

Review

Aristolochic acids in herbal medicine: Public health concerns for consumption and poor regulation of botanical products in Nigeria and West Africa

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Aristolochic acids are naturally occurring biomolecules found in plants of the genus *Aristolochia* and *Asarum* belonging to the family *Aristolochiaceae*. They are reported to be carcinogenic and nephrotoxic; and are implicated in kidney diseases, aristolochic acid nephropathy (AAN) which may result in kidney failure, other health complications and possibly death. Aristolochic acids are highly genotoxic and are linked to upper urothelial cancer in animals and humans. Some *Aristolochia* species are used in traditional medicine practice in Nigeria and other West African countries without regard to safety concerns. Several countries, especially in the Western world, have banned the use and importation of herbal products containing aristolochic acids. There is need for warning and strict regulation on the importation and consumption of aristolochic acids-containing botanical products in Nigeria. This study aims to review the availability of aristolochic acids, their toxicity, circulation, as well as the quantitative analytical techniques and regulations. It analyzes the herbal products containing aristolochic acids, and aristolochaceae plants grown in Nigeria in respect to public health implications. It highlights the importance of doing an extensive study on indigenous plants producing aristolochic acids and imported herbal products used as weight loss supplements marketed in Nigeria. There is need to emphasize the labeling of herbal products containing aristolochic acids.

Key words: Aristolochic acid, herbal medicine, Nigeria, aristolochaceae, toxicity, regulation.

INTRODUCTION

Medicinal plants are used in combating multiple and complex disease conditions affecting humans because of their popularity, accessibility, affordability and claimed

efficacy (Ayodele et al., 2010). Developing countries depend on ethno-medicines especially at the most basic level of health care due to perceived ease of accessibility

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and affordability. Adulteration and safety of botanical products continue to be of great concern (Mustapha, 2013).

Aristolochic acids are a group of naturally occurring compounds produced by aristolochaceae family of plants; they are reported to have various physiological effects on living tissues (Bode and Dong, 2015). Aristolochic acids refer to a mixture of structurally related nitrophenanthrene carboxylic acids (Gbadamosi and Egunyomi, 2012). The most abundant aristolochic acid is aristolochic acid I (1), followed by aristolochic acid II (2) (Shibutani et al., 2007). Aristolochic acids are carcinogens (National Toxicological Program, 2008).

Most plants reported to contain aristolochic acids belong to the genus *Aristolochia* or *Asarum* of the family Aristolochaceae (Arlt et al., 2002a, b). The use of *Aristolochia* species in traditional medicines and herbal products has been of global concern since the 1990s after a toxic herbal slimming product used in a Belgium clinic was found to contain *Aristolochia fangchi* instead of *Stephaniae tetrandrae*. After consuming the product, more than 100 patients were admitted to hospitals with renal failure and severe atrophy of the proximal renal tubules (Ekor, 2014).

Aristolochia fangchi was reported to contain aristolochic acids, whereas *Stephaniae tetrandrae* did not (Ekor, 2014). This inadvertent exchange of plant species containing aristolochic acids ultimately resulted in many patients suffering from end-stage renal failure and urethral damage (Heinrich et al., 2009). There are over twenty aristolochic acids and analogues known, that are produced by plants (Center for Food Safety and Applied Nutrition, 2001). Many dietary supplements containing aristolochic acids have been reported (AHPA Botanical Identity References Compendium, 2017).

Plants belonging to *Aristolochaceae* family known to produce aristolochic acids are widely cultivated in Nigeria and actively used in traditional medicine practice to treat various ailments without recourse to their toxicity and public health hazards. Also, while the regulatory bodies of many countries have made definite laws and regulations concerning aristolochic acids, there are no strict governmental regulations and restrictions on the importation and consumption of aristolochic acids-containing botanical products in Nigeria and other West African countries.

The aim of this review was to show that consuming aristolochaceae plants and herbal products containing aristolochic acids is dangerous to health and stresses the need for regulatory action to be taken in Nigeria and West Africa.

ARISTOLOCHIC ACIDS AND ANALOGUES

Aristolochic acids I and II

Aristolochic acid I (aristolochic acid A) is a crystalline

solid. Its molar extinction coefficient (ϵ) in ethanol is 6,500 at 390 nm, 12,000 at 318 nm, and 27,000 at 250 nm. Aristolochic acid II is also called Aristolochic acid B (Kumar et al., 2003).

