REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Received: Revised: Accepted:

A Mechanistic Evaluation of *Agad Tantra* Formulations as Targeted Proto-Nanomedicines and the Evolution of *Sookshma Guna*

Dr.Neelanjana Sen[1*], Dr. Anushka Chauhan [2], Dr.Piyush Solanki[3], Prof. (Dr.) Ritu Kapoor[4], Dr.Praveen Kumar[5]

[1*]PG Scholar, PG Dept. Of Agadtantra evum vidhi vaidhyka, Post Graduate Institute of Ayurveda, DSRRAU Jodhpur Rajasthan
[2] PG Scholar, PG Dept. Of Agadtantra evum vidhi vaidhyka, Post Graduate Institute of Ayurveda, DSRRAU Jodhpur Rajasthan
[3] PG Scholar, PG Dept. Of Agadtantra evum vidhi vaidhyka, Post Graduate Institute of Ayurveda, DSRRAU Jodhpur Rajasthan
[4] Professor and HoD PG Dept. Of Agadtantra evum vidhi vaidhyka, Post Graduate Institute of Ayurveda, DSRRAU Jodhpur Rajasthan
[5]Associate Professor & PhD Scholar ,PG Dept. Of Agadtantra evum vidhi vaidhyka, Post Graduate Institute of Ayurveda, DSRRAU Jodhpur Rajasthan

***Corresponding Author-** Dr.Neelanjana sen
(neelanjana117@gmail.com)

Abstract
Background: Agad Tantra, one of the eight specialized branches of Ayurveda (*Ashtanga Ayurveda*), employs unique pharmaceutical processes to enhance drug delivery in toxicological emergencies.
Objective: This study evaluates the classical concept of *Sookshma Guna* (subtlety/minuteness) as a historical precursor to modern nanomedicine, specifically examining herbo-mineral and metallic formulations.
Methods: A comparative analysis was conducted between traditional pharmaceutical processes (*Shodhana*, *Bhavana*, *Bhasmikarana*) and modern nanoparticle synthesis techniques. Primary texts (*Charaka*, *Sushruta*, *Ashtanga Hridaya*, *Sharngadhara*, and *Rasaratna Samuccaya*) were screened for toxicokinetic descriptors.
Results: Findings indicate that processes like *Bhavana* achieve top-down particle size reduction to the sub-micron level, creating "bio-nanoparticles" with high surface-to-volume ratios. Formulations like *Bilwadi Agad* and *Swarna Bhasma* function as proto-nanomedicines, offering targeted, rapid-acting toxin neutralization that aligns with modern pharmacokinetics, including blood-brain barrier (BBB) penetration.
Conclusion: Re-evaluating the *Visha Gunas* through material science provides a pathway for naturally biocompatible, targeted antitoxins.

1. Introduction

The global burden of envenomation remains a critical public health challenge, with approximately 50,000 snakebite deaths annually in India alone. While contemporary pharmacology often relies on synthetic nanocarriers for targeted drug delivery, Agad Tantra has utilized bio-mineral complexes for millennia to achieve similar ends. The core of this efficacy lies in *Sookshma Guna* the quality of minuteness.[i]

In Ayurvedic toxicological logic, a toxin (*Visha*) spreads with lethal velocity due to its *Vyavayi* (pervasive) and *Vikasi* (tissue-loosening) properties, allowing it to bypass standard metabolic pathways and vitiate the *Rasadi Dhatus*. To counteract this, an antitoxin (*Agada*) must match or exceed the poison's kinetics to reach vital organs (*Marmas*) and cellular junctions (*Srotas*) first. This paper hypothesizes that the traditional processing of *Agadas* transforms bulk materials into proto-nanomedicines capable of crossing the tightest biological barriers.[ii]

2. Toxicokinetics: Mapping *Visha Gunas* to Modern Science

The *Charaka Samhita* defines ten attributes that render a poison effective. These properties are functional equivalents of modern toxicokinetic parameters.[iii]

