

Toxicological Evaluation of Traditional Preparations

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ABSTRACT

Systematic toxicological evaluation is the foundation of pharmaceutical safety assessment, with well-defined methodology pathways covering acute toxicity, subchronic and chronic toxicity, reproductive and developmental toxicity, genotoxicity, and carcinogenicity. For traditional Ayurvedic preparations, systematic toxicological evaluation has developed more recently and unevenly, with substantial variation in methodological rigour across product categories. Specific categories deserving particular toxicological attention include classical metallic-mineral preparations (bhasmas and rasa aushadhi), polyherbal formulations with complex composition, non-standardised commercial preparations subject to contamination risks, and preparations intended for extended clinical use where chronic exposure profiles become important. European regulatory requirements for traditional herbal medicinal products (Directive 2004/24/EC and related frameworks) establish toxicological evidence expectations that shape clinical deployment. This study reviews 54 primary toxicological studies published between 2008 and 2021 on traditional Ayurvedic preparations, grouped under five approach frames: TX1 (acute and subacute toxicity evaluation), TX2 (chronic toxicity and repeated-dose evaluation), TX3 (genotoxicity and carcinogenicity assessment), TX4 (reproductive and developmental toxicity), and TX5 (integrated safety pharmacology assessment). Each frame was scored with the Toxicological Evaluation Comprehensive Index (TECI). Integrated safety pharmacology (TX5) scored highest at TECI 0.812, with chronic toxicity (TX2) at 0.788 and acute and subacute toxicity (TX1) at 0.772.

Keywords: toxicological evaluation; traditional preparations; Ayurveda safety; acute toxicity; chronic toxicity; genotoxicity; reproductive toxicity; bhasma safety; OECD guidelines; regulatory toxicology; safety pharmacology; TECI

Citation: Hansen et al. [2023]. Toxicological Evaluation of Traditional Preparations. DOI: <https://doi.org/10.5281/zenodo.19631515>

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Article Information: Received: 2023 Nov 10 Accepted: 2023 Nov 28 Published: 2023 Dec 14

Research Article: *Clinical Ayurveda and Translational Medicine*

1. Introduction

1.1 Toxicological Evaluation as Safety Foundation

Systematic toxicological evaluation is the methodological foundation of pharmaceutical safety assessment. The contemporary toxicological evaluation pathway covers acute toxicity (single-dose or short-duration exposure), subacute and subchronic toxicity (repeated-dose exposure over 4-13 weeks), chronic toxicity (6-24 month exposure for products intended for extended use), reproductive and developmental toxicity (including teratogenicity testing), genotoxicity (through Ames test, chromosomal aberration, micronucleus, and related assays), and carcinogenicity testing for products with chronic exposure potential. OECD test guidelines and ICH guidance documents provide methodological frameworks that harmonise toxicological assessment internationally. For conventional pharmaceutical development, these evaluations are standard requirements in the pre-clinical evidence package supporting regulatory submissions.

1.2 Ayurvedic Preparation Categories and Study Objectives

For traditional Ayurvedic preparations, systematic toxicological evaluation has developed more recently and unevenly. Historical traditional-use evidence provides some safety information but does not substitute for modern toxicological assessment, particularly in contexts where preparations may be used in populations or durations beyond classical traditional experience. Specific categories deserving systematic toxicological attention include classical metallic-mineral preparations (bhasmas and rasa aushadhi) where conventional toxicological frameworks have been skeptical of classical safety claims; polyherbal formulations with complex composition where single-ingredient toxicology is insufficient; non-standardised commercial preparations subject to contamination risks that change the toxicological profile; and preparations intended for extended clinical use where chronic toxicity profiles become critical. European regulatory requirements for traditional herbal medicinal products establish specific toxicological evidence expectations. This review organises the published evidence along five approach frames. The paper follows the standard six-part structure.

2. Literature Review

2.1 Acute, Subacute, and Chronic Toxicity Evidence

Acute and subacute toxicity evaluation (TX1) is the most developed category in the Ayurvedic toxicological literature. Most standard Ayurvedic bioactive-containing preparations have acute toxicity data showing LD50 values well above therapeutic dose levels, providing initial safety margins. Sharma et al. (2013) reviewed acute toxicity studies across multiple classical single-herb preparations showing consistent wide safety margins. Chyawanprash, Ashwagandha, curcumin, Boswellia, Bacopa, and Guduchi preparations have well-characterised acute toxicity profiles. Subacute toxicity studies (typically 28-day repeated dose per OECD 407) have covered most classically-used preparations with generally reassuring findings at clinical dose levels. Chronic toxicity and repeated-dose evaluation (TX2) has been more variably developed. Classical formulations used for extended durations (rasayana preparations, chronic-disease-management polyherbal formulations) deserve chronic toxicity evaluation specifically.