Analogues of aristolochic acid

In addition to aristolochic acids I and II, other chemically related compounds found in Aristolochaceae family of plants (Figure 1) include aristolochic acid I methyl ester (3), 7-hydroxy aristolochic acid I (aristolochic acid Ia) (4), aristolochic acid II methyl ester (5), aristolochic acid III (6), aristolochic acid IIIa (aristolochic acid C) (7), aristolochic acid III methyl ester (8), aristolochic acid IV (9), aristolochic acid IVa (aristolochic acid D) (10), aristolochic acid IV methyl ester (11), aristolochic acid V (12), aristolochic acid Va (13), aristolochic acid VIa (14), aristolochic acid VII (15), aristolochic acid VIIa (16), aristolochic acid E (17), aristolactams (18) and dioxaporphines (19) (National Toxicological Program, 2008; Kumar et al., 2003; Cosyns JP. 2003). Krumbiegel et al (1987) identified aristolactam I (20), aristolactam Ia (21), aristolochic acid Ia (4), aristolochic acid I (22) and 3,4-methylenedioxy-8-hydroxy-1-phenanthrenecarboxylic acid (23) in rodents (Figure 1).

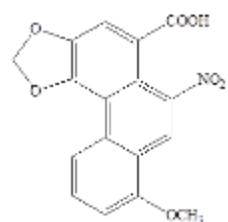
NATURAL SOURCES OF ARISTOLOCHIC ACIDS

Biosynthesis of aristolochic acids

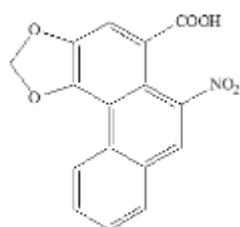
The biosynthetic pathway of aristolochic acids is not clear. However, a biogenetic relationship between aristolochic acids and the aporphine alkaloids has been postulated based on structural similarities. Magnoflorine, an aporphine alkaloid, is associated with aristolochic acids in various *Aristolochia* species (Comer et al., 1969; Schutteu et al., 1967). An aporphine alkaloid, 4,5-dioxaporphine (24), is found mostly among the Aristolochiaceae family of plants and regarded as possible intermediates of the precursors of aristolochic acids and aristolactams (Kumar et al., 2003). A total of about seventeen aporphine alkaloids have been characterized from *Aristolochia* species. Aristolactams were thought to originate from the cyclization condensation reaction of the reduction products of aristolochic acids. Twelve aristolactams have been reported from *Aristolochia* species (Kuo et al., 2012).

Distribution and occurrence of aristolochic acids

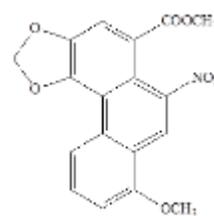
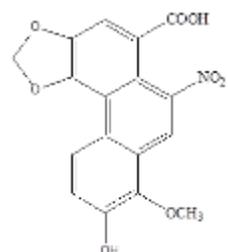
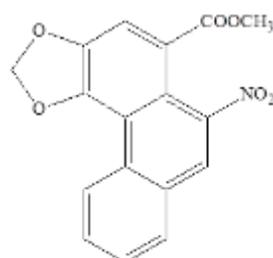
Aristolochic acids are nitro-compounds, non-alkaloidal constituents of different parts of a wide range of species of the family *Aristolochiaceae* (National Toxicological Program, 2008; Heinrich et al., 2009). Some *Aristolochia* species are native to Brazil but introduced into West



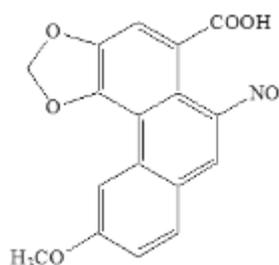
Aristolochic acid I (1)



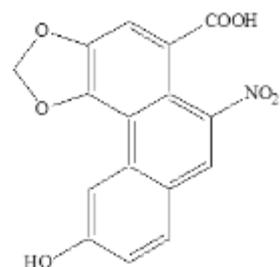
Aristolochic acid II (2)

Aristolochic acid I methyl ester
(3)7-Hydroxy aristolochic acid I
(4)

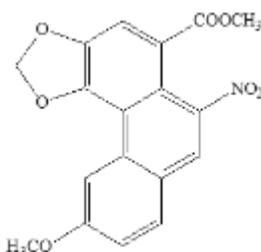
Aristolochic acid II methyl ester (5)



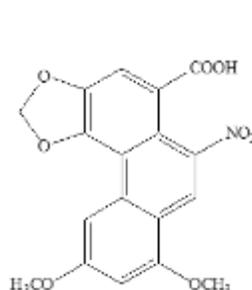
Aristolochic acid III (6)



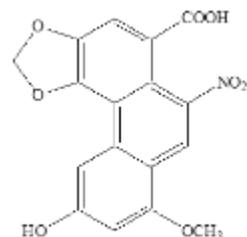
Aristolochic acid IIIa (7)



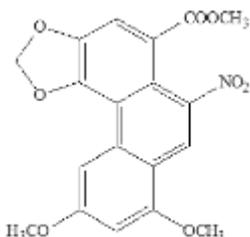
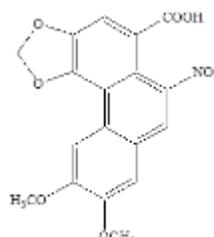
Aristolochic acid III methyl ester (8)



Aristolochic acid IV (9)



Aristolochic acid Iva (10)

Aristolochic acid IV methyl ester
(11)

Aristolochic acid V (12)

Figure 1. Chemical structures of aristolochic acids and analogues.