Table 1: Technical Comparison of *Visha Gunas* and Modern Pharmacokinetics

Ayurvedic Guna	Definition/Action	Modern Pharmacological Correlate
Vyavayi	Spreads throughout the body before digestion.	Rapid Systemic Distribution; bypasses 1st pass metabolism.
Vikasi	Causes looseness of joints and tissue pores; destroys Ojas.	High Tissue Permeability; enzymatic lysis of interstitial bonds.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Received: Revised: Accepted:

Sookshma	Ability to enter minute pores and micro-channels (*Srotas*).	Nanoscale Dimensions (1–100 nm); ability to cross BBB.
Ashu	Rapid onset of action; fast acting.	Expedited Kinetics; immediate receptor binding.
Tikshna	Piercing nature; disrupts vital organs (*Marmas*).	High Potency; pierces cellular junctions.
Yogavahi	Enhances the quality of other drugs and acts as a vector.	Bio-enhancer / Molecular Vector; catalytic vectoring.
Laghu	Lightness; makes management difficult.	Low Molecular Weight; facilitates rapid absorption.

Acharya Sharngadhara further refined this in the *Sharngadhara Samhita* by adding *Chedi* (penetrating nature) and *Madavaha* (narcotic effect), which modern science correlates with increased systemic secretions (saliva, sweat, gastric acid) and neurotoxicity.[iv]

3. Pathogenesis (Role of Biological Barriers)

The pathology of poisoning, or *Visha Prabhavam*, begins when the toxin enters the bloodstream, vitiating the *Raktha Dhatu* first, followed by the progressive disturbance of *Kapha*, *Pitta*, and *Vata* along with their respective anatomical seats. If the poison does not mix with the blood, its systemic harm is minimized; however, once it enters the circulation, it spreads "like oil in water". The vitiated blood then obstructs the minute channels of respiration, known as *Pranavahasrotas*, leading to unconsciousness and eventually death.[v]

The most formidable barrier to both toxins and therapeutic agents is the blood-brain barrier (BBB), an anatomical structure composed of endothelial cells fused by tight junctions and supported by astrocytes. Traditional *Agad Tantra* recognizes that for an antitoxin to be effective against neurotoxic venoms, it must match the "subtle" or *Sookshma* property of the poison. This realization led to the development of unique pharmaceutical processes designed to reduce medicine to the nanoscale, facilitating the delivery of active phytoconstituents across these biological hurdles.[vi]

4. Pharmaceutical Synthesis: Engineering at the Nanoscale

The preparation of *Agada* involves qualitative improvements (*Samskaras*) that transition bulk materials into bioavailable nano-forms.

4.1 *Shodhana* & *Bhavana*: Top-Down Synthesis

4.1.1 *Shodhana*: The Process of Surface Functionalization

Shodhana is often simplified as "purification," yet its mechanism is deeply chemical. It involves the repeated heating and quenching of bulk materials in organic media such as herbal decoctions, oils, or animal urines. Mechanistically, this cycle of thermal stress and immersion results in several critical changes:

1. **Physical Size Reduction**: Thermal shock creates cracks and crevices in minerals, increasing the surface area for subsequent reactions.
2. **Detoxification**: Impurities that are soluble in the quenching media are removed, and toxic metallic components are often converted into less harmful chemical forms.
3. **Impregnation of Organic Ligands**: The material absorbs properties from the media, turning an inorganic substance into a complex herbo-mineral matrix that is more biocompatible.

Table 2: *Shodhana* Method

Shodhana Method	**Description**	**Mechanistic Implication**
Nirvapa	Repeated heating and quenching in liquid media.	Increases brittleness and induces surface etching.
Swedana	Boiling in a cloth bundle (Pottali) within medicinal fluids.	Facilitates deep chemical penetration and cleaning.
Mardana	Milling or grinding with specific organic substances.	Mechanical energy for initial size reduction.
Bharjana	Frying or roasting with media like Ghee or herbal oils.	Surface activation and modification of lipid affinity.

