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SAFETY DETERMINANTS OF HERBO-MINERAL FORMULATIONS

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Abstract: This review systematically evaluates the safety determinants of herbo-mineral formulations (Rasaushadhi) by integrating classical processing parameters, metal speciation dynamics, gut microbiome interactions, and organ-specific toxicity profiles. Formulation safety depends critically on the quality of Shodhana and Marana processes, bioaccessibility of transformed metal species, and host-microbiome interactions, rather than on metallic content alone. Classical processing demonstrably alters metal physicochemical form and biological reactivity; for instance, purification and incineration reduced acute toxicity compared to crude materials, and finished formulations such as Chondrokola Rosh and Sidh Makardhwaj showed no remarkable organ toxicity in rodent models. Furthermore, the gut microbiome critically mediates heavy metal bioavailability, with gnotobiotic mouse models revealing that microbiome depletion significantly increases arsenic bioaccumulation and mortality, while probiotic supplementation with Lactobacillus plantarum CCFM8610 reduces tissue cadmium accumulation. Classical texts describe Anupana as modifying drug action through five mechanistic themes, and a human pharmacokinetic study of Swarna Bhasma showed that honey produced the highest early blood gold exposure with systemic bioavailability below 0.01 percent. Integration of biomarker data identified urinary kidney injury molecule-1 as a highly sensitive early marker of subclinical renal injury. Properly processed formulations demonstrate minimal overt organ toxicity, whereas contaminated or improperly prepared products pose significant hepatorenal risk.

***Keywords*:** *Rasaushadhi, Shodhana, Marana, Metal-speciation, Bioaccessibility, Gut-microbiome, Bioaccumulation, Anupana, Bioavailability, Hepatorenal-risk.*

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INTRODUCTION

The use of herbo-mineral formulations, known as Rasaushadhi in the Ayurvedic system of medicine, represents a sophisticated therapeutic tradition that has been practiced for centuries. These formulations intentionally incorporate processed metals and minerals, such as mercury, iron, copper, and arsenic, combined with herbal extracts, with the aim of achieving potent pharmacological effects at low doses. However, the presence of heavy metals in these products has generated significant and persistent controversy within the global biomedical community. Case reports have documented severe hepatotoxicity and nephrotoxicity associated with the consumption of certain herbo-mineral products, leading to widespread concerns about their safety [1]. Conversely, a substantial body of preclinical evidence indicates that properly manufactured formulations, such as Sidh Makardhwaj and Chondrokola Rosh, do not induce overt organ toxicity in animal models, even at multiples of the human-equivalent dose. This apparent contradiction suggests that the safety of these formulations is not an inherent property of their metallic constituents but is instead critically dependent on a complex interplay of manufacturing quality, biological transformation, and host factors.

The central hypothesis of this research posits that the safety of herbo-mineral formulations is determined by the quality of classical purification (Shodhana) and incineration (Marana) processes, the bioaccessibility and speciation of transformed metal species, and the modulating role of the host gut microbiome. Classical processing is not merely a physical reduction step; it is a multi-step procedure involving repeated heating, quenching, and trituration with herbal media that fundamentally alters the physicochemical form and biological reactivity of the starting materials [2]. For example, the Shodhana of Croton tiglium seeds has been shown to decrease cytotoxic concentration and alter genotoxicity outcomes, while the structural investigation of Lauha Bhasma confirmed that multi-step processing changes the metal phase and particle structure. Furthermore, the gut microbiome has emerged as a critical mediator of heavy metal bioavailability and toxicity. Gnotobiotic mouse models have demonstrated that microbiome depletion significantly increases arsenic bioaccumulation and mortality, while the presence of specific taxa, such as Faecalibacterium prausnitzii, is protective [3]. Dietary co-factors, including the use of adjuvant vehicles (Anupana), further modify systemic exposure and toxicity profiles.

The safety evaluation of herbo-mineral formulations has historically been dominated by two conflicting narratives. On one hand, clinical case reports have linked the consumption of certain Ayurvedic products to severe hepatorenal toxicity, raising alarms about the inherent danger of heavy metal exposure [4]. On the other hand, a growing body of preclinical evidence suggests that properly processed formulations, such as Sidh Makardhwaj and Chondrokola Rosh, do not induce overt organ damage in animal models even at multiples of the human-equivalent dose [5]. This paradox has motivated researchers to move beyond simple heavy metal quantification and instead investigate the physicochemical transformations that occur during classical processing.

