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Toxic pyrrolizidine alkaloids provide a warning sign to overuse of the ethnomedicine *Arnebia benthamii*

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ABSTRACT

Ethnopharmacological relevance: From early times man has used medicinal plants for the treatment of various ailments and basic health care needs. The use of herbal medicines has increased day by day and with this, so do reports of adverse events, poisoning, and suspected toxicity. Similarly, the indigenous communities of Neelum Valley in Azad Kashmir commonly use *Arnebia benthamii* (Wall. ex G.Don) I.M.Johnst. for medicinal purposes to treat various human aliments. Besides their medicinal uses, it also contains hepatotoxic pyrrolizidine alkaloids (PAs).

Aim of the study: This explorative study underscores two major aspects about this herbal medicine. Firstly we aimed to document the traditional therapeutic uses of *Arnebia benthamii* in Neelum Valley, Azad Kashmir. Secondly, to determine the presence or absence of hepatotoxic pyrrolizidine alkaloids and if they are within the suggested limit for the use of herbs in excess.

Materials and methods: Interviews, group discussions, and inquiries were carried out from July to September 2016 with local indigenous and elder people. In the laboratory, the plant was investigated for pyrrolizidine alkaloids by using high performance liquid chromatography (HPLC).

Results: A total of 30 respondents were interviewed. They explained the preferred preparation, parts used, and treatment indications. Treatment of fever along with kidney and liver problems are the three principle uses. Among the different parts of *Arnebia benthamii*, 43% respondents preferred aerial parts for the herbal formulation, followed by whole plants, and leaves. Decoction was the major mode of preparation and all herbal preparations were administrated orally. This study reports, for the first time according to our literature review, a study of *Arnebia benthamii* with regard to PA determination. By using column Zorbax SB-Aq and acetonitrile-water gradient as the mobile phase, HPLC results showed that the aerial parts of the plant were PA positive, and (1) Europine, Heliotrine (2), Lycopsamine (3), and Echimidine (4) were identified.

Conclusions: This study has revealed two new findings of significance to herbal medicine producers, practitioners, and consumers of *Arnebia benthamii* First, local knowledge regarding the medicinal uses of *Arnebia benthamii* were documented in five sites of Neelum Valley, Azad Kashmir. The use of this plant by a large part of the population in the study area shows the importance for their therapeutic benefits. Unfortunately, the second finding of this study shows that *Arnebia benthamii* contains hepatotoxic PAs. Hence, we advised to the government regulatory authorities and non-governmental organizations that use of this plant as herbal medicine should be excluded before more accurate quality control tests.

1. Introduction

The interaction of humans and plants form a long history that also extends into today. Humans depend on botanical resources to fulfill their needs such as food, clothing, shelter, transportation and medicine (Ahmad et al., 2006). Medicinal plants have played a critical role in human healthcare throughout history (Letsyo et al., 2017). While used historically, they also form a major part of present-day human healthcare (Sadat-Hosseini et al., 2017). The World Health Organization reported that approximately 65% of people throughout the world and 90% of people in developing countries depend on traditional or herbal medicine for primary healthcare (Vandebroek et al., 2008). Even if one does not rely on herbal medicine, their healthcare is still touched by plants. Medicinal plants are a major source of secondary metabolites used in drug discovery. Some estimates assert that plants and their derivatives are involved in 25% of

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commercial drugs (Bodeker and Burford, 2007). This shows their place in the lives of traditional and herbal medicine users, and even non-herbal medicine users.

Studies on the place of plants in the lives of humans lands in the field of ethnobotany. Ethnobotany includes studies on this interaction of humans and botanical resources (Rodrigues, 2007). Knowledge of these interactions is often encapsulated in traditional knowledge, but it can and is lost rapidly, especially in areas of social, cultural and environmental transformations (da Costa et al., 2017). While this knowledge is certainly valuable at a local scale, it is also important for scientific knowledge in general, and especially for conservation initiatives (Cussy-Poma et al., 2017; Muthu et al., 2006). To prevent losses, and provide benefit to all parties, ethnobotanists play a critical role in analyzing potential mechanisms of conservation, sustainable, and safe use of botanical resources (Gandolfo and Hanazaki, 2011).

Consumers of herbal medicine, often called ethnomedicine, provide alternatives for treatment. People often seek these ethnomedicines in hopes that they have less side effects and be safer than pharmaceutical medications (Rates, 2001). However, ethnomedicines can be considered to have harmful or toxic effects if taken in excess (Ahmad et al., 2015). Unfortunately, the testing and critical role of pharmacovigilance in local ethnomedicinal markets is often lacking to non-existent (Debbie et al., 2012). These dangers can be magnified with issues of product quality, processing methods, correct identification of species, and knowledge of potential physiological effects (Shaw, 2010).