4.1.2 *Bhavana*

Bhavana is perhaps the most critical stage in the creation of proto-nanomedicines. It is the process of continuous wet trituration, where a powdered drug is ground in a mortar and pestle with liquid media until the liquid is completely

evaporated and absorbed. This process represents a classical "top-down" synthesis approach, where mechanical pressure in the presence of liquid achieves sub-micron particle sizes.[vii]
The process of *Bhavana* contributes significantly to the final drug's efficacy through:

- **Micro-Thermal Cavities**: The grinding action creates localized high-pressure and high-temperature zones, activating secondary plant metabolites to act as chelating or capping agents.
- **Prevention of Agglomeration**: Organic ligands from the herbal juices adsorb onto the surface of the reduced particles, acting as stabilizers that prevent the particles from clumping back together, thereby maintaining a high surface-to-volume ratio.
- **Potentiation (Gunantara)**: The medicine absorbs the virtues (Guna-Karma) of the liquid media, which often reduces the required therapeutic dose by increasing bioavailability.

The number of *Bhavana* cycles is directly related to the refinement of the particle size. Studies indicate that repeated trituration ensures uniformity and a sharper particle size distribution peak.

5. *Bhasmikarana*: Thermal Synthesis of Crystalline Nanoparticles

Bhasmikarana is a sophisticated "bottom-up" thermal synthesis method used to create Bhasmas, which are incinerated ashes of metals or minerals. This process involves the Puta system, where materials are kept in a closed crucible (Sharava Samputa) and exposed to specific quanta of heat in specialized furnaces.

Table 3: *Ayurvedic* Process Vs Modern Nanotech

***Ayurvedic* Process**	**Modern Nanotech Equivalent**	**Functional Benefit**
Shodhana	Surface Etching / Activation	Increases reactivity and reduces innate toxicity.
Bhavana	Wet Milling / Levigation	Achieves top-down size reduction and capping.
Bhasmikarana	High-Temperature Calcination	Formation of metallic oxide nanoparticles.
Jarana	Controlled Oxidation	Stabilization of crystalline phases (e.g., ZnO in Yashada Bhasma).

The end product is a lusterless (Nishchandratvam), extremely light (Varitara), and fine powder that can float on water, indicating it has achieved a particle size comparable to pollen grains. Modern analysis via X-ray Diffraction (XRD) and Transmission Electron Microscopy (TEM) has confirmed that Bhasmas typically consist of polycrystalline nanoparticles ranging from 10 to 100 nm.

6. Mechanistic Evaluation of *Agad Tantra* Formulations

1. Swarna Bhasma (Gold Nanomedicine)[viii]

Gold has been utilized in Ayurveda as a supreme *Vishahara* (antitoxin) and *Rasayana* (rejuvenator). Modern physicochemical characterization has demonstrated that *Swarna Bhasma* consists of globular, monatomic colloidal gold nanoparticles (AuNPs).

1.1 Physical and Chemical Characterization

Analysis of *Swarna Bhasma* using advanced analytical tools reveals a high degree of precision in its ancient manufacturing protocols.

Table 3: Physical and Chemical Characterization[ix]

Analytical Tool	**Characterization Finding**	**Scientific Implication**
XRD Analysis	Peaks at = 37.170^, 44.350^, 64.530^ and 77.500^.	Confirms a face-centered cubic (fcc) crystalline structure.
Crystallite Size	Measured at 10.00 nm to 40 nm.	Nanoscale dimensions optimal for cellular and nuclear entry.
SEM Analysis	Spherical/irregular agglomerated particles (2-4mm).	High surface area for adsorption of phytochemical ligands.
EDX (Purity)	90%-99.40% pure gold; trace elements: Na, Fe, K, Ca.	Trace elements enhance biocompatibility and prevent adverse reactions.
PSA	Mean particle size around 2.6mm with low polydispersity.	Consistency across batches from traditional manufacturers.

The use of Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) has further shown that *Swarna Bhasma* remains free of toxic heavy metals like lead and cadmium when prepared correctly, confirming its safety for internal consumption.

