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Toxic pyrrolizidine alkaloids in herbal medicines commonly used in Ghana

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ABSTRACT

Ethnopharmacological relevance: Herbal medicines have been used for centuries for the management and treatment of various ailments due to the belief that they pose only little or no health risk and side effects, and also, in part, due to their availability, affordability and/or self-supply. However, the increasing information over the recent years on the occurrence of pyrrolizidine alkaloids (PAs) in honey, herbal food and tea products has raised concerns about the safety of herbal medicines with respect to contamination. To this day, little is known on the occurrence of toxic PAs in herbal medicines, especially in tropical West Africa. The aim of this study was therefore to determine the PA content of 70 well-known and widely patronized plant-derived medicinal preparations, which are commercialized in Ghana and some West African countries, in order to ascertain their potential health risk.

Materials and methods: PAs of the herbal medicinal products, sourced from specialized drugstores and mostly regulatory approved, were analyzed for their PA content by a HPLC-ESI-MS/MS sum parameter method.

Results: The results show that a total of 60% of the analyzed herbal products were PA positive, indicating an average PA-concentration of 25.0 µg/kg. The maximum PA level (1290.0 µg/kg) was attributed to a regulatory-approved herbal medicine not known, according to the list of declared ingredients, to contain PA-plant parts. Interestingly, higher PA content (average, 30.2 µg/kg) was detected in regulatory-approved herbal medicines, in contrast to lower amount (average, 8.0 µg/kg) detected in non-regulatory-approved products.

Conclusion: The findings of this study clearly demonstrate that herbal medicines containing PA plants as ingredients, as well as some of those containing plant species not known to produce PAs, are likely to contain hepatotoxic PA at levels higher than the daily dose in food and herbal medicinal products proposed by the European Medicines Agency (i.e. 0.35 µg PA per day for 50 kg adult and 0.14 µg PA per day for 20 kg children). Hence, regulatory authorities are advised to carry out more rigorous quality control tests with respect to PAs so as to minimize the exposure of the consumers to these toxic compounds.

1. Introduction

Herbal medicines have played a significant role in health care delivery for centuries, essentially for the treatment and/or management of various diseases and ailments. Their usage has maintained popularity around the world, particularly in West Africa where an estimation between 70% and 80% of the populations rely on them to meet their primary health care needs (WHO, 2000). A similar survey suggested that 60% of children with high fever resulting from malaria use herbal medicines at home as the first line of treatment in three West African countries: Ghana, Mali and Nigeria (Aschwanden, 2001). The widespread use of commercially available herbal medicines in West Africa is partly due to the belief people have about these medicines in effectively managing a wide spectrum of diseases some of which may not be effectively managed using synthetic medicines (Lawal and Banjo, 2007). Also, in part, because of their abundance, accessibility (i.e. no medical prescription required), or inexpensiveness compared to synthetic medicines.

Herbal medicines used traditionally in Ghana and as in most African countries are usually sold either as crude herbal products or commercial herbal preparations (Fig. 1) with no restraint as non-prescription herbal products (World Intellectual Property

Abbreviations: HPLC-ESI-MS/MS, high performance liquid chromatography-electrospray ionization tandem mass spectrometry; PAs, pyrrolizidine alkaloids; HVOD, hepatic veno-occlusive disease; BfR, Bundesamt für Risikobewertung/German Federal Institute for Risk Assessment; EFSA, European Food Safety Authority; COT, Committee on Toxicity; IST, internal standard; CEP, cell entrance potential; RE, retronecine equivalents; bw, body weight; THF, tetrahydrofuran; MRM, multiple reaction monitoring; rpm, revolution per minutes; Mw, molecular weight; PANOs, pyrrolizidine alkaloid-*N*-oxides; CAD, collisionally activated dissociation; DP, declustering potential; CXP, collision cell exit potential

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Fig. 1. Crude (a) and commercial (b) herbal medicinal products sold in Ghana.