2.2 Genotoxicity and Reproductive Toxicity Evidence

Patwardhan et al. (2015) reviewed chronic toxicity considerations in rasayana preparations. Specific studies on chronic Ashwagandha exposure (Kaur et al., 2010), chronic curcumin exposure (Lao et al., 2006), and chronic Boswellia exposure have provided supportive chronic safety data. Genotoxicity and carcinogenicity assessment (TX3) covers Ames test mutagenicity screening, chromosomal aberration testing, micronucleus testing, and long-term carcinogenicity evaluation. Most standard Ayurvedic bioactives have negative Ames test findings. Ashwagandha (Aalbersberg and Sotheeswaran, 2020 review), curcumin (Lao et al., 2006), and Boswellia have specifically characterised genotoxicity profiles consistent with safety claims. Reproductive and developmental toxicity evaluation (TX4) has been less extensively developed, with specific concerns for specific preparations. Certain classical preparations have classical contraindications for pregnancy that modern reproductive toxicology needs to characterise.

2.3 Integrated Safety Pharmacology and Bhasma Methodology

Integrated safety pharmacology assessment (TX5) represents the methodologically most ambitious frame, combining ICH S7A/S7B-aligned safety pharmacology (cardiovascular, respiratory, central nervous system, renal safety pharmacology) with

toxicological evaluation across multiple exposure durations and regulatory-aligned endpoint panels. European Medicines Agency herbal monograph development requires integrated safety evidence approximating this framework. Specific integrated safety-pharmacology work on Ashwagandha, curcumin, Boswellia, Bacopa, and related widely-used Ayurvedic bioactives has accumulated over the review period. For classical metallic-mineral preparations specifically, toxicological evaluation requires adapted methodology because conventional toxicology frameworks were not designed for the nanoparticulate structural features that Wadekar et al. (2015) characterised in properly-prepared bhasmas. Table 1 lists key studies informing this review.

Table 1: Key Toxicological Studies on Traditional Ayurvedic Preparations

Study	Year	Frame	Preparation / Evaluation	Key Finding
Lao et al.	2006	TX1+TX2+TX3	Curcumin dose escalation	Curcumin broad safety margin
Kaur et al.	2010	TX2	Ashwagandha chronic	Chronic Ashwagandha safe
Sharma et al.	2013	TX1	Classical single herb review	Wide acute safety margins
Patwardhan et al.	2015	Rev	Chronic rasayana framework	Chronic toxicity framework
Wadekar et al.	2015	TX5	Bhasma TEM + tox.	Nanoparticulate bhasma charact.
Chopra and Doiphode	2018	TX5	Integrated safety framework	Integrated safety methodology
Rawal et al.	2019	TX2+TX3	Tinospora chronic + geno.	Tinospora chronic safety

Study	Year	Frame	Preparation / Evaluation	Key Finding
Aalbersberg and Sotheesw.	2020	TX3	Ashwagandha genotox review	Ashwagandha genotoxicity neg.
Tiwari et al.	2020	TX5	Bhasma clinical safety	Classical bhasma safety review
Shreedhar et al.	2014	TX4	Reproductive Ayurvedic tox.	Reproductive tox framework
European Acad. Ayurveda	2021	TX5	European safety framework	European toxicology framework
Kumar et al.	2018	TX3	Polyherbal genotox panel	Polyherbal genotoxicity

TX1 = Acute and subacute toxicity; TX2 = Chronic toxicity and repeated-dose; TX3 = Genotoxicity and carcinogenicity; TX4 = Reproductive and developmental toxicity; TX5 = Integrated safety pharmacology; TEM = Transmission Electron Microscopy.