1.2 Mechanism of Action and Neuroprotection

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Received: Revised: Accepted:

The *Sookshma Guna of Swarna Bhasma* allows it to bypass the blood-brain barrier, a feat that remains a significant challenge for modern pharmacological drug delivery. In the context of neurotoxicity, these gold nanoparticles are hypothesized to act as a "chemical sponge," adsorbing venom proteins and preventing them from binding to vital receptors in the central nervous system.[x]

Experimental research has validated these neuroprotective effects in zebrafish behavioral models. In studies involving rotenone-induced Parkinson's-like symptoms, Swarna Bhasma treatment demonstrated:

- **Behavioral Stabilization**: Significant reduction in freezing behavior and maintenance of normal swimming speed and angular velocity.
- **Proteomic Protection**: Maintenance of normal expression levels for proteins such as sncgb and uchl1, which are typically altered in neurodegenerative conditions.
- **Dopamine Preservation**: Protection of dopaminergic neurons, preventing the neurotransmitter depletion that characterizes neurotoxic injury.

2. *Bilwadi Agad*: A Multi-Herbal Synergistic Antitoxin

Bilwadi Agad (or Bilwadi Gutika) is a classical polyherbal formulation referenced in the Ashtanga Hridaya as the primary intervention for acute envenomation from snakes (*Bhujanga Visha*), spiders (*Loota Visha*), and scorpions (*Vrischika Visha*).[xi]

2.1.Composition and Synergistic Rationale

The formulation consists of thirteen ingredients, primarily herbal, which are triturated with goat urine (Basta Mootra) for six Yaama (approximately 18 hours) to achieve an extremely fine consistency.[xii]

Table 3: Therapeutic Role[xiii]

Ingredient	Botanical Name	Known Therapeutic Role
Bilwa	*Aegle marmelos*	Anti-inflammatory, hepatoprotective, and Vata-Kapha balancing.
Surasa (Tulsi)	*Ocimum sanctum*	Antimicrobial, respiratory support, and anti-toxic agent.
Karanja	*Pongamia pinnata*	Analgesic, blood purifier, and anti-toxic in skin diseases.
Haridra	*Curcuma longa*	Strong antioxidant; curcuminoids inhibit venom enzymes.
Daruharidra	*Berberis aristata*	Antimicrobial and liver-cleansing properties.
Triphala	*Terminalia chebula* etc.	Gentle detoxification and immune system support.
Trikatu	*Piper nigrum* etc.	Bio-enhancer (Yogavahi); improves absorption of other herbs.
Basta Mootra	Goat Urine	Acts as a reducing agent and size-reduction medium.

The choice of goat urine as the Bhavana medium is scientifically significant. It contains various bio-organic compounds and minerals that act as a vehicle for sub-micron drug delivery, while the mechanical trituration achieves the Su-sookshma Pishtam (fine paste) required for rapid systemic action.

2.2. Enzymatic Inhibition: The PLA2 Mechanism

The lethality of cobra venom is largely attributed to Phospholipase A-2 (PLA2), an enzyme that hydrolyzes membrane phospholipids, releasing arachidonic acid and causing massive inflammation, hemolysis, and neurotoxicity. Phytochemical ligands within Bilwadi Agad, specifically curcuminoids and various alkaloids, serve as competitive inhibitors of PLA2.

Molecular docking studies using AutoDock version 4.2.6 have identified several lead compounds from the herbs in Bilwadi Agad that exhibit strong binding affinity for the PLA2 enzyme (PDB: 2QOG).

- **Gymnemic Acid**: Showed the highest binding affinity at 12.33 kcal/mol and an inhibition constant (of 923.65 pM, forming multiple hydrogen bonds with active site residues like leucine and aspartate.
- **Scutellarein**: A flavonoid with a binding energy of 8.67kcal/mol.
- **Aristolochic Acid**: Exhibited a binding affinity of 7.29 kcal/mol; its anti-inflammatory properties further counteract venom-induced edema.
- **Curcumin**: Demonstrated the ability to bind deep within the hydrophobic channel of the enzyme, blocking the access of fatty acids necessary for toxin activation.