African gardens, hence found in Nigeria and Cameroon. The prominent species widely distributed in West Tropical Africa include *Aristolochia abida*, *Aristolochia bracteolata*, *Aristolochia clegans*, *Aristolochia gibbosa*, among others (Oladipupo, 2000).

About thirty *Aristolochia* species are native to the

United States; the most widely distributed species include *A. serpentaria* (Virginia snakeroot), *A. tomentosa* (wooly Dutchman's pipe), *A. macrophylla* (pipevine) and *A. clematitis* (birthwort) (National Toxicological Program, 2008). Aristolactams have been reported in Aristolochiaceae and related plant families, including the genus *Piper* (family Piperaceae), *Stephania*

(Menispermaceae) and *Schefferomitra* (Annonaceae) (National Toxicological Program, 2008). Aristolochic acids are found in several species of butterflies that feed on *Aristolochia* plants (National Toxicological Program, 2008). Other herbs identified as producing aristolochic acid and botanicals which may be adulterated with aristolochic acid have been reported (U.S FDA/ FDA, 2001; U.S. Food and Drug Administration/Center for Food Safety and Applied Nutrition 2001; National Toxicological Program, 2008).

Out of 16 samples of slimming pills and powders studied, the principal component was aristolochic acid I in *Aristolochia fang chi* with content ranging from 437 to 668 ppm; whereas aristolochic acid II was the principal component in *Aristolochia contorta*. Twelve of the samples were reported to contain aristolochic acids I and/or II. The principal component of majority of the slimming products was aristolochic acid II with content ranging from less than 1 to 148 ppm. Aristolochic acids I and II were detected in all the plants from the genus *Aristolochia* and at trace levels in some plants from the genus *Asarum* (National Toxicological Program, 2008).

Many herbal products advertised on the internet have been reported to contain aristolochic acids (National Toxicological Program, 2008; Center for Food Safety and Applied Nutrition, 2001). Guang fang ji (*Aristolochia fang chi*) was one of three types of fang ji (Chinese herbs) sold, but known to contain aristolochic acids. The root of Han fang ji (*Stephania tetrandra*) and mu fang ji (*Cocculus trilobus*) can be mistakenly substituted with guang fang ji because of similarities in their appearance (Center for Food Safety and Applied Nutrition, 2001). Despite extensive warnings on the dangers of aristolochic acids, aristolochic acid-containing Chinese herbal products, like Mu Tong, which has been associated with an increased risk of cancer of the bladder in humans, can still be purchased on the internet as an antibiotic and a remedy to improve cardiac function (Bode and Dong, 2015).

Occurrence of aristolochic acids in foods

Extracts from *Asarum canadense* (Canadian snakeroot or wild ginger) and *Aristolochia serpentaria* (Virginia snakeroot) are used as flavoring agents in foods or alcoholic beverages (National Toxicological Program, 2008).

Occurrence of aristolochic acids in insects

Aristolochic acids can be found in larvae of several species of butterflies, particularly those of the genera *Atrophaneura*, *Battus*, *Pachliopta*, and *Troides*, which feed on *Aristolochia* plants (Shibutani et al, 2007; Fordyce, 2000; Rothschild et al., 1972).

Anatomical characteristics of *Aristolochia* species

Aristolochia is a large plant genus with over 500 species (Minari and Idris, 2015). *Aristolochia* species can be perennial shrubs, lianas, or herbs bearing essential oils. The morphology of the whole plant varies from silica bodies to non-silica bodies, and climbing to self-supporting. The leaves could be alternate, spiral or flat and do not usually possess conspicuous aggregations; they are usually dorsiventral or bifacial with hairs present and possessing minor leaf veins without phloem cells. The plants stems are axial, with the presence of cork cambium. The fruits are usually non-fleshy dehiscent fruits while the seeds usually contain alkaloids and flavonoids as secondary metabolites, but lacking cyanogen. The supposed basic chromosome number of the family Aristolochiaceae is seven. *Aristolochia* species reproduce by either pollination of the flowers or by non-pollination and usually grow more in temperate regions but less in sub-tropical to tropical regions (Watson, 1992).