One common source of herbal product toxicity comes from pyrrolizidine alkaloids. Pyrrolizidine alkaloids (PAs) are secondary metabolites which are accumulated in plants that may cause hepatotoxicity in humans, and be hazardous to livestock and other wildlife (Avula et al., 2015). These accumulate in plants as PA-*N*-oxides that serve as defense compounds. They are produced by around 6000 plant species, which represents 3% of all angiosperms (Letsyo et al., 2017). Of these, the three families of Boraginaceae, Fabaceae, and Asteraceae contain largest number of PA producing species (Coulombe, 2003). Of these, the Boraginaceae has particular fame of having toxic PAs and their oxides (El-Shazly and Wink, 2014). According to previous studies, PAs are mutagenic, carcinogenic, and hepatoxic. This affects humans, wildlife, and livestock who ingest them (Mattocks, 1986; Prakash et al., 1999). The presence of PAs in herbal products and foodstuffs could lead to liver damage and then subsequently, hepatic veno-occlusive disease (Chen and Huo, 2010). If ingestion of these herbal products or foodstuffs with elevated concentrations of PAs is constant, death can result. Unfortunately, in animals and humans symptoms are not seen immediately. So the toxic effects are often diagnosed when it is too late. Exposure can come from direct consumption of foodstuffs, dietary supplements, herbal teas, or herbal products contaminated with PA containing plant material (Zhu et al., 2016). Sometimes, they can enter the food supply as a result of co-harvesting PA containing plant material with edible grains. (El-Shazly and Wink, 2014). Worldwide, around 10,000 PA poisoning cases have been reported (Dai et al., 2007; Fu et al., 2004).

Moreover, recently numerous cases of intoxications due to PAs are on the rise and researchers are pointing to PAs in herbal medicines (IRIN Asia, 2008a, 2008b; Molyneux et al., 2011; Edgar et al., 2011). The European Medicines Agency (EMA), implemented a limit of oral ingestion of herbal products containing PAs. This limit is 1 μg/day as a transitional measure for three years, then the threshold will be lowered to 0.35 μg/day for adults and 0.14 μg/day for children following a limit of 0.007 μg/kg body weight (BfR, 2011; EMA, 2016). For instance, the German Federal Health Administration limits internal exposure to no more than 1 μg/day (0.017 μg/kg/day for a 60 kg human) of PAs for no more than six weeks (German Federal Health Bureau, 1992). Furthermore, Belgium recommends a limit for PAs in herbs at 1 ppm (Dharmanada, 2013). Considering the risk of human exposure to these alkaloids, the World Health Organization (WHO) established the Health and Safety Guide (International Programme on Chemical Safety, 1989) on PAs and provided guidelines on the prevention of exposure with the dose limit of 15 μg/kg/day (IPCS, 1989).

One potential route of PA toxicity comes from *Arnebia* Forsskål (1775: 62) (Boraginaceae, *Lithospermeae*) with ca. 30 species distributed in the Himalayan region, Northeast Africa, Central and Southwestern Asia, and the Southeast Mediterranean region (Coppi et al., 2015). *Arnebia benthamii* (Wall. ex G.Don) I.M.Johnst. is a monocarpic perennial herb up to 2.7 feet tall with a swollen base. Its thick roots exude a red or purplish dye. It's simple, hollow, and solitary stem arises from a cluster of large basal leaves that are covered with long hairs that are 2–2.6 mm long. In Pakistan, it is distributed in the Pan-Himalayan region like in the Neelum Valley, Dir, Hazara (Flora of Pakistan, 1989). The present study has three aims: (1) Determine the presence of PAs in *Arnebia benthamii*. (2) Investigate and document the ethnobotany and use practices of *Arnebia benthamii* in the Neelum Valley, Pakistan (3) Assess if cultural practices fall within the EMA, IPCS, and German Federal Health Administration guidelines or if safety recommendations must be issued.

2. Materials and methods

2.1. Study area

This explorative study was carried out in Neelum Valley, Azad Kashmir, Pakistan. Neelum Valley is the largest district of Azad Kashmir having an area of 3621 square kilometers. It lies between 73–75°E longitude and 32–35°N latitude at an altitude of 900-6325 m above sea level (Ahmad et al., 2012; Mahmood et al., 2011; Dar, 2003).

The climate of Neelum Valley is temperate with cold winters and severe weather from mid-November until then end of April when temperatures average − 2.0 °C. While summers are moderate and very short lasting from June to August with an average temperature of 37.0 °C. The average rainfall is 165 cm annually (Ahmad et al., 2012; Qamar et al., 2010). The majority of the area is covered with thick vegetation, dominated by wild *Ficus palmata* Forssk. forests (Khan, 2008).

The main ethnic groups of the study area are the *Gujars, Syed, Kashmiris*, and *Awan*. Frequently spoken languages are *Kashmiri*, *Hindko*, and *Gujri* (Ishtiaq et al., 2012) (Fig. 1)

2.2. Ethno-pharmacological data collection and interviews with local people

Ethnobotanical data were collected from local respondents through open and semi-structured interviews (Martin, 1995; Cotton, 1996) between July and September of 2016. We conducted all interviews with prior informed consent regarding our academic research following the International Society of Ethnobiology Guidelines (2006). Due to the Line of Control (LOC), security risk, and the dangerous situation in this disputed area of Kashmir, we consulted with the security agency; Pakistan Army, local police and Forestry Department; and it was decided to conduct the research in five main localities (Athmuqam, Keren, Sharda, Kel, and Tao Butt). With the help of the local forestry department and inhabitants, 51 traditional healers and knowledgeable herbalists were identified in the five main localities. Of these, 30 agreed to voluntary interviews.

We interviewed the local people in public areas such as hotels and shops. Due to cultural and language differences, we selected the national language, Urdu, for interviews. For these ethnobotanical interviews, we conducted group discussions and asked questions regarding the local name of the plants, ethnomedicinal use, parts of the plants used, collection times, modes of preparation, route of administration, herbal preparation, and side effects if any.