2.3. Experimental Validation in Wistar Albino Rats

The anti-venom activity of *Bilwadi Gutika* (specifically the Visha Bilwadi Gutika variant) has been validated in in vivo models using cobra venom injected into Wistar rats and mice.

The experimental results confirmed that Bilwadi Gutika:

1. **Prolongs Survival**: In mice challenged with $2\times$ the median lethal dose (LD50) of cobra venom, the formulation prevented immediate mortality for the first two hours, providing a critical window for hospital-based intervention.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Received: Revised: Accepted:

2. **Reverses Hematological Vitiation**: Venom-induced thrombocytopenia (low platelets) and anemia were moderately reversed, likely due to the antioxidant and anti-toxic effects of Tulsi and Karanja.
3. **Offers Organ Protection**: The formulation showed a significant reduction in histopathological damage to the liver and kidney, reversing elevated creatinine levels and degenerative changes.
4. **Maintains Biochemical Homeostasis**: The treatment prevented the sharp decline in cholesterol and protein levels typically seen after cobra envenomation.

3. *Dooshivishari Agada*: The Primary Antitoxic Agent

Dooshivishari Agada is a herbo-mineral formulation composed of twelve ingredients, including *Pippali, Jatamansi, Lodhra, Kushtha, and Gairika* (purified red ochre). This formulation is designed to neutralize latent poisons and clear the blood (*Raktashodhana*).

The formulation's mechanism of action is supported by several research findings:

- **Antioxidant and Scavenging Capacity**: The methanolic fraction of Dooshivishari Agada is rich in phenolic compounds that neutralize free radicals (ROS), preventing the cellular injury and DNA damage caused by chronic environmental pollutants.
- **Metal Chelation**: In ferric ion reducing assays, the formulation demonstrated "marked reduction potential," suggesting it can bind and facilitate the excretion of heavy metals like lead and arsenic.
- **Reproductive Protection**: Research on Monosodium Glutamate (MSG)-induced toxicity in Wistar rats showed that Dooshivishari Agada significantly protected ovarian structures, increased primary follicle counts, and stimulated essential oocyte genes like GDF9 and BMP15.
- **Neuroprotection**: Ingredients such as Tagara and Pippali correct the neurodegeneration and hormonal imbalances caused by MSG, effectively rebalancing the Hypothalamus-Pituitary-Ovarian axis.

7. Challenges in Standardization and Heavy Metal Toxicity

A significant obstacle to the integration of herbo-mineral *Agadas* into mainstream medicine is the risk associated with improperly processed metallic preparations. If the *Shodhana* and *Bhasmikarana* cycles are incomplete, the resulting "*Ashodhit*" metals can cause severe systemic toxicity.[xiv]

Metal	Ayurvedic Toxicity Symptoms	Modern Toxicological Findings
Mercury (*Parada*)	Paralysis (Pakshaghat), tremors, and skin discoloration.	Neurotoxicity, renal failure, and respiratory distress.
Copper (*Tamra*)	Anorexia, giddiness (Bhrama), and fainting.	Gastrointestinal erosion and hepatotoxicity.
Lead (*Naag*)	Jaundice (Kamala) and profound anemia.	Chronic nephropathy and peripheral neuropathy.
Arsenic (*Haratala*)	Skin lesions and transverse white nail lines (Mee's lines).	Carcinogenicity and cardiovascular disease.

The resolution of these concerns lies in modern characterization and standardization protocols. Recent studies using Atomic Absorption Spectroscopy (AAS) and Dynamic Light Scattering (DLS) confirm that when prepared by reputable manufacturers according to classical texts, Bhasmas achieve a highly soluble and non-toxic state. Standardizing the particle size distribution and ensuring the absence of free heavy metals is crucial for building clinical confidence.

8. Conclusion

Agad Tantra formulations are sophisticated examples of proto-nanotechnology. The concept of *Sookshma Guna* provides a theoretical framework that anticipates modern challenges in drug delivery and targeting. By re-evaluating formulations like *Bilwadi Agad* and *Swarna Bhasma* through the lens of modern material science, we can unlock new pathways for targeted, fast-acting antitoxins that are naturally biocompatible and effective against acute envenomation and chronic environmental toxicity.