Uses of *Aristolochia* species

The U.S. Food and Drug Administration's "Approved Drug Products with Therapeutic Equivalence Evaluations" ("Orange Book") does not list any prescription or over-the-counter products (current or discontinued) that contain aristolochic acids (National Toxicological Program, 2008). The name *Aristolochia* means "the best delivery or birth" thus reflects centuries of use in traditional birth (Frei et al., 1985). Plant products containing aristolochic acids have been used extensively in traditional herbal medicine for various illnesses. They have been used as antirheumatics, as diuretics, in the treatment of edema, in wound healing, to facilitate childbirth, and for less common conditions such as hemorrhoids, cough and asthma (Li et al., 2005).

Various *Aristolochia* and *Asarum* species have been used in herbal medicines since ancient times in obstetrics and in treatment of snakebite, wounds and tumors, and they are still in use today, particularly in Chinese herbal medicine (Arlt et al., 2002b; Jiménez-Ferrer et al., 2005). Aristolochic acids have been reported to have antibacterial, antiviral, antifungal, and antitumor effects and in more recent times, have been used in conventional pharmaceuticals (Kupchan and Doskotch, 1962; Zhang et al., 2004). Herbal remedies containing aristolochic acids have been used for different illnesses such as hepatitis, urinary tract infection, vaginitis, oral ulcer, upper respiratory tract infection, eczema, headache, dysmenorrhea, arthralgia, neuralgia, hypertension, cerebrovascular accident, bronchitis, pneumonia, heart failure and edema (Li et al., 2005a).

The leaves and bark of *Aristolochia indica* were used in gastrointestinal disturbances and intermittent fever in children (Kumar et al., 2015).

In Africa, the use of *Aristolochia* had been reported. A decoction of *Aristolochia ringens* with *Picralima nitida* seed was used as stimulant for men by herb sellers at Adeleye market, Bariga, Lagos State and Oke Aje market, Ijebu Ode, Ogun State, Nigeria (Minari and Idris, 2015; Idu et al., 2010). *Aristolochia albida* Duch has been used by Zimbabweans to combat malaria (Ngarivhume et al., 2015). *A. albida*, *Aristolochia bracteolata* and *Aristolochia repens* have been used in Nigeria for the management of diabetes and other diseases (Ezuruike and Prieto, 2014). Overall, members of the *Aristolochia* genus seem to have a long history of medicinal use in Europe, Asia (including China), Africa, and Central America, which was also exemplified in studies on indigenous Mexican medicine (Heinrich et al., 2009).

Aristolochic acids and aristolochaceae in Nigeria

Aristolochic acid 1 is extracted from the rhizome of *A. albida* in Nigeria (Haruna and Ilyas 2000). The root bark, stem and root of *A. bracteolata* Lam known as Ga-daukuka (Hausa, Nigeria) are used as spices in Nigeria (Kayode and Ogunleye, 2008). The phytochemical constituents and antimicrobial activity of *A. ringens* are reported (Fasola et al., 2015; Oladoye et al., 2014). The tuber of *A. albida* Duch (known as kaucin kasa in Hausa) and the aerial part of *Aristolochia* spp (commonly known as Madakin kasa or kiwaye tsamiya in Hausa) are used in the management cancer (Ayodele et al., 2010; Ngulde et al., 2015). *A. albida*, *Aristolochia bracteolata* and *Aristolochia repens* are used in Nigeria for the management of diabetes and other diseases (Ezuruike and Prieto, 2014; Soladoye et al., 2012; Gbadamosi and Egunyomi, 2012; Idu et al., 2010; IARC, 2012; Wooltorton, 2004). Harmful effect of *A. ringens* has been reported by Sulyman et al. (2017). *A. repens* Mill. (Ako igun in Yoruba) is used by the people of Abeokuta in traditional healthcare for deworming (Idu et al., 2010). *A. albida* Duch is abundantly available in Nigeria and very much used by the traditional herbalist for a variety of purposes which include treatment of abdominal colic and management of snake bites (Oladipupo, 2000). *A. ringens* Mills is used for the traditional management of infantile dermatitis in Odeda, South Western Nigeria (Minari and Idris 2015; Erinoso et al., 2016).

LEGISLATIVE AND REGULATORY ACTIONS ON ARISTOLOCHIC ACIDS

FDA documentations on aristolochic acid

Consumption of products containing aristolochic acids is associated with permanent nephropathy, which may result in kidney failure and other complications, hence in May 2000, U.S. Food and Drug Administration (FDA) alerted consumers to discontinue use of botanical

products containing or suspected to contain aristolochic acid (U.S FDA/ FDA, 2001). Due to the potent carcinogenicity and nephrotoxicity of aristolochic acids, the agency also issued alerts to manufacturers, distributors, importers and health professionals of dietary supplements urging them to review their manufacturing procedures to ensure that botanicals are free of aristolochic acids (Center for Food Safety and Applied Nutrition, 2001; U.S. Food and Drug Administration/ Center for Food Safety and Applied Nutrition, 2001).

