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Is aristolochic acid nephropathy a widespread problem in developing countries? ☆
A case study of *Aristolochia indica* L. in Bangladesh using an ethnobotanical–phytochemical approach

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ABSTRACT

Ethnopharmacological relevance: Species of *Aristolochia* are associated with aristolochic acid nephropathy (AAN), a renal interstitial fibrosis and upper urinary tract cancer (UUC). Aristolochic acid nephropathy has been reported in ten countries but its true incidence is unknown and most likely underestimated. By combining an ethnobotanical and phytochemical approach we provide evidence for the risk of AAN occurring in Bangladesh. More specifically, we assess the intra-specific variation of aristolochic acid analogues in medicinally used *Aristolochia indica* samples from Bangladesh.

Materials and methods: Ethnobotanical information was collected from 16 *kavirajes* (traditional healers) in different study locations in Bangladesh. Plant samples were obtained from native habitats, botanical gardens, herbal markets and pharmaceutical companies. The samples were extracted using 70% methanol and were analysed using LC-DAD-MS and ^{1}H-NMR.

Results: Roots as well as leaves are commonly used for symptoms such as snake bites and sexual problems. Among the informants knowledge about toxicity or side effects is very limited and *Aristolochia indica* is often administered in very high doses. Replacement of *Aristolochia indica* with other medicinal plants such as *Rauvolfia serpentina* (L.) Benth. ex Kurz was common. *Aristolochia indica* samples contained a variety of aristolochic acid analogues such as aristolochic acid I, aristolochic acid II, cepharadione A and related compounds.

Conclusions: AAN cases are likely to occur in Bangladesh and more awareness needs to be raised about the health risks associated with the use of *Aristolochia indica* and other species of *Aristolochia* as herbal medicines.

Abbreviations: A, alstonine; AA, aristolochic acid; AAA, aristolochic acid analogue; AAN, aristolochic acid nephropathy; AL, aristolactam; BA, benzoic acid; BEN, Balkan endemic nephropathy; COSY, correlation spectroscopy; CP, commercial product; D_2O, deuterated water; EBC, economic botany collection; ESI, electrospray ionisation; LC-DAD-MS, liquid chromatography-diode array detector-mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; GNP, gross national product; HMBC, heteronuclear multiple-bond correlation spectroscopy; HMQC, heteronuclear multiple-quantum correlation spectroscopy; K_2HPO_4, di-potassium hydrogen orthophosphate; KH_2PO_4, potassium dihydrogen orthophosphate; J-RES, *J*-resolved; MeOD, deuterated methanol; MS, market sample; *m/z*, mass-to-charge-ratio; NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser effect spectroscopy; PC, principle component; PCA, principle component analysis; PPM, parts per million; PTFE, polyfluoroethylene; RBG, Royal Botanic Gardens, Kew; RPM, revolutions per minute; S, serpentine; t_R, retention time; TSP, 3-(tetramethylsilyl)propionic-2,2,3,3-*d*4 acid, sodium salt; UUC, upper urinary tract cancer; UV, ultraviolet

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1. Introduction

Species of Aristolochia are known to cause aristolochic acid nephropathy (AAN), a renal interstitial fibrosis, which is associated with a high incidence of upper urinary tract cancer (UUC) (Nortier et al., 2000). It was initially reported in a Belgian cohort of over 100 patients after the intake of slimming pills containing a Chinese herb, *Aristolochia fangchi* Wu ex L.D. Chow & S.M. Hwang (Vanherweghem, 1998). Following this tragic incidence, species of *Aristolochia* were banned in many countries, including Germany, the UK, USA, Australia and Canada. However, drugs and medical preparations from species of *Aristolochia* are used widely (and often legally) in many countries and can be bought via the Internet (Gold and Slone, 2003; Schaneberg and Khan, 2004; Heinrich et al., 2009). So far AAN has only been reported in 15 countries but its true incidence is unknown and probably underestimated (National Toxicology Program, 2008).

Evidence for AAN being a major public health problem has only been provided for two small regions worldwide, specifically the Balkan region and Taiwan. In the Balkan region, the dietary intake of flour contaminated with *Aristolochia clematitis* L. seeds is proposed as the environmental causal factor for Balkan Endemic Nephropathy (BEN) (Grollman et al., 2007; Jelakovic et al., 2012). This disease has affected thousands of patients in the Danube basin and its clinical expressions and pathological lesions are strikingly similar to AAN (De Broe, 2012). Furthermore, recent research has shown that exposure to species of *Aristolochia* used in traditional Chinese medicines contributes significantly to the incidence of upper urothelial cancer (UUC) in Taiwan (Chen et al., 2012). However, it is likely that AAN and UUC are also prevalent in other countries where species of *Aristolochia* are used (Heinrich et al., 2009), creating a global public health problem of considerable but largely unknown magnitude (Debelle et al., 2008; Grollman et al., 2009; Grollman, 2013).