9. References

1. [i] Lochan, K. (Ed.). (2014). *Madhava-Nidanam: An Ancient Text on Ayurvedic Diagnostics* (Volume II, Chapter 69: Visha Nidana, pp. 1050–1124). Varanasi, India: Chaukhamba Surbharati Prakashan.
2. [ii] Murthy, K. R. S. (Ed.). (2016). *Ashtanga Samgraha of Vagbhata: Uttara Tantra* (Chapter 48: Prativisha Vijnaniya, pp. 451–481). Varanasi, India: Chaukhamba Orientalia.
3. [iii] Shastri, K., & Chaturvedi, G. (Eds.). (2020). *Charaka Samhita of Agnivesha: Chikitsa Sthana* (Chapter 23: Visha Chikitsa Adhyaya, pp. 574–601). Varanasi, India: Chaukhamba Bharati Academy.
4. [iv] Angadi, R. (Ed.). (2017). *Sarngadhara Samhita of Acarya Sarngadhara: Madhyama Khanda* (Chapter 9: Sneha Kalpana, pp. 284–287). Varanasi, India: Chaukhamba Surbharati Prakashan.

5. [v] Sajayan, J. (2024). *Illustrated Textbook of Agada Tantra Evam Vidhi Vaidyaka: Toxicology, Forensic Medicine & Medical Jurisprudence*. Varanasi, India: Chaukhamba Surbharati Prakashan.
6. [vi] Shastri, A. D. (Ed.). (2009). *Sushruta Samhita of Maharshi Sushruta: Kalpa Sthana* (Chapter 2: Sthavara Visha Vijnaniya, p. 9; Chapter 5: Sarpa-dasta Kalpa, pp. 64–65). Varanasi, India: Chaukhamba Sanskrit Bhawan Publications.
7. [vii] Pawar, S. H. (Ed.). (2024). *Nanotechnology Applications in Medicinal Plants and their Bionanocomposites: An Ayurvedic Approach*. Cambridge, UK: Cambridge Scholars Publishing.
8. [viii] Singh, Y. (2024). Swarn Bhasm is a traditional ayurvedic medicine, synthesis, characterization and uses of Swarn Bhasm as a nanoparticles. *International Engineering Research Journal*, *10*(4), 116–122.(https://doi.org/10.21276/IERJ24499752170659)
9. [ix] Netam, A. K., & Bhargava, V. P. (2021). Physico-chemical characterization of ayurvedic swarna bhasma by using SEM, EDAX, XRD and PSA. *Journal of Complementary Medicine Research*, *12*(2), 204. https://doi.org/10.5455/jcmr.2021.12.02.23
10. [x] Satpute, A. D. (Ed.). (2010). *Rasaratna Samuchchaya (I-XI Chapters)* (pp. 18–316). Varanasi, India: Chaukhamba Sanskrit Pratishthan.
11. [xi] Gopakumar, S., & Hussain, G. (2020). An experimental study on anti venom activity of Visha Bilwadi Gutika in cobra venom injected Wistar albino rats. *Annals of Ayurvedic Medicine*, *9*(4), 256–266. https://doi.org/10.5455/AAM.83360
12. [xii] Kamal, G. C., et al. (2021). A review on Bilwadi Agada and its indications. *World Journal of Pharmacy and Pharmaceutical Sciences*, *10*(3), 339–350. https://doi.org/10.20959/wjpps20213-18364
13. [xiii] Chunekar, K. C. (Ed.). (2015). *Bhavaprakash Nighantu of Shri Bhavamishra* (Haritakyadi Varga, 1/101, p. 42; Swarna Varga, Verse 7, p. 423). Varanasi, India: Chowkhamba Bharati Academy.
14. [xiv] Rajchal, B., Bhatta, P., & Adhikari, R. (2025). Exploring the nanomedicinal features of ayurvedic bhasmas: Insights from traditional medicine. *International Journal of Complementary Medicine*, *1*(1), 10–13. https://doi.org/10.14302/issn.3070-3360.ijco-24-5294