The Shodhana and Marana procedures are central to this transformation. These multi-step processes involve repeated heating, quenching in herbal media, and trituration, which fundamentally alter the crystalline structure, particle size, and oxidation state of the starting metals [6]. For instance, X-ray absorption spectroscopy studies have shown that the mercury in Sidh Makardhwaj exists predominantly as a mercury-sulfur complex rather than as elemental mercury, a form that is significantly less bioavailable and less toxic [7]. Similarly, the iron in Lauha Bhasma is transformed from metallic iron to a mixture of iron oxides and iron-carbide phases, which exhibit reduced reactivity in biological systems [6]. These structural changes are not merely cosmetic; they directly impact the bioaccessibility of the metals in the gastrointestinal tract. In vitro digestion models have demonstrated that the release of metals from properly processed bhasmas is substantially lower than from crude mineral powders, suggesting that classical processing reduces the fraction of metal available for systemic absorption [8].

The gut microbiome has emerged as a critical, yet often overlooked, modulator of heavy metal toxicity. The intestinal microbiota can directly alter the speciation and bioavailability of ingested metals through several mechanisms, including reduction, methylation, and thiolation [9]. For example, germ-free mice and antibiotic-treated mice exhibit significantly higher arsenic bioaccumulation and mortality compared to conventionally colonized mice when exposed to the same dose of arsenic [9]. This protective effect is partially attributable to specific bacterial taxa, such as Faecalibacterium prausnitzii, which can sequester arsenic and reduce its absorption across the intestinal epithelium [10]. Furthermore, the gut microbiome can methylate inorganic arsenic to monomethylarsonic acid and dimethylarsinic acid, which are more readily excreted in urine, thereby reducing the body burden of the more toxic inorganic forms [9]. Probiotic interventions have also shown promise; Lactobacillus plantarum CCFM8610, which possesses cadmium-binding capacity, has been shown to reduce tissue cadmium accumulation and improve gut barrier integrity in rodent models [11].

Dietary co-factors, particularly the use of Anupana (adjuvant vehicles), further modulate the pharmacokinetics and toxicity of herbo-mineral formulations. Classical Ayurvedic texts describe Anupana as a critical component that modifies drug action through five recurring mechanistic themes: carrier and bioenhancer function, digestive facilitation, channel-level transport, toxicity moderation, and dosha-directed pharmacology [12]. A human pharmacokinetic study of Swarna Bhasma (gold ash) administered with different Anupana media—honey, ghee, and black pepper—demonstrated that honey at 2.5 g produced the highest early blood gold exposure, with an average maximum plasma concentration of 0.002333 µg/L at a time to maximum concentration of 3 hours. However, the overall systemic bioavailability was less than 0.01% over the 5-hour sampling window, indicating that the vast majority of the administered gold was not absorbed [13]. Notably, honey and ghee taken together in equal weight are considered incompatible and potentially harmful according to classical texts, highlighting the importance of proper Anupana selection.

The assessment of organ toxicity in herbo-mineral formulation research has traditionally relied on conventional biomarkers such as serum creatinine, blood urea nitrogen, and liver enzymes. However, these markers are insensitive to early, subclinical injury. Urinary kidney injury molecule-1 (KIM-1) has emerged as a highly sensitive and specific early marker of tubular damage, capable of detecting injury before serum creatinine becomes abnormal [14]. In Sri Lankan populations with chronic kidney disease of unknown etiology, urinary KIM-1 identified early kidney injury even in cases with normal conventional markers and was more sensitive than neutrophil gelatinase-associated lipocalin (NGAL) for early tubular damage [15]. For herbo-mineral formulations specifically, the Chondrokola Rosh rat study with 90-day chronic oral administration at 100 mg/kg/day reported no remarkable changes in liver enzymes, with bilirubin decreasing and albumin increasing, indicating subclinical or no overt biochemical hepatotoxicity [16]. The Sidh Makardhwaj study with 28-day administration at up to five times the human-equivalent dose reported no toxicological effects on rat liver or kidney [5]. Human hair and nail analysis is supported as a useful exposure biomarker for toxic metals, though no retrieved study has linked hair or nail metal levels to KIM-1, NGAL, liver elastography, or cumulative Rasaushadhi exposure [17].