Legislative and regulatory actions taken on aristolochic acids by different countries

Germany 1981: The German Federal Health Office withdrew all preparations containing aristolochic acids from the national market following demonstration of their carcinogenic potential in a three-month toxicity study in rats; banned branded drugs containing aristolochic acid as well as herbal preparations or extracts prepared from plants belonging to the aristolochiaceae family, with the exemption of homeopathic preparations prepared to a dilution of at least 1:100,000,000,000. Aristolochic acid was identified as potent carcinogen even after dosage discontinuation (United Nations Publication 2005).

Austria 1981: The inherent risk associated with the use of preparations containing aristolochic acid led the Australian Federal Ministry of Health and Environmental Protection to instruct pharmacists against its use in Austria (United Nations Publication 2005).

Egypt 1982: Demonstration of carcinogenicity in rats resulted in products containing aristolochic acid being withdrawn in Egypt (United Nations Publication 2005).

USA 2001: The FDA cautioned consumers against consuming any dietary supplement or traditional medicine containing aristolochic acids (United Nations Publication 2005).

France 2001: All homeopathic preparations containing *Aristolochia brasiliensis* and homeopathic preparations containing products belonging to Aristolochiaceae or related plant families were withdrawn due to risks of nephrotoxicity and carcinogenicity associated with aristolochic acids (United Nations Publication 2005).

Oman 2001: Oman prohibited importation and marketing of aristolochic acids or products containing plants from aristolochiaceae family, due to kidney toxicity and urinary tract cancer associated with preparations containing aristolochic acids (United Nations Publication 2005).

Canada 2001: Health Canada issued a Customs Alert to ban the sale and import of products containing aristolochic acid. Manufacturers, retailers and importers were requested to withdraw from the market all existing

products containing *aristolochia* and aristolochic acids (United Nations Publication 2005).

Australia 2001: A traditional herbal product named Longdan Qiegan Wan (Wetness Heat Pill) containing aristolochic acids was removed from the Australian Register of Therapeutic Goods (United Nations Publication 2005).

Venezuela: Aristolochic acids containing products are not approved for use and/or sale in Venezuela (United Nations Publication, 2005).

ANALYTICAL METHODS FOR DETERMINATION OF ARISTOLOCHIC ACIDS

Several methods have been studied in analysing aristolochic acids in botanical samples and human tissues. Detection methods vary over time, with ultraviolet (UV) light absorption being most common, mass spectrometry (MS), electrochemical detection (ED), diode-array detection (DAD), laser-induced fluorescence (LIF) detection, fluorescence detection, and other methods have also been reported in more recent publications (National Toxicological Program, 2008; Chang et al., 2007a; Chang et al., 2007b). The United States Food and Drug Administration (FDA) issued a Laboratory Information Bulletin for the determination of aristolochic acids in traditional Chinese medicines and dietary supplements (National Toxicological Program 2008).

High performance liquid chromatography (HPLC)

The concentrations of aristolochic acids in botanical products were determined by high-performance liquid chromatography (HPLC) with UV absorption detection at 390 nm (IARC, 2002, Trujillo et al., 2006). To detect and quantify aristolochic acid in human tissues, a hollow fiber liquid-phase microextraction technique in conjunction with high-performance liquid chromatography was used (Heinrich et al., 2009). Aristolochic acids have been determined in medicinal plants and slimming products using HPLC with RP-18 reversed phase column. An average recovery of 97.8% was obtained when aristolochic acids in *Aristolochia* plant samples were quantified by reversed-phase HPLC method involving extraction with methanol and chromatographic separation with a mobile phase of acetonitrile–water–trifluoroacetic acid–tetrahydrofuran in the ratio of 50:50:1:1, using photodiode array detection. The limit of detection was 0.10 g per injection with a 5 µl injection volume (National Toxicological Program, 2008; Heinrich et al., 2009; Li et al., 2005a).

Ultra-high-performance liquid chromatography-multistage fragmentation mass spectrometry (UHPLC/MS)

This is a hyphenated technique applied to determine aristolochic acid I in herbal dietary supplements (Yang et al., 2014). Furthermore, ultraperformance liquid chromatography-triple quadrupole mass spectrometry is a noninvasive and efficient method developed to detect aristolactam-DNA adducts in exfoliated urothelial cells (Yang et al., 2014).

Liquid chromatography-mass spectroscopy

This method with limit of quantitation equivalent to 140 mg/ml has been applied to determine aristolochic acids in botanical samples as well as in renal cortex, using either an ion-trap mass spectrometer or a triple quadrupole mass spectrometer (Heinrich et al., 2009; IARC, 2002; Rao et al., 1975).