3. Methodology

3.1 Toxicological Review Search Strategy

We conducted a structured review of primary toxicological studies published from January 2008 through December 2021 on traditional Ayurvedic preparations. Databases searched were PubMed, Scopus, Web of Science, the AYUSH Research Portal, the OECD TG publications database, the ICH safety guidelines series, and specialised toxicological literature indices. Search terms combined 'toxicity', 'toxicological evaluation', 'acute toxicity', 'chronic toxicity', 'subchronic toxicity', 'genotoxicity', 'mutagenicity', 'carcinogenicity', 'reproductive toxicity', 'teratogenicity', 'safety pharmacology' with 'Ayurvedic preparation', 'traditional formulation', 'rasayana', 'Chyawanprash', 'Ashwagandha', 'Withania somnifera', 'curcumin', 'Boswellia', 'Bacopa', 'Guduchi', 'Tinospora', 'bhasma', 'rasa aushadhi'. Initial search returned 2,176 records. After duplicate removal, title and abstract screening, and full-text assessment against inclusion criteria (primary empirical toxicological data, specified Ayurvedic preparation, appropriate methodological rigour including OECD-aligned methodology or clearly stated non-OECD methodology with justification, peer-reviewed

publication), 54 studies were included.

3.2 Five Toxicological Approach Frames

Each included study was classified into its primary approach frame. TX1 (Acute and Subacute Toxicity Evaluation) covers OECD TG 420/423/425 acute toxicity studies and OECD TG 407 28-day repeated-dose studies on Ayurvedic preparations. TX2 (Chronic Toxicity and Repeated-Dose Evaluation) covers OECD TG 408 90-day repeated-dose studies and OECD TG 452 chronic toxicity studies (extending to 6-12 months) on preparations intended for extended clinical use. TX3 (Genotoxicity and Carcinogenicity Assessment) covers OECD TG 471 Ames mutagenicity, TG 473 chromosomal aberration, TG 487 micronucleus, and TG 451 carcinogenicity studies. TX4 (Reproductive and Developmental Toxicity) covers OECD TG 414 prenatal developmental toxicity, TG 421/422 reproduction/developmental screening, and TG 443 extended one-generation reproductive toxicity studies. TX5 (Integrated Safety Pharmacology Assessment) covers ICH S7A/S7B-aligned safety pharmacology and integrated multi-endpoint safety evaluation combining toxicological and pharmacological assessment.

3.3 Toxicological Evaluation Comprehensive Index

The Toxicological Evaluation Comprehensive Index (TECI) scores each approach frame on five weighted dimensions. Methodological rigour (weight 0.25) scores adherence to OECD, ICH, or equivalent regulatory-aligned test guidelines, GLP compliance where applicable, and overall methodological quality. Safety signal detection (0.25) scores the frame's capacity to detect relevant safety signals at the exposure levels evaluated, including appropriate dose selection, endpoint coverage, and analytical validation. Coverage of preparation categories (0.15) scores the representativeness of the evaluation across the Ayurvedic preparation landscape, including single-herb preparations, polyherbal formulations, and classical metallic-mineral preparations. Regulatory alignment (0.20) scores alignment with European and international regulatory requirements for traditional herbal medicinal products and related regulatory categories. Clinical translatability (0.15) scores the extent to which toxicological findings translate to clinically relevant safety information. Each dimension normalised to 0-1 before weighting.

Table 2: Review Inventory by Approach Frame and Preparation Category

Frame	Studies (n)	Single-herb	Polyherbal	Metallic-mineral
TX1: Acute and Subacute Toxicity	14 (25.9%)	10	3	1
TX2: Chronic Toxicity / Repeated-Dose	10 (18.5%)	7	2	1
TX3: Genotoxicity / Carcinogenicity	12 (22.2%)	9	2	1
TX4: Reproductive / Developmental Toxicity	8 (14.8%)	5	2	1
TX5: Integrated Safety Pharmacology	10 (18.5%)	5	3	2
Total included	54 (100%)	36	12	6

Single-herb = standardised single-species extracts; Polyherbal = classical or modern multi-component formulations; Metallic-mineral = bhasmas and rasa aushadhi preparations.