PROCESSING PARAMETERS AND METAL SPECIATION

Classical Ayurvedic processing, specifically Shodhana (purification) and Marana (incineration), demonstrably alters the physicochemical form and biological reactivity of metals, thereby constituting the foundational safety determinant for herbo-mineral formulations. A toxicity-focused study on a metal preparation reported that purification and incineration reduced acute toxicity compared to the crude material, although detailed processing variables and speciation data were not provided in the retrieved snippet [18]. Similarly, a study on Shodhana of Croton tiglium seeds found that purification decreased cytotoxic concentration and altered genotoxicity outcomes, indicating that classical processing can materially change toxicity profiles [19]. A review of Bhasmas and Tibetan medicines noted that lead- and arsenic-

containing Ayurvedic medicines can show high bioaccessibility, with some compounded products having very high lead content and complete bioaccessibility [20].

Structural investigation of Lauha Bhasma (iron-based) confirmed that the multi-step processing involving repeated heating and cooling changes the metal phase and particle structure [6]. The finished product of Chondrokola Rosh (CKR), a formulation containing roasted mercury and copper with medicinal herbs, showed no remarkable liver toxicity in rats despite containing traces of transformed heavy metals [16]. Sidh Makardhwaj, a mercury-based formulation, was reported to have no toxicological effects on rat brain, liver, or kidney at up to five times the human-equivalent dose for 28 days [5]. Manikya Bhasma, an incinerated herbo-metallic preparation of purified ruby and arsenic sulfide, was studied mainly for its cytotoxic and apoptotic effects, not for kidney or liver biomarker profiling [21].

The literature supports the general hypothesis that classical processing can reduce toxicity and alter metal behavior, and that bioaccessibility is a key determinant of exposure. However, the evidence is insufficient to build a quantitative regression or ANOVA dataset for correlating Shodhana and Marana parameters with speciation and in vitro toxicity outcomes. A practical extraction template for future studies includes columns for study identifier, formulation name, Shodhana type (media, cycles, temperature, duration, L:S ratio), Marana parameters (Puta cycles, Puta type, peak temperature, fuel, Bhavana media/cycles, particle size, total time), speciation technique (XANES, EXAFS, XPS, XRD, TEM-EDX), bioaccessibility assay, cytotoxicity (cell line, IC50, assay), transcriptomics, and oxidative stress markers.

GUT MICROBIOME MODULATION OF HEAVY METAL BIOAVAILABILITY

The gut microbiome emerges as a critical mediator of heavy metal bioavailability and toxicity, with evidence drawn primarily from non-Ayurvedic heavy-metal exposure models that are highly relevant to herbo-mineral formulations. The strongest evidence supports a model where microbiome composition and stability are major determinants of metal bioavailability, fecal elimination, and organ toxicity, especially for arsenic and lead [22].

Gnotobiotic mouse models have provided compelling evidence for the protective role of the gut microbiome. Coryell et al. demonstrated that microbiome depletion via germ-free status or antibiotic treatment significantly increases arsenic bioaccumulation and mortality, while a stable microbiome, particularly with Faecalibacterium prausnitzii, is protective [10]. Specifically, germ-free mice excreted less arsenic in stool and accumulated more in organs, leading to increased mortality compared to conventionally colonized mice [10]. The gut microbiome alters arsenic oxidation state, methylation, and thiolation, thereby changing its toxicity and internal burden [23]. Disruption of the gut microbiome was shown to alter arsenic biotransformation and increase liver toxicity in mice [9].

Dietary co-factors significantly modify susceptibility to heavy metal toxicity. A high-fat diet increased liver and kidney metal accumulation and worsened toxicity while reducing fecal excretion in mice exposed to arsenic, cadmium, and lead [22]. This study reported that high-fat diet-fed mice accumulated more metals in liver and kidney and excreted less in feces, with specific microbial taxa such as Bacteroides, Lactobacillus, Butyricimonas, Dorea, Desulfovibrio, and Roseburia varying by metal and diet [22]. Probiotic supplementation with Lactobacillus plantarum CCFM8610, which possesses cadmium-binding capacity, reduced tissue cadmium accumulation and improved gut barrier integrity in mice. This intervention increased fecal cadmium excretion and decreased tissue cadmium accumulation while reducing intestinal permeability [11].

The protective signatures center on stable microbiomes, Faecalibacterium prausnitzii, and probiotic Lactobacillus strains, whereas susceptibility is linked to microbiome depletion, high-fat diet, and dysbiosis-associated taxa such as Desulfovibrio and some exposure-responsive Bacteroides patterns [22]. Chronic multi-metal exposure in human residents near mining and smelting areas has been associated with microbiome remodeling, including changes in Lachnospiraceae, Eubacterium eligens, and Ruminococcaceae [25]. Lead exposure in mice has been shown to enrich metal-resistance functions in gut taxa, indicating that the microbiome can adapt to heavy metal stress [26].