Targeted liquid chromatography/serial mass spectrometry (LC/MS/MS)

This method has been employed in detecting aristolochic acids I and II in multi-component herbal remedies, using a quadrupole ion-trap mass spectrometer. Aristolochic acids were determined to be between 250 pg and 2.5 ng on-column within a matrix containing compounds extracted from 2 mg of herbal remedy (National Toxicological Program, 2008).

Capillary zone electrophoresis (CZE)

Capillary zone electrophoresis (CZE) was used for the analysis of aristolochic acids in medicinal plants. The limits of detection for aristolochic acids I and II were 30 and 22.5 mg/kg, respectively (IARC, 2002).

Enzyme-linked immunosorbent assay (ELISA)

This has been used and reported to have limit of detection (LOD) for aristolochic acid I of 0.7 ng/ml, or ~ 2×10^{-9} M, but its LOD for aristolochic acid II was similar to the other methods previously mentioned (18 ng/ml, or ~ 6×10^{-8} M) (National Toxicological Program, 2008).

Pressurized liquid extraction method

Extraction and analysis of aristolochic acids I and II in medicinal plants (*Radix aristolochiae*) using pressurized liquid extraction method reportedly gave better result than ultrasonic and soxhlet extraction methods (IARC, 2002).

P-post-labeling and ultra performance liquid chromatography–electrospray ionization/multistage mass spectrometry (UPLC-ESI/MSⁿ)

P-post-labeling technique was the most widely employed method for detecting putative DNA adducts in humans. It was used to analyze aristolochic acid–DNA adducts in the kidneys of CHN patients (National Toxicological Program, 2008; Arlt et al., 2001a, b; Schmeiser et al., 1996).

ABSORPTION, METABOLISM AND EXCRETION OF ARISTOLOCHIC ACIDS

Pharmacokinetics studies

Aristolochic acids are absorbed from the gastrointestinal tract and distributed unchanged and/or in metabolized form throughout the body (IARC, 2012; Lunn et al., 2008). They are metabolized by oxidation and reduction pathways called phase I metabolism. Aristolactam I which is the product of aristolochic acid I (AA-I) reduction was observed in urine. Further metabolism by O-demethylation of aristolactam I resulted in aristolactam IA as the primary metabolite (IARC, 2002). Nitroreduction yielded an N-acylnitrenium ion, an important ion in mutagenicity of aristolochic acid I (IARC, 2012; Lunn et al., 2008). AA-I was reportedly metabolized along two major pathways; aerobic demethylation to 8-hydroxyaristolochic acid-I (aristolochic acid Ia) which, in turn, was metabolized by phase II glucuronide or sulphate conjugation reactions. An alternative pathway was by enzymatic reduction of the nitro group to generate the biologically inactive aristolactam-I (Shibutani et al., 2007; IARC, 2002), which was further subjected to phase II conjugation. Aristolochic acid II (AA-II), which lacks the O-methoxy group, was reduced to aristolactam-II (L-II) and further hydroxylated at C-8 to form 8-hydroxyaristolactam 1a (L-Ia). Excretion of aristolochic acids and their metabolites is through the urine (Shibutani et al., 2007; Lunn et al., 2008).

Metabolites

Aristolochic acid I and II metabolites such as aristolactam I, aristolactam Ia, aristolochic acid Ia, aristolochic acid I (**22**); and 3,4-methylenedioxy-8-hydroxy-1-phenanthrenecarboxylic acid (**23**) were identified in rodents following the oral administration of aristolochic acid I and aristolochic acid II. In rats, the major metabolite was aristolactam Ia (46% of the dose in urine and 37% in the faeces). In both rats and mice, the metabolites of aristolochic acid II were identified as aristolactam II, aristolactam Ia, and 3,4-methylenedioxy-1-phenanthrenecarboxylic acid. Generally, fewer

metabolites were observed in beagle dogs, rabbits, guinea-pigs, and humans than in rodents. Among the metabolites of aristolochic acids I and II, only aristolactam I and aristolactam II were identified in human urine samples collected from 6 healthy volunteers to whom a mixture of aristolochic acids I and II were given over several days (National Toxicological Program, 2008; IARC, 2012).

Metabonomic studies

The renal proximal tubule is reported as the principal target of aristolochic acids in rats. Nephrotoxicity has been reported in male rats by identifying elevated serum urea and creatinine levels, and urinary protein and glucose. Furthermore, increased activity of the enzymes; gamma glutamyl transferase (γ -GT) and *N*-acetyl- β -D-glucosaminidase (NAG) was observed in rats exposed to aristolochic acids, which was interpreted as resulting from a lesion of the renal duct epithelial cells (National Toxicological Program, 2008).