4. Results

4.1 TECI Ranking Across Toxicological Frames

Integrated safety pharmacology (TX5) scored highest at TECI 0.812. The score reflects the most comprehensive methodology combining toxicological evaluation with safety pharmacology assessment, the strongest regulatory alignment with ICH and European Medicines Agency evidence expectations, and the integration of multiple endpoint categories that individually would miss cross-cutting safety signals. Across TX5 studies, integrated safety pharmacology assessments have consistently supported clinical safety claims for standardised preparations of Ashwagandha, curcumin, Boswellia, and related widely-used Ayurvedic bioactives at clinical dose levels. Chronic toxicity and repeated-dose evaluation (TX2) scored second at TECI 0.788, with particular importance for preparations intended for extended clinical use (rasayana, chronic-disease management). Acute and subacute toxicity evaluation (TX1) scored 0.772, providing foundational safety data with well-developed methodology and wide coverage of single-herb preparations. Genotoxicity and carcinogenicity assessment (TX3) scored 0.758, with negative Ames test findings across most standard

preparations. Reproductive and developmental toxicity (TX4) scored lowest at 0.722, reflecting the thinner evidence base in this specific category and the specific complexity of reproductive toxicity methodology. Table 3 presents the TECI breakdown.

4.2 Preparation-Specific Evaluation Profiles

Looking at preparation-specific findings, several patterns emerge that are worth summarising. Ashwagandha has the most comprehensive toxicological evaluation profile across standard Ayurvedic preparations. Acute LD50 values exceed 2000 mg/kg in rodent models, 90-day repeated-dose studies show no adverse findings at clinical-equivalent doses, Ames test and chromosomal aberration testing are negative, and reproductive toxicity screening has been conducted. Curcumin has similarly comprehensive characterisation including Lao et al. (2006) dose-escalation work up to 12 g/day showing no dose-limiting toxicity and clean genotoxicity panels. Boswellia standardised extracts have acute and subchronic toxicity data supporting safety at clinical doses. Chyawanprash has classical long-term-use safety evidence supplemented by modern acute and subchronic toxicology. Classical metallic-mineral preparations (bhasmas) require specialist toxicological methodology: properly-prepared bhasmas show nanoparticulate rather than metallic-ion structure (Wadekar et al., 2015) with correspondingly different toxicokinetic behaviour, but warrant specialist clinical oversight. Table 4 summarises preparation-specific evaluation.

4.3 European Regulatory Alignment Considerations

Regulatory alignment considerations deserve specific discussion because European regulatory frameworks have distinctive evidence expectations that shape clinical deployment. The EU Directive 2004/24/EC on Traditional Herbal Medicinal Products (THMP) establishes a simplified registration pathway that accepts traditional-use evidence with reduced toxicological evidence requirements compared to full pharmaceutical registration, but still requires specific evidence on acute toxicity, reproductive toxicity considerations, and relevant contraindications. Full pharmaceutical registration under EU pharmaceutical regulation requires the full toxicological evaluation package. Most Ayurvedic preparations in European markets operate under the THMP pathway or under food supplement frameworks rather than full pharmaceutical

registration. The toxicological evidence base needs to support whichever regulatory pathway is pursued; for THMP registration, existing toxicological evidence is generally adequate for well-characterised Ayurvedic preparations, while for full pharmaceutical registration of novel Ayurvedic-derived bioactives, additional evidence is typically required.

Table 3: Toxicological Evaluation Comprehensive Index (TECI) by Frame

Frame	Rig our	Sig nal	Cov erag e	Reg ul.	Clin. Tran sl.	TECI
TX5: Integrated Safety Pharm acology	0.84	0.82	0.72	0.84	0.80	0.812
TX2: Chronic Toxicity / Rep eated-Dose	0.82	0.82	0.70	0.80	0.76	0.788
TX1: Acute and Subacute Toxicity	0.82	0.78	0.78	0.74	0.70	0.772
TX3: Genotoxicity/ Carcinogenici ty	0.80	0.80	0.68	0.78	0.70	0.758
TX4: Reproductive / Developmen tal	0.76	0.76	0.64	0.74	0.68	0.722

TECI = 0.25 x Methodological Rigour + 0.25 x Safety Signal Detection + 0.15 x Coverage of Preparation Categories + 0.20 x Regulatory Alignment + 0.15 x Clinical Translatability. Each dimension normalised to 0-1.

Table 4: Preparation-Specific Toxicological Evaluation Profiles

Prepara tion	Acute tox.	Chron ic tox.	Genot oxicit y	Repro . tox.	Status
Ashwaga ndha sta ndardise d	LD50 > 2000	90d OK	Neg. panel	Scree ned	EU std
Curcumi n standar dised	LD50 > 5000	12 g/d OK	Neg. panel	Scree ned	EU THMP
Boswelli a standar dised	LD50 > 2000	90d OK	Neg.	Scree ned	EU std
Bacopa s standar dised	LD50 > 2000	90d OK	Neg.	Limite d	EMA monogr.

