Bangladesh and identified by taxonomists at the Bangladesh National Herbarium, where a voucher specimen (DACB 43377) has been deposited for future reference. The air-dried shoots were powdered and extracted by maceration with ethanol at room temperature for 72 h. The crude extract was obtained after filtration and evaporation of the solvent using a rotary evaporator, followed by freeze drying (yield 1.83% w/w).

2.2. Animals

Adult Swiss-albino mice of 4–5 weeks age (20–25 g) were obtained from the Animal Resources Branch of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICCDR,B). The mice were kept in polypropylene cages under pathogen free conditions at an ambient temperature of 24 ± 1 °C; 12 h light/dark cycle and 55 ± 5% controlled relative humidity. The animals were fed with a standard pellet diet and water ad libitum. The experimental protocol followed the guidelines and regulations of the Animal Ethics Committee, Khulna University, Bangladesh (Reference no.: KU/PHARM/AEC/15/006/017).

2.3. Acute toxicity test

An acute toxicity study of the ethanolic shoot extract of *B. arundinacea* was conducted following a standard protocol (Lorke, 1983) as described in the Supplementary materials.

2.4. Grouping and extract dosing

Animals of either sex were randomly divided into 4 groups (n = 5 mice). The negative control group received vehicle (1% Tween 80 in water) orally at a volume of 10 mL/kg b.w., while the positive control group received castor oil (0.3 mL/animal, p.o.). The other two groups (test groups, 2 × 5 mice) were treated orally with the *B. arundinacea* extracts (dissolved in 1% Tween 80 in water), one at a dose of 250 mg/kg b.w. and the other at a dose of 500 mg/kg b.w., respectively.

2.5. Effect of B. arundinacea shoot ethanol extract on faecal consistency

Faecal consistency activity of the ethanol shoot extract was measured utilising a standard method (Méité et al., 2010), with some modification, as described in the Supplementary materials.

2.6. Effect of B. arundinacea ethanol shoot extract on intestinal transit

Effect of *B. arundinacea* shoot extract on intestinal transit was measured as per an established method (Méité et al., 2010; Vogel et al., 2002), with minor modification, as described in the Supplementary materials.

2.7. Effect of B. arundinacea ethanol shoot extract on entero-pooling

Entero-pooling activity of the *B. arundinacea* shoot ethanol extract was determined according to a standard protocol (Robert et al., 1976; Samuel et al., 2015), with minor modification, as described in the Supplementary materials.

2.8. Phytochemical investigation of B. arundinacea shoot extract

The extract was subjected to phytochemical investigation using ultrahigh-pressure liquid chromatography electrospray ionisation triple-quadrupole mass spectrometry (UHPLC-ESI-QqQ MS) and ultrahigh-pressure liquid chromatography electrospray ionisation Orbitrap mass spectrometry (UHPLC-ESI-Orbitrap MS) analysis as described in the Supplementary materials.

2.9. Statistical analysis

All data exhibited normal distribution and the results were expressed as mean ± SEM. We performed unpaired student's *t*-test for statistical analysis using GraphPad Prism 5.0, USA software and results were considered significant when $p < 0.05$ compared to the control. We also then performed one-way ANOVA for analysis of variance of each experiment (reported in the Supplementary material as Table S1) to identify where these effects lie. ANOVA analysis was performed using InVivoStat 3.6, UK software.

3. Results

3.1. Acute toxicity study of B. arundinacea ethanol shoot extract

No mortality or symptoms of toxicity were observed in mice for the *B. arundinacea* shoot ethanol extracts under investigation even at the highest dose (0.5 g/kg) tested.

3.2. Effect of B. arundinacea shoot ethanol extract on faecal consistency

The results of the faecal consistency assay are reported in Fig. 1. In this study, the *B. arundinacea* ethanol shoot extract showed a dose dependent increase in % wet faeces of mice (variation in the degrees of 'wet faeces' were seen) with results being statistically significant as compared to the negative control (see Fig. 1). However, the effect of the shoot extract was not as strong as the castor oil (positive control) (% wet faeces was > 50% and 50–20% up to 4 h, for castor oil and test extracts, respectively).

3.3. Effect of B. arundinacea ethanol shoot extract on intestinal transit

Apart from the increase in watery feaces, the ethanol shoot extract of *B. arundinacea* significantly increased the propulsion of $BaSO_4$ through the gastrointestinal tract in a dose-dependent manner

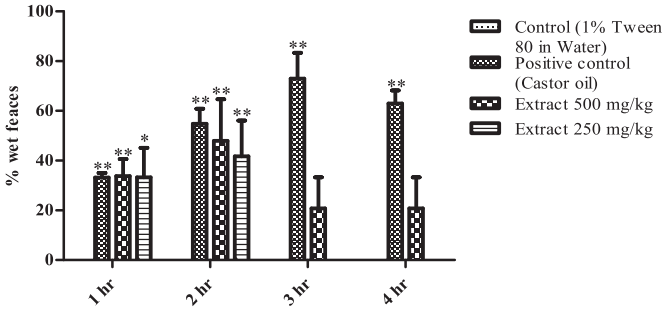

Fig. 1. Effect of *B. arundinacea* shoot ethanol extract on faecal consistency in mice. Results are expressed as mean ± SEM. $^{*}p < 0.05$ vs. control, $^{**}p < 0.001$ vs. control.