Organization, 2014). The former are sold in the open markets in the form of fresh or dried roots, leaves, seeds, stems, fruits or barks, whereas the latter, which are usually in the form of herbal tablets, decoctions or capsules, are more commonly sold in drug/chemical stores, pharmacies and herbal shops. The commercial herbal products, in particular, often vary in content of chemical constituents from batch-to-batch, thus resulting in differences in bioavailability and pharmacological activity in humans (Stickel et al., 2005). Moreover, West Africa is located within the tropical climate and it is a known fact that plants accumulate secondary metabolites as a natural means of surviving in a hostile environment. These metabolites may act individually, additively, or in synergy to improve health. However, plants containing pyrrolizidine alkaloid (PA) as secondary metabolites have been specifically found to contaminate the food chain (EFSA, 2011), and hence have generated much interest in recent times due to their potential toxic health effects (i.e. chronic genotoxic and carcinogenic effects) in humans. PAs are accumulated in plants predominantly as PA-*N*-oxides (PANOs), and are produced by over 6000 plant species (representing 3% of all flowering plants) as defense compounds against herbivores. PANOs *per se* show low or no acute toxicity but *in vivo* they are metabolized in the liver into the toxic tertiary PAs. Three plant families (e.g. Asteraceae, Boraginaceae and Fabaceae) are specifically the most important sources of these compounds (Dewick, 2002). Of the Asteraceae, PAs occur mainly in the tribes Senecioneae and Eupatorieae, while of the Boraginaceae (in many genera) and Fabaceae the genus Crotalaria is representative of PAs (Ober and Hartmann, 1999). The 1,2-unsaturated PAs, in particular, have been widely associated with hepatotoxicity in herbal remedies consumption (Röder, 1995; Dharmananda, 2007), herbal teas (BfR, 2013; Mädge et al., 2015) and in honeys collected by bees visiting PA-containing plants (Kempf et al., 2008; Dübecke et al., 2011). In fact, PA-containing honey has been suggested to be the source of PA contamination for other foodstuffs containing honey as ingredient (Kempf et al., 2011) or medical honey (Cramer and Beuerle, 2012). The presence of PAs in these foodstuffs could lead to liver damage then subsequently to hepatic veno-occlusive disease (HVOD) (Chen and Huo, 2010) which could also potentially result in acute and chronic effects on the liver in both humans and animals (IPCS, 1989). According to Röder (1995), these diseases are more profound in developing countries where consumption of herbs in folk medicine plays a more important role than in highly industrialized countries.

Notwithstanding this, previous studies have identified *Ageratum conyzoides* (L.) L., *Aspilia africana* (Pers.) C.D.Adams, *Bidens pilosa* L., *Chromolaena odorata* (L.) R.M.King & H.Rob., *Erigeron floribundus* (Kunth) Sch. Bip., *Emilia praetermissa* Milne-Redh., as well as *Heliotropium indicum* L., *Synedrella nodiflora* (L.) Gaertn., *Tridax procumbens* (L.) L., *Vernonia cinerea* (L.) Less., and *Vernonia colorata* (Willd.) Drake as the commonly used PA-containing plants in West African herbal medicinal preparations (Burkill, 2000; Okoli et al., 2007; Kone and Kande, 2012; Avula et al., 2015; Komlaga et al., 2015). Indeed, some of these plants have resulted in several fatal cases and severe liver diseases related to the consumption of herbal medicines in the past; with notable cases being the occurrence of liver tumors in the natives of Central and South Africa, former Soviet Union, Northwest Afghanistan and Tajikistan. These cases have been specifically attributed to the consumption of PA-containing herbs/bread of the genera *Crotalaria*, *Cynoglossum*, *Heliotropium* and *Senecio* (Dharmananda, 2001; Röder, 1995). Besides concerns of contamination of herbal medicines by PA plants, Letsyo et al. (unpublished data) have recently reported significant amounts of PAs in retail honeys from Ghana and some West African countries which could also be a potential source of PA contamination of herbal products marketed in the sub-region. This finding is significant since honeys are frequently used in commercial herbal preparations in the country, besides possible medicinal properties, to sweeten the herbal preparations thus making it more pleasant for the patient. Consequently, the continuous use of PA-producing plants and/or honey in herbal medicines to treat various ailments/diseases may be injurious to health.

In view of the numerous cases of intoxications and diseases reported from many parts of the globe, European Medicines Agency (EMA) and several European countries have implemented a limit of intake of PAs from herbal medicinal products (i.e. 1 μg/day) as a transitional measure for 3 years, after which the threshold will be set to 0.007 μg of 1,2-unsaturated PA/kg body weight (b.w.) (i.e. 0.35 μg PA per day for a 50 kg adult and 0.14 μg PA per day for 20 kg children) (BfR, 2011; EMA, 2016). In spite of these regulations and a previous study by Steenkamp et al. (2000) which implicated PAs in herbal medicines for the high rates of liver cancer and cirrhosis in Africa, several plant species containing these compounds continue to be used in herbal preparations, particularly in developing countries where access to modern health facilities is limited. Furthermore, despite their widespread use and sufficiently demonstrated potential toxicity, most investigations on herbal products in the sub-region have, however, focused on microbiological profiling, and trace metal and qualitative analysis (Ampofo et al., 2012; Anim et al., 2012). This has resulted in little or no quantitative data, up to date, on PA content of herbal plants/medicines used in tropical Ghana/West Africa. These data are essential in identifying possible sources of PA-contamination and potentially harmful products on the market so as to develop interventions to reduce PA intoxications of herbal products or, if possible, recommending a complete withdrawal of these products from the supply chain. This study, therefore, analyzes the PA contents of herbal medicines (mainly sourced from Ghana) prepared from various tropical plant species/honey commonly used in West African herbal medicinal preparations in order to assess the potential health risk posed by exposure to these toxic compounds.

2. Material and methods

2.1. Chemicals and reagents

Strong cation exchanger solid phase extraction cartridges (SCX-