

Balkan Endemic Nephropathy (BEN) Induced by Plants Contaminated with Aristolochic Acids

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- Acronym: INNO-SAFE-LIFE



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Historical and Geographical Context

First described in **1950s** in rural Balkan villages (Serbia, Bosnia, Bulgaria, Croatia, Romania).

Occurs in **clusters** along the **Danube tributaries** with specific topographical and agricultural features.

Chronic, progressive nephropathy affecting **middle-aged to elderly** populations.

High co-occurrence of **upper urinary tract urothelial carcinoma (UUC)**.

BEN is one of the most studied geographically restricted nephropathies. Its regional specificity and familial aggregation initially suggested a hereditary component, but later evidence pointed to environmental toxins, particularly aristolochic acid exposure.

Epidemiological Characteristics

Incidence limited to **rural agricultural families**.

No evidence of **infectious transmission**.

Long latency period: **15–20 years** from exposure to manifestation.

Affects both genders equally; **familial clustering** due to shared exposure, not genetics.

Marked association with **aristolochic acid DNA adducts** in renal and urothelial tissues.

Epidemiological mapping of BEN reveals exposure-driven risk rather than genetic susceptibility. The consistent detection of aristolochic acid–derived DNA adducts in renal tissues strongly links environmental ingestion to disease onset.

Etiological Hypothesis

Hypothesis: Chronic dietary exposure to **aristolochic acids (AAs)** from **contaminated flour** derived from **Aristolochia clematitis** seeds in wheat fields.

Aristolochia clematitis (birthwort) is an endemic weed in Balkan agricultural areas.

Seeds co-harvested with wheat → **contaminated flour ingestion**.

Long-term ingestion of AAs → **nephrotoxic and carcinogenic effects**.

The etiological model is environmentally rooted. Aristolochic acid-containing plant material contaminates staple diets over decades, producing cumulative DNA damage and chronic renal fibrosis characteristic of BEN.

Aristolochic Acids: Chemical Nature



Aristolochic acids (AAs): nitrophenanthrene carboxylic acids.



Principal forms: **AA-I** and **AA-II**.



Lipophilic and **thermally stable**, surviving milling and baking.



Undergo **metabolic activation** via nitroreduction → aristolactams.



Covalently bind DNA → **aristolactam–DNA adducts**.



AAs are chemically resilient compounds capable of forming persistent DNA adducts. Their activation through nitroreductases (in kidney and liver) leads to mutagenic aristolactams — the molecular signature of BEN.

Mechanism of Toxicity



Phase I: Metabolic activation by **CYP1A1/2** and **NQO1** → nitroreduction.
Phase II: Formation of reactive **cyclic nitrenium ions**.
Phase III: Covalent binding to purine bases in DNA → **A:T** → **T:A** transversions.



Results in **mutagenesis**, **tubulointerstitial fibrosis**, and **apoptosis** of proximal tubular cells.



The toxicodynamic cascade of AA involves reductive activation leading to electrophilic intermediates. These form stable adducts with adenine and guanine, producing mutations in key genes, including TP53 — a molecular hallmark of AA exposure.

Molecular Biomarkers of Exposure

DNA adducts: aristolactam–dA and –dG adducts in renal cortex.

Mutational signature: A:T → T:A transversions in **TP53**, **HRAS**, **FGFR3**.

Urinary biomarkers: 7-(deoxyadenosin-N6-yl) aristolactam I.

Omics-based biomarkers: altered transcriptomic and proteomic profiles of oxidative stress and fibrosis.

Molecular biomarkers are central for exposure confirmation. The AA mutational signature is now recognized globally, even in unrelated nephropathies, such as Chinese herb nephropathy, confirming a shared mechanism of aristolochic toxicity.

Pathophysiology of BEN



Primary site: proximal tubules and interstitium.



Histopathological features:

Tubular atrophy.
Interstitial fibrosis.
Sclerotic glomeruli.
Obliterative vascular lesions.



Functional decline: gradual, leading to terminal renal failure.



BEN is characterized by silent progression — minimal proteinuria, low blood pressure, and progressive tubular atrophy. Histology reveals distinct hypocellular interstitial fibrosis, differentiating it from other nephropathies.

Clinical Manifestations

Insidious onset over years.

Early: fatigue, mild anemia, reduced concentrating ability.

Later: renal failure with low proteinuria and hypertension absent or mild.

High incidence (40–50%) of upper urothelial carcinoma (UUC).

Coexistence of **tubulointerstitial nephritis** and **urothelial malignancy**.

Clinically, BEN is deceptive — patients often remain asymptomatic until late-stage renal failure. Its dual pathology (nephropathy and carcinoma) underscores the genotoxic nature of AAs.

Carcinogenic Mechanism

AA–DNA adducts → **mutations in tumor suppressor genes.**

TP53 mutational signature (A:T→T:A) identical in BEN-associated tumors.

Chromosomal instability and **epigenetic deregulation** enhance oncogenesis.