These findings have direct implications for herbo-mineral formulation safety. The gut microbiome may serve as a biological barrier that limits the systemic absorption of metals released from bhasmas during gastrointestinal transit. Conversely, individuals with dysbiosis-whether due to antibiotic use, high-fat diet, or other factors-may be at increased risk of metal bioaccumulation and toxicity. This suggests that the safety of herbo-mineral formulations is not solely determined by the formulation itself but is also contingent on the host's microbiome status. Future studies should therefore consider incorporating microbiome analysis as a standard component of safety evaluation for herbo-mineral formulations, and probiotic interventions may offer a strategy to mitigate potential risks in susceptible populations.

ANUPANA: CLASSICAL MECHANISMS AND PHARMACOKINETIC EFFECTS

Classical Ayurvedic texts describe Anupana (adjuvant vehicle) as a critical component that modifies drug action, with five recurring mechanistic themes: carrier and bioenhancer function, digestive facilitation, channel-level transport, toxicity moderation, and dosha-directed pharmacology [12,27,28]. These themes are not merely theoretical; they provide a framework for understanding how the choice of vehicle can fundamentally alter the pharmacokinetic and toxicokinetic profile of a herbo-mineral formulation. The classical roles of common Anupana substances, along with their proposed modern pharmacokinetic and toxicokinetic effects, are detailed in Table 01.

Table 01: Classical roles and proposed modern pharmacokinetic and toxicokinetic effects of commonly used Anupana substances in Ayurveda.

Anupana Substance	Classical Role	Proposed Modern Pharmacokinetic Effect	Proposed Toxicokinetic Effect
Madhu (Honey)	Yogavahi, Srotoshodhana, Deepana, Pachana	Enhances absorption, dispersion, and systemic delivery; may alter intestinal uptake of fine particles	Reduces accumulation in channels; supports elimination of toxic residues
Ghrita (Ghee)	Yogavahi, Sneha, Srotas-anugata	Lipid vehicle may facilitate transport of lipophilic constituents and modulate distribution	May protect tissues, soften sharpness of potent drugs, and reduce irritation/toxicity
Jala (Water)	Anulomana, Anupana, universal supportive medium	Assists dissolution and oral administration; may aid transit and timing of absorption	Gentle elimination support; generally low toxicity risk
Ksheera (Milk)	Balya, Sheeta, Pitta/Rakta pacifying	May slow absorption and buffer mucosal irritation; supports tolerability	Tissue-soothing and protective effect, especially in hot/sharp conditions
Kwatha/Yusha/Swarasa	Srotoshodhana, Pachana, Anulomana	Can alter gastric emptying, solubilization, and drug milieu; may change metal dispersion and uptake	Supports detoxification and digestion, reducing harmful retention

The classical texts also specify safety modifiers that are critical for clinical practice. Honey and ghee taken together in equal weight are considered incompatible (Viruddha Ahara) and potentially harmful, a warning that has been supported by modern toxicological studies [29,30]. Dosha-specific selection is emphasized: honey is favored in Kapha-dominant contexts, ghee in Pitta-dominant contexts, and warm or rough liquids in Kapha states, while sweet and cooling liquids are used in Pitta and Rakta states. This individualized approach to vehicle selection is a hallmark of Ayurvedic pharmacology and underscores the importance of considering host factors in safety assessment.

Quantitative pharmacokinetic data on Anupana effects are limited but concentrated in Swarna Bhasma (gold) studies. A human bioavailability study with 30 healthy participants compared four different Anupana media for Swarna Bhasma at a 30 mg oral dose [13]. The results showed that honey at 2.5 g produced the highest early blood gold exposure, with an average maximum plasma concentration (Cmax) of 0.002333 µg/L at a time to maximum concentration (Tmax) of 3 hours. The overall systemic bioavailability was reported as less than 0.01% over the 5-hour sampling window, indicating that the vast majority of the administered gold was not absorbed into systemic circulation [13]. The black pepper-ghee combination, lactose, and glucose were also tested, but honey was the most effective in enhancing absorption [13,30]. No toxicity outcomes were reported in this study. A narrative review suggested that ghee may enhance absorption and bioassimilation, especially for lipophilic compounds, but provided no metal-specific pharmacokinetic parameters [31].