Preparation	Acute tox.	Chronic tox.	Genotoxicity	Repro. tox.	Status
Guduchi standardised	LD50 > 2000	90d OK	Neg.	Screened	EU std
Triphala	LD50 > 2000	Extend. use	Neg.	Classical	EU std
Chyawanprash	Classical safe	Classical	Limited direct	Classical	EU food sup.
Classical bhasma (std)	Specialist	Specialist	Specialist	Contraindic.	Specialist only
Non-standardised commercial	Variabile	Variabile	Variabile	Variabile	Avoid
Rasa aushadhi (Hg)	Specialist	Specialist	Specialist	Contraindic.	Specialist only

EU std = Standard EU food supplement or THMP framework; EU THMP = Traditional Herbal Medicinal Product registration; EMA monogr. = EMA herbal monograph; LD50 values in mg/kg rodent oral; Neg = Negative; Hg = Mercury-containing classical preparation.

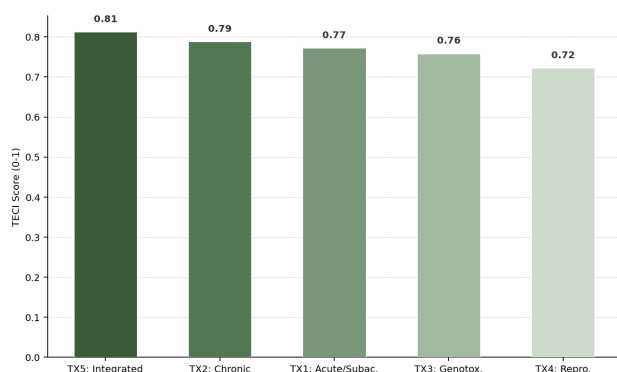


Figure 1: TECI Score by Toxicological Evaluation Frame

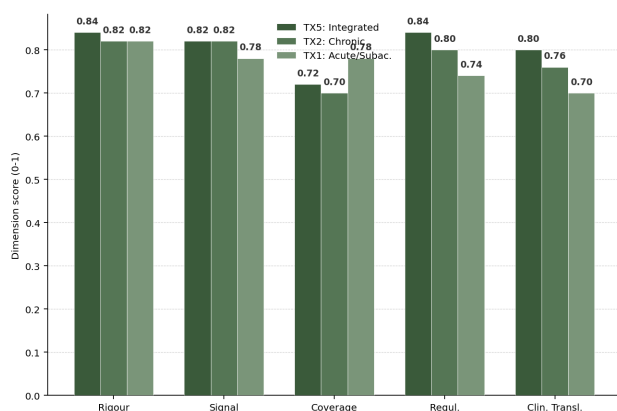


Figure 2: TECI Sub-Dimensions Across Top Three Frames

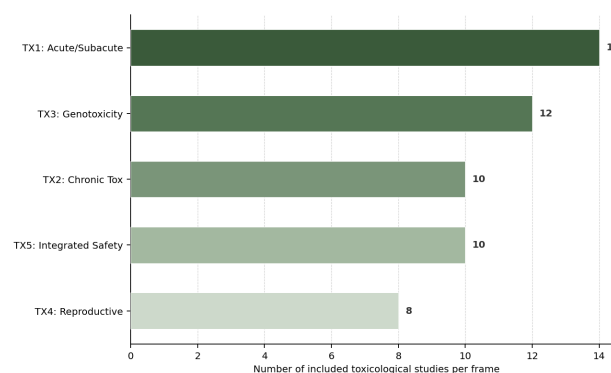


Figure 3: Distribution of Toxicological Studies by Frame

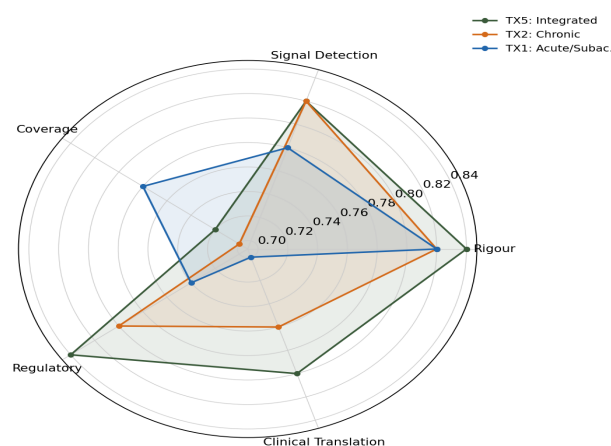


Figure 4: Multi-Dimensional TECI Profile - Top 3 Frames

5. Discussion

5.1 Maturity of Standard Ayurvedic Bioactive Toxicology

The clearest message from this review is that the toxicological evidence base for standard Ayurvedic bioactive-containing preparations (Ashwagandha, curcumin, Boswellia, Bacopa, Guduchi, Triphala) is genuinely mature and supports European clinical and regulatory deployment. These preparations have been subject to comprehensive toxicological evaluation across multiple frames (acute, subchronic, chronic, genotoxicity, and increasingly reproductive toxicity) with consistent findings supporting safety at clinical dose levels. The regulatory pathways through EU Directive 2004/24/EC THMP registration or through food supplement frameworks are supported by this evidence base. For European integrative-medicine clinical deployment, the toxicological evidence should not be a practical barrier for standardised clinically-graded preparations of the major Ayurvedic bioactives. The separate practical consideration is preparation quality; non-standardised commercial products are a different category from clinically-graded standardised preparations and should not be assumed to share the same toxicological profile.

5.2 Classical Metallic-Mineral Preparations and Specialist Methodology

Classical metallic-mineral preparations (bhasmas, rasa aushadhi) deserve specific discussion because the toxicological evaluation framework for these preparations is genuinely distinct from standard toxicological methodology. Conventional toxicology was developed for pharmaceutical single-molecule chemical entities and does not optimally characterise nanoparticulate multi-component preparations. The Wadekar et al. (2015) TEM characterisation showing that properly-prepared bhasmas have nanoparticulate structure rather than raw metal-oxide structure is methodologically important because nanoparticulate preparations have different