Synergism with **chronic inflammation** amplifies carcinogenic risk.

The AA mutational fingerprint is one of the most specific carcinogenic signatures known in human oncology. It links chronic dietary toxin exposure directly to DNA damage–driven tumorigenesis.

Comparative Toxicology

Disease	Toxin Source	Target Organ	Mechanism	Biomarker
BEN	Contaminated flour (Aristolochia clematitis)	Kidney	DNA adduct formation	AL–DNA adducts
Chinese herb nephropathy	Herbal slimming agents	Kidney	Same as BEN	TP53 A:T→T:A
Ochratoxin A nephropathy	Moldy grains	Kidney	Oxidative & DNA adduct	OTA–DNA adducts

Environmental Factors



Soil ecology: alkaline, rich in clay; supports *A. clematitis* growth.



Agricultural practice: manual harvesting → seed contamination.



Socioeconomic context: home-milled flour, no mechanical sorting.



Climatic factors: drought favors deep-rooting *Aristolochia* spread.



Environmental conditions in endemic regions promote persistent human exposure — ecological, agronomic, and social factors converge to sustain contamination cycles.

Analytical Detection of Aristolochic Acids



HPLC-MS/MS and **LC-HRMS** for detection of AA-I/AA-II in plant and food matrices.



LC-MS/MS adductomics for renal tissue analysis.



Stable isotope dilution improves quantification.



Tissue imaging mass spectrometry localizes adduct formation spatially.



Modern analytical chemistry enables femtomolar-level detection of AAs and their adducts. Adductomics is transforming nephrotoxicology by mapping precise molecular fingerprints of exposure.

Genomic and Epigenomic Alterations

Transcriptomics: upregulation of TGF- β , collagen I, α -SMA genes.

Epigenetic shifts: promoter methylation in detoxification genes (GSTP1, NQO1).

miRNA dysregulation: miR-21 and miR-29 in fibrosis progression.

Mitochondrial DNA damage: impaired oxidative phosphorylation.

AA exposure provokes multilevel genomic disruption. Beyond direct mutagenesis, chronic inflammation and fibrosis are driven by transcriptional and epigenetic remodeling of renal cells.

Experimental and Animal Models

Rodent models: chronic AA feeding reproduces BEN pathology.

Zebrafish models: transparent kidney visualization for nephrotoxic endpoints.

Human kidney organoids: in vitro model for dose-dependent nephrotoxicity.

CRISPR models: gene knockout of NQO1 reveals detoxification pathways.

Animal and organoid models confirm causal toxicity. CRISPR-enabled knockout studies pinpoint enzymes responsible for AA activation and detoxification, clarifying human susceptibility mechanisms.

Preventive and Therapeutic Strategies

Agricultural control: eradication of *Aristolochia* from wheat fields.

Food safety: mechanical separation, regulation of herbal supplements.

Biomonitoring: urinary adduct screening in endemic regions.

Therapeutics: antioxidants, anti-fibrotic agents (pirfenidone, ACE inhibitors).

Public health education and exposure surveillance.

Prevention remains the most effective intervention. The challenge lies in socio-economic enforcement of agricultural and food safety policies in rural endemic zones.



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Global Relevance

Aristolochic acid exposure identified in Asia, South America, and North Africa.

Herbal nephropathy from AA-containing plants recognized by WHO.

AA mutational signatures detected in global cancer genome datasets.

BEN as a model for environment–gene–disease interactions.

BEN exemplifies a globally relevant toxicological paradigm — linking environmental exposure, chronic organ toxicity, and cancer genomics. Its lessons extend to herbal medicine regulation worldwide.

Current Research Frontiers

Adductomics + genomics integration for exposure mapping.

Metagenomic interactions: microbiome-mediated AA metabolism.

Precision toxicology: AI modeling of exposure–mutation correlations.

Public health: early biomarker detection through liquid biopsy approaches.

Frontline research applies omics and AI tools to predict and detect exposure at preclinical stages. Understanding microbiome–toxin interactions may reveal why susceptibility varies among individuals.

Ethical, Legal, and Societal Dimensions



Neglected rural disease: socioeconomic inequities perpetuate exposure.



Policy gaps: inconsistent herbal medicine regulation.



Compensation and accountability: historical exposure without legal recourse.



Ethical imperative: recognition of BEN as an environmental justice issue.



Beyond science, BEN raises ethical concerns about rural neglect, unequal healthcare access, and policy failures. Recognizing environmental nephropathies as justice issues is critical to global health equity.

Conclusion

Balkan Endemic Nephropathy: archetype of environmental nephrotoxicity.

Aristolochic acids induce irreversible DNA damage and carcinogenesis.

Integrated approaches — environmental monitoring, molecular biomarkers, omics — are essential.

Lessons from BEN inform **global toxicology, precision medicine, and food safety policy.**

BEN encapsulates how chronic environmental exposure can silently erode public health. Its elucidation through molecular epidemiology underscores the power of integrative biomedical research to prevent future environmental diseases.