The pharmacokinetic data from the Swarna Bhasma study are particularly instructive. The extremely low systemic bioavailability (<0.01%) suggests that the vast majority of the administered gold is either excreted in feces or retained in the gastrointestinal tract without being absorbed [13]. This finding is consistent with the hypothesis that properly processed bhasmas are designed to deliver their therapeutic effects locally within the gastrointestinal tract or through transient interactions with the gut-associated lymphoid tissue, rather than through systemic absorption. The fact that honey enhanced absorption, albeit to a very low absolute level, suggests that the choice of Anupana can modulate the fraction of metal that becomes bioavailable [13]. This modulation may be clinically significant, as even small changes in systemic exposure could accumulate over repeated dosing.

The classical concept of Yogavahi-the property of an Anupana to carry the drug to its target tissue—is supported by the differential absorption observed with different vehicles. Honey, with its high sugar content and viscosity, may alter gastric emptying time and intestinal transit, thereby increasing the contact time between the bhasma particles and the absorptive epithelium. Ghee, as a lipid vehicle, may facilitate the transport of lipophilic constituents or metal complexes that partition into lipid phases [31]. The black pepper-ghee combination, which includes piperine, a known bioenhancer, did not outperform honey in this study, suggesting that the bioenhancing effects of piperine may be formulation-specific or dose-dependent [13,30].

The toxicokinetic implications of Phyto-adjuvant selection are equally important. Classical texts describe certain Anupana as having toxicity-moderating properties, meaning they can reduce the harmful effects of potent drugs. For

example, milk is described as having a tissue-soothing and protective effect, particularly in conditions of heat or sharpness (Pitta and Rakta). This suggests that milk may buffer the mucosal irritation caused by certain metal formulations, thereby reducing local toxicity. Similarly, honey is described as supporting the elimination of toxic residues, which may correspond to enhanced fecal excretion of unabsorbed metals. These classical observations align with modern concepts of toxicokinetics, where the vehicle can influence not only the rate and extent of absorption but also the distribution and elimination of the toxicant.

The integration of classical Anupana mechanisms with modern pharmacokinetic data provides a nuanced understanding of how adjuvant vehicles modulate the safety profile of herbo-mineral formulations. The choice of Anupana is not a trivial matter; it can alter the fraction of metal that becomes systemically available, the rate at which it appears in the bloodstream, and potentially its tissue distribution and elimination. Future studies should prioritize the systematic evaluation of Anupana effects on the pharmacokinetics and toxicokinetics of a broader range of herbo-mineral formulations, including those containing mercury, arsenic, and lead. Such studies should employ longer sampling windows (beyond 5 hours) to capture the full absorption profile and should include measurements of metal speciation in blood and urine to understand the form in which the metal is circulating. Furthermore, the classical warnings about incompatible combinations, such as honey and ghee, should be investigated using modern toxicological methods to validate their clinical relevance.

EARLY SUBCLINICAL ORGAN INJURY BIOMARKERS

The integration of multi-omics and biomarker data for early injury profiling reveals that urinary kidney injury molecule-1 (KIM-1) is a highly sensitive early marker of subclinical renal injury, particularly for detecting tubular damage before conventional markers such as serum creatinine become abnormal [14]. In Sri Lankan populations with chronic kidney disease of unknown etiology (CKDu), urinary KIM-1 identified early kidney injury, including cases with normal conventional markers, and was more sensitive than neutrophil gelatinase-associated lipocalin (NGAL) for early tubular damage [15]. In postoperative acute kidney injury (AKI) literature, KIM-1 showed high diagnostic performance and an earlier rise than creatinine, with studies reporting that KIM-1 levels increase within hours of injury, whereas creatinine may take days to become abnormal [32,33]. This temporal advantage makes KIM-1 a particularly valuable tool for monitoring potential nephrotoxicity in the context of herbo-mineral formulation use, where cumulative low-level exposure may cause subtle tubular damage that escapes detection by standard clinical chemistry.

For herbo-mineral formulations specifically, the evidence for early subclinical injury biomarkers is sparse but instructive. The Chondrokola Rosh (CKR) rat study, involving 90-day chronic oral administration at 100 mg/kg/day, reported no remarkable changes in liver enzymes (sGOT, sGPT, ALP), with bilirubin decreasing and albumin increasing, indicating subclinical or no overt biochemical hepatotoxicity [16]. The Sidh Makardhwaj study, with 28-day administration at up to five times the human-equivalent dose, reported no toxicological effects on rat liver or kidney [5]. These findings suggest that properly manufactured formulations, despite containing transformed heavy metals, do not induce overt organ injury in rodent models. However, neither study employed early subclinical biomarkers such as KIM-1 or NGAL, leaving open the possibility that subtle, subclinical tubular damage may have occurred without detection.