Ethnobotanical studies indicate that the Indian subcontinent is one of the hot spots for *Aristolochia* use (Heinrich et al., 2009). *Aristolochia indica* L. was found to be the most frequently cited species in the literature. A possible association between chronic interstitial fibrosis in Indians and the intake of *Aristolochia* has been proposed before (Vanherweghem, 1997; Debelle et al., 2008; Grollman et al., 2009). Therefore, it is likely that the medicinal use of *Aristolochia indica* results in a large number of undiagnosed AAN and UUC cases on the Indian subcontinent.

This work focuses specifically on the uses of *Aristolochia indica* in Bangladesh and their implications on public health. Bangladesh was chosen as a case study since *Aristolochia indica,* the most frequently cited Aristolochia species is native there. However, only fewer ethnobotanical reports on *Aristolochia indica* uses exist in comparison to India. While four species of *Aristolochia* are found in Bangladesh (*Aristolochia elegans* Mast., *Aristolochia indica* L., *Aristolochia saccata* Wall. and *Aristolochia acuminata* Lam., Ahmed et al., 2007), *Aristolochia indica* is the most widely distributed species and has the greatest importance as a medicinal plant (Heinrich et al., 2009; Mollik et al., 2010).

In Bangladesh only 1.5% of the gross national product (GNP) is spent on health care (Rahman, 2005). Non-communicable diseases including kidney disease are not priorities and patient care is deficient. The number of nephrologists in Bangladesh is limited and renal disease care is only available in six public hospitals and 10 private hospitals (Rashid, 2004). An overburdened health-care system with few renal facilities means that recording cases is not a priority. Since there are few statistics regarding kidney disease (Jha, 2009) it is not surprising that no records of AAN cases in Bangladesh exist. In addition to limited data recording there is a lack of knowledge regarding the condition and its causes. Furthermore, the medical care offered is rarely integrated with herbal medicine and it is unlikely that the connection between the use of *Aristolochia* as a medicine and kidney disease would to be made.

The use of herbal medicine in Bangladesh is widespread with estimates of up to 75% of the population using complementary and alternative medicines to manage their health needs (Ghani and Pasha, 2004). In addition to allopathic medicine a range of medical practices exist (and overlap) in Bangladesh including Ayurvedic, Unani, homoeopathy, popular and spiritual medicines. *Kavirajes* are 'folk' or 'traditional' healers that use plant preparations to treat various ailments (Rahman et al., 2010). The practices of *kavirajes* are varied as they draw on Ayurvedic, Unami and spiritual models of healing (either in combination or exclusively). They are frequently found across Bangladesh catering to the primary health care needs of a large proportion of the population (Mollik et al., 2010).

The aim of this study was to assess the risk of AAN occurring in Bangladesh by combining an ethnobotanical and phytochemical approach. More specifically, we assessed the importance of *Aristolochia indica*, its medicinal uses and the knowledge about the health risks associated with its use by carrying out interviews with healers (*kavirajes*) in different study locations in Bangladesh. Furthermore, we studied the intraspecific phytochemical variation of *Aristolochia indica* samples obtained in Bangladesh using metabolomic methods based on LC-DAD-MS and ^{1}H -NMR (Michl et al., 2011). We especially focused on the content of aristolochic acid analogues (AAAs), the group of compounds known to be responsible for the nephrotoxic and carcinogenic effects of *Aristolochia* species (National Toxicology Program, 2011; Kumar et al., 2003).

2. Materials and methods

2.1. Ethnobotanical survey

Prior to the start of the fieldwork ethical approval was obtained from the School of Pharmacy's (University of London, UK) Ethics Committee (January 2012).

Ethnobotanical information was collected during two field studies in February 2012 and August 2012. Semi-structured interviews were carried out with 16 healers (*kavirajes*) in different study locations in Bangladesh (Rajshahi, Dhaka, Natore, Tangail, Bandarban and Sylhet, Fig. 1) in order to obtain data representative for the whole of Bangladesh. The interviews were carried out in Bengali with the help of a translator and generally took place in public spaces such as markets. Informant consent was obtained prior to the interviews. However, the aims of the study relating to the toxicity of *Aristolochia indica* could not be fully disclosed prior to the interview. Informants were asked to list medicinal uses of *Aristolochia indica* and were then asked to provide more detailed information about these uses. The questions covered in the interviews included vernacular names of the species, the parts used (leaves, roots, stems, fruits, seeds), the mode of preparation and administration, dose, duration of treatment and knowledge about toxicity or side effects. All informants were male, however they belonged to different ethnic groups: Bengali (10), Marma (1), Santal (2), Tripura (1), Mandai (1) and Bongshi (1). All informants were asked whether they would be willing to provide a sample of *Aristolochia indica* or to identify the plant in photographs if plant material was not available.

2.2. Plant material and herbal formulations

Aristolochia indica samples were collected either in their natural habitat or were obtained from botanical gardens. Voucher specimens of samples 3 (J. Michl BI-21582) and 4 (J.Michl