TOXICITY OF ARISTOLOCHIC ACIDS

Nephrotoxicity

Three main terms have been used to designate the renal disease due to consumption of herbs. These are Chinese herb nephropathy (CHN), aristolochic acid nephropathy (AAN), phytotherapy-associated interstitial nephritis (PAIN) and Endemic (Balkan) nephropathy (BEN) (National Toxicological Program, 2008; Heinrich et al., 2009).

The ingestion of herbal remedies containing aristolochic acids is associated with the development of a chronic, progressive renal disease, termed aristolochic acid nephropathy (AAN) (National Toxicological Program, 2008; Ekor, 2014; Debelle et al., 2008).

Nephrotoxicity is reflected by gradual and progressive atrophy of renal proximal tubules and development of a characteristic form of interstitial fibrosis involving the outer renal cortex and progressing toward the medulla, while the glomeruli are spared. This nephropathy is associated with less inflammation than most types of interstitial nephritis. Steady progression of the disease leads to chronic renal failure and a strong association with transitional cell (urothelial) carcinoma of the upper urinary tract (National Toxicological Program, 2008; Heinrich et al., 2009).

Although both aristolochic acid I and II (AA-I and AA-II) are cytotoxic to cells in culture, AA-I is solely responsible for the nephrotoxicity associated with AAN in mice (National Toxicological Program, 2008). Endemic (Balkan) nephropathy affected people in rural areas of Bosnia, Bulgaria, Croatia, Romania, and Serbia, and has been linked to the consumption of aristolochic acids

containing products (Heinrich et al., 2009).

In 1992, it was reported in Brussels, Belgium that a Chinese herbal product containing *Aristolochia fangchi* (Guang Fang Ji) was mistakenly labelled as containing *Stephania tetrandra* (Han Fang Ji), a Chinese slimming regimen. This unintentional substitution was confirmed by phytochemical analysis of 12 different batches of the herb powders. Only one batch was found to contain tetrandrine and not aristolochic acids I and II; one contained both tetrandrine and aristolochic acids and 10 contained aristolochic acids only (National Toxicological Program, 2008). Similar incidence of substitution was also reported in Hong Kong (Liang et al., 2006). Consequently, patients who took the regimen developed interstitial renal fibrosis and subsequently, end-stage renal disease (Comer et al., 1969). It is also documented that about 5% of the exposed population (patients taking the weight-loss regimen from May 1990 to October 1992) developed renal disease. The mean average exposure per patient was about 900 mg of powder per day for 6 to 12 months (National Toxicological Program, 2008). To avoid unintentional substitution involving aristolochic acid-producing plant, nomenclature by pharmaceutical name was recommended (Wu et al., 2007).

The presence of aristolactam-DNA adducts formed by aristolochic acid was confirmed in series of kidney samples obtained from 38 patients with AAN six years after their exposure to the so-called *Stephania tetrandra* powder (actually, *Aristolochia fangchi*). These adducts were absent in kidney tissues obtained from eight patients with renal disease of other origin (National Toxicological Program, 2008; Ekor, 2014).

Similar cases of aristolochic acid nephropathy were reported in many other countries: four cases in France resulting from the intake of slimming pills labeled as containing *Stephania tetrandra* which was, in fact, *A. fangchi* (Arlt et al., 2004); One case in Spain after chronic consumption of a tea made with a mixture of herbs containing *Aristolochia pistolochia*, two cases in the United Kingdom after treatment of eczema with Mu Tong containing aristolochic acid (Lord et al., 2001), 12 cases in Taiwan related to the use of various unidentified herbal medications for different purposes (Chang et al., 2001; Yang, 2000), one case in the USA after intake of herbal medicine containing aristolochic acid for low back pain (Stewart et al., 2003), and 12 cases in Japan, five of which were herbal medicines containing aristolochic acid; in the other cases there was confusion of *Mokutsu* (*Akebia quinata*) with *Kan-Mokutsu* (*Aristolochia manshuriensis*) and *Boui* (*Sinomenium acutum*) with *Kou Boui* (*Aristolochia fangchi*) or *Kanchu-Boui* (*Aristolochia heterophylla*) (National Toxicological Program, 2008; Ekor, 2014).

In Japan, the cases of Chinese herb nephropathy often presented with adult-onset Fanconi syndrome. A similar case was reported in Germany after intake of a purported *Akebia* preparation containing aristolochic acid (National

Toxicological Program, 2008; Ekor, 2014).

Recently, it was estimated that about 100,000 individuals were at risk of Endemic (Balkan) nephropathy (BEN), while about 25,000 have developed the disease with the highest prevalence rates in Serbia, Bulgaria, Romania, Bosnia and Herzegovina and Croatia (Jadot et al., 2017).