Human hair and nail analysis is supported as a useful exposure biomarker for toxic metals, providing a cumulative measure of long-term exposure that is not subject to the diurnal variation of blood or urine levels [17,34,35]. However, no retrieved study linked hair or nail metal levels to KIM-1, NGAL, liver elastography, or cumulative Rasaushadhi exposure. This represents a significant gap in the literature, as such a linkage would provide a direct method for monitoring the safety of long-term herbo-mineral formulation use in human populations. The absence of this data is particularly notable given that hair and nail analysis is non-invasive, relatively inexpensive, and can provide a historical record of exposure over months to years.

No retrieved study provided the full multi-omics package of quantitative speciation, bioaccessibility, HK-2/HepG2 transcriptomics, rodent metallomics-metabolomics-epigenetics, and human longitudinal biomarker-outcome linkage for herbo-mineral formulations [18,19,20]. The strongest directly usable biomarker signal is that urinary KIM-1 is a sensitive early marker of subclinical renal injury, while finished, properly manufactured Ayurvedic metal preparations in the retrieved animal studies showed minimal overt liver or kidney toxicity despite containing transformed metal content [5,14,16]. This disconnect between the sensitivity of KIM-1 in other contexts and its absence in herbo-mineral formulation studies underscores the need for prospective studies that incorporate these biomarkers into the safety evaluation framework.

The implications of these findings for clinical practice and research are substantial. First, the incorporation of KIM-1 and NGAL into routine safety monitoring for patients using herbo-mineral formulations could enable the early detection of subclinical renal injury, allowing for timely intervention before irreversible damage occurs. Second, the use of hair and nail analysis as a cumulative exposure biomarker could provide a quantitative measure of long-term metal burden, which could be correlated with clinical outcomes and biomarker levels. Third, the absence of overt toxicity in properly manufactured formulations, combined with the sensitivity of KIM-1, suggests that the safety margin for these products may be wider than commonly assumed, provided that manufacturing quality is assured. Future studies should prioritize the integration of these biomarkers into both preclinical and clinical study designs, with particular attention to the correlation between metal speciation, bioaccessibility, and biomarker responses.

CONCLUSION

This review establishes that the safety of herbo-mineral formulations is determined by a complex interplay of classical processing quality, metal speciation and bioaccessibility, gut microbiome composition, and host dietary factors, rather than by metallic content alone. Our integrated analysis demonstrates that properly executed Shodhana and Marana procedures fundamentally alter metal physicochemical forms and reduce biological reactivity, with finished formulations such as Sidh Makardhwaj and Chondrokola Rosh showing no overt organ toxicity in rodent models at multiples of human-equivalent doses. The gut microbiome emerges as a critical protective barrier, with gnotobiotic models revealing that microbiome depletion significantly increases metal bioaccumulation and mortality, while probiotic supplementation reduces tissue cadmium accumulation. Furthermore, Anupana selection modulates pharmacokinetic profiles in a vehicle-specific manner, with honey enhancing early gold absorption while overall systemic bioavailability remains below 0.01 percent. These findings challenge the prevailing assumption that metallic content alone determines toxicity and instead reframe the safety discourse around manufacturing quality, host microbiome status, and adjuvant vehicle selection as the primary determinants of clinical risk.

Future research should prioritize prospective human studies that integrate urinary KIM-1 and NGAL as early subclinical injury biomarkers alongside hair and nail elemental analysis for cumulative exposure assessment, with careful documentation of processing parameters, Anupana selection, and dietary co-factors. The gut microbiome's role warrants investigation through randomized controlled trials comparing formulation safety with and without probiotic supplementation, using metagenomic sequencing to identify protective taxa. There is a pressing need for standardized reporting guidelines for Ayurvedic formulation studies that mandate disclosure of Shodhana and Marana parameters, particle characterization data, and bioaccessibility assay results to enable meaningful cross-study comparisons. The classical warnings about incompatible Anupana combinations, such as honey and ghee, also merit systematic toxicological investigation using modern pharmacokinetic methods. By establishing this multi-dimensional safety framework, our work reconciles the conflicting clinical observations of severe hepatotoxicity from contaminated products versus minimal toxicity from properly manufactured formulations, thereby providing a scientific foundation for evidence-based regulation and clinical practice in herbo-mineral medicine.

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CONFLICT OF INTEREST

Nil

INFORMED CONSENT

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ETHICAL STATEMENT

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