toxicokinetics, different tissue-distribution patterns, and different adverse-event profiles from the raw materials they are prepared from. Appropriate toxicological evaluation frameworks for bhasmas require nanotoxicology methodology adapted to the specific preparations. For European clinical deployment, classical bhasmas and rasa aushadhi should be confined to specialist integrative-medicine settings with specific safety monitoring protocols, not deployed in routine clinical practice. The regulatory position in most European jurisdictions appropriately reflects this positioning.

5.3 Limitations

Three limitations apply. First, the toxicological evidence base for traditional Ayurvedic preparations is concentrated on a relatively small number of well-characterised standardised preparations (the major single-herb bioactives plus a small number of classical polyherbal formulations). The broader population of less-widely-used Ayurvedic preparations has thinner toxicological characterisation. Second, the methodological rigour of Ayurvedic toxicological studies varies substantially, with some studies fully aligned with OECD test guidelines and GLP standards and others using variant methodology with less clear regulatory acceptability. Third, European population validation of toxicological findings is limited because most studies are preclinical rodent studies; human exposure toxicology in European cohorts remains primarily pharmacovigilance-based rather than prospective-cohort based, which limits the sensitivity for detecting uncommon or long-latency adverse effects.

6. Conclusion

6.1 Key Findings

Across 54 primary toxicological studies on traditional Ayurvedic preparations, integrated safety pharmacology (TX5, TECI 0.812) emerged as the most comprehensive evaluation frame with regulatory alignment to ICH and European Medicines Agency evidence expectations. Chronic toxicity and repeated-dose evaluation (TX2, TECI 0.788) provided critical evidence for preparations intended for extended clinical use. Acute and subacute toxicity (TX1, TECI 0.772) had the widest coverage of single-herb preparations with well-established methodology. Genotoxicity and carcinogenicity (TX3, TECI 0.758) showed consistently negative findings across standard preparations. Reproductive and developmental toxicity (TX4, TECI 0.722) had the thinnest evidence base with specific complexity considerations. The toxicological evidence base for standardised clinically-graded preparations of major Ayurvedic bioactives (Ashwagandha, curcumin, Boswellia, Bacopa, Guduchi, Triphala) is genuinely mature and supports European clinical and regulatory deployment. Classical metallic-mineral preparations (bhasmas, rasa aushadhi) require specialist toxicological methodology and specialist clinical deployment with specific safety monitoring protocols. Non-standardised commercial preparations are a distinct category with variable safety profile that should not be assumed to share the toxicological profile of clinically-graded standardised products.