Natural food chain contamination by root uptake

Another possible pathway of aristolochic acid exposure to human bodies has recently been proposed and is known as natural food chain contamination by root uptake. When *Aristolochia spp* grows, degenerates and decomposes, it may leave deposits of aristolochic acid in the soil which is accumulated by root uptake in other crops grown successively. Some authors proved that the roots of maize plant and cucumber were capable of absorbing aristolochic acids from the soil. To strengthen this proposed intoxication pathway, AAs were subsequently identified in corn, wheat grain and soil samples collected from the endemic village of Kutles in Serbia (Jadot et al., 2017).

Carcinogenicity

Aristolochic acid is considered a potent human carcinogen (IARC, 2012) and listed among the most potent 2% of carcinogens known (Wooltorton, 2004). It was classified as a human carcinogen class I by the World Health Organization International Agency for Research on Cancer (Jadot et al., 2017). Aristolochic acid nephropathy was associated with a high prevalence of urothelial cell carcinoma (National Toxicological Program, 2008; Kuo et al. 2012), which often occurs years after the onset of chronic renal disease and tend to develop in the upper urinary tract unlike most other urothelial cell tumors (National Toxicological Program, 2008). The carcinogenic effects of aristolochic acids are thought to be a result of mutation of the tumor suppressor gene *TP53*, which is unique to aristolochic acid-associated carcinogenesis (Go'kmen et al., 2013). Aristolochic acid-associated cases of urothelial cancer were reported in many countries including China (Li et al., 2005).

Genotoxicity

Following metabolic activation, aristolochic acid reacts with DNA to form aristolactam (AL)-DNA adducts lesions which concentrate in the renal cortex as a sensitive and specific biomarker of exposure (Kuo et al. 2012). Several mammalian enzymes have been shown to be capable of activating both AAI and AAIi *in vitro* and in cells (Arlt et al., 2002a). Aristolochic acid I (AAI) and aristolochic acid

II (AAII) have genotoxic and carcinogenic effects as deduced by their ability to form AA-DNA adducts in target organs and tissues of intoxicated mice (Jadot et al., 2017). 7-(deoxyadenosin-N6-yl)-aristolactam I, a distinct DNA adduct, was an established biomarker of AA exposure that can be persistent decades after AA exposure, thus attesting to the role of AA in human urothelial malignancy (Schmeiser et al., 2014).

Aristolochic acids structure-activity relationships

Studies conducted in mice regarding structure–activity relationships revealed that the carboxyl group aristolochic acid 1 is an absolute structural requirement for its transport and high affinity interaction with organic anion transporter (OAT) 1, 2 and 3, while the nitro group is only required by OAT1; the O-methoxy group present at the 8-position may be a functional key determinant for AA-induced toxicity in mice but was not involved in transport (Jadot et al., 2017). Consequently, it was demonstrated that only AAI was capable of inducing nephrotoxicity as evidenced by tubular damage and development of interstitial fibrosis in AAI-treated mice (Jadot et al., 2017).

FUTURE SCOPE

Future scope of research and regulatory concerns on exposure to aristolochic acid include: Are there incidences of unreported cases aristolochic acid nephropathy in regions of Nigeria where *Aristolochia* species are used in treating ailments? Could more *Aristolochia* species than already studied be growing in Nigeria but yet to be identified due to poor coverage and inadequate taxonomy? Is there a possible correlation between the recent upsurge in incidences of chronic kidney disease (CKD) and bladder carcinoma in Nigeria, and the consumption of aristolochic acid producing plants, herbal supplements adulterated with *Aristolochia* spp and imported botanical products? What regulations are put in place for the production and use of products containing *Aristolochia* spp in Nigeria and West African sub-region? This is need for proper identification and comprehensive study of Nigerian local herbs belonging to aristolochaceae family, particularly *Aristolochia* spp and related plants for possible aristolochic acids content. Appropriate regulatory bodies should conduct extensive investigation of imported herbal products and weight loss supplements marketed in Nigeria for possible adulteration with aristolochic acids. Local and imported herbal products label should contain information on aristolochic acid content.

CONCLUSION

Aristolochia species belonging to Aristolochaceae family

of plants known to produce aristolochic acid are not native to Nigeria and other African countries; they are widely cultivated in Nigeria and are actively used in traditional medicine practice to treat various ailments without recourse to their toxicity. This review exposed the fact that not many studies have been done on aristolochic acids and aristolochic acids-producing plants grown in Nigeria with respect to their toxicity profiles. In the course of this review, it was observed that while the regulatory bodies of many countries have made definite laws and regulations concerning aristolochic acids, there are no strict governmental regulations and restrictions on the importation and consumption of aristolochic acids-containing botanical products in Nigeria. As obtainable in other countries, the Nigerian Federal Ministry of Health should issue a warning on the danger of consuming aristolochic acids, botanical products containing aristolochic acids or herbal products containing plants belonging to aristolochaceae or related families.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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