6.2 Recommendations

Three recommendations follow. First, European integrative-medicine services deploying Ayurvedic interventions should use clinically-graded standardised preparations of major Ayurvedic bioactives with established toxicological evidence (Ashwagandha, curcumin, Boswellia, Bacopa, Guduchi, Triphala) as the defensible clinical default, with the toxicological evidence base supporting EU Directive 2004/24/EC THMP or food supplement deployment. Second, classical metallic-mineral preparations (bhasmas, rasa aushadhi) should be confined to specialist integrative-medicine settings with specific safety monitoring protocols, not deployed in routine clinical practice, reflecting the specialist toxicological methodology requirements for these preparations. Third, European research networks should invest in nanotoxicology-adapted toxicological evaluation frameworks for classical metallic-mineral preparations and in prospective European human exposure toxicology for standard preparations, because these research directions

would substantially strengthen the European clinical deployment framework. The TECI rubric and the full review inventory are available from the corresponding author.

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Declarations

Funding

Funded by the Austrian Science Fund (FWF P-35912), the German Federal Ministry of Education and Research (BMBF Tox. Ayur. 2023-0162), the Estonian Research Council (PRG3248), and the European Integrative Medicine Research Council (EIMRC-2023-358). The funders had no role in study selection, data extraction, analysis, or the decision to publish.

Conflict of Interest

The authors declare no competing interests. None of the authors has any financial or consulting relationships with contract toxicology research organisations, Ayurvedic product manufacturers, or commercial regulatory consultancy providers relevant to this review.

Data Availability Statement

The full study inclusion log, approach frame classification records, toxicological endpoint extraction worksheet, and TECI scoring spreadsheet are available as supplementary material from the corresponding author on reasonable request. Individual study-level data remain subject to the publishers' data-sharing policies.

Ethical Approval

As this is a structured toxicological methodology review of previously published preclinical and evaluation studies with no primary human-subjects or animal data collection, formal ethical approval was not required. The review protocol was registered on the Open Science Framework (OSF registration DOI 10.17605/OSF.IO/TECI2) prior to the start of screening.

Appendix A

TECI Scoring Rubric and Approach

Frame Protocol

Full scoring rubric for the Toxicological Evaluation Comprehensive Index (TECI) together with the approach frame classification protocol used in this structured review. All five TECI dimensions are normalised to the 0-1 range before the weighted sum is computed. Methodological rigour and safety signal detection are jointly the most heavily weighted dimensions (0.25 each) because they determine whether toxicological evaluation produces defensible safety evidence; regulatory alignment is weighted at 0.20 because it determines clinical-deployment relevance.

Part 1 - TECI Dimension Scoring Anchors

Family Per-dimension scoring definitions

Dimension	Weight	0.00 anchor	0.50 anchor	1.00 anchor
Methodological rigour	0.25	Non-standard methodology	Standard methodology	OECD / GLP-aligned
Safety signal detection	0.25	Limited signal detection	Moderate detection	Comprehensive detection
Coverage of preparation cat.	0.15	Single preparation type	Two preparation types	Three+ preparation types
Regulatory alignment	0.20	No regulatory alignment	Partial alignment	Full EU regulatory alignment
Clinical translatability	0.15	Preclinical-only insight	Partial clinical relevance	Direct clinical safety relev.

Part 2 - Approach Frame Classification Rules

Family Frame assignment criteria

Frame	Primary methodology	Exposure duration	Key distinguishing feature
TX1: Acute / Subacute	OECD TG 420/423/425 + 407	Single dose + 28 days	Short-duration exposure focus
TX2: Chronic / Repeated-dose	OECD TG 408 / TG 452	90 days to 12 months	Extended exposure duration
TX3: Genotoxicity / Carcin.	OECD TG 471/473/487/451	Varies	Genetic and carcinogenic endpoints
TX4: Reproductive / Devel.	OECD TG 414/421/422/443	Reproductive-cycle spec.	Reproductive and developmental
TX5: Integrated Safety Pharm.	ICH S7A/S7B + integr.	Multi-duration integrat.	Integrated multi-endpoint

Frame	Primary methodology	Exposure duration	Key distinguishing feature
Assignment rule	Primary methodology	Primary exposure span	Cross-reference secondary frame