Preparation and Characterization of Harmaline-Loaded Silver Nanoparticles

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ABSTRACT

The present study aimed to prepare and characterize Harmaline-loaded silver nanoparticles (H-AgNPs) using a green synthesis approach. Harmaline, a biologically active β-carboline alkaloid obtained from *Peganum harmala*, was utilized as both reducing and stabilizing agent for the synthesis of silver nanoparticles. The formation of nanoparticles was initially confirmed by visual color change from pale yellow to dark brown due to surface plasmon resonance. The synthesized nanoparticles were characterized using UV–Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Scanning Electron Microscopy (SEM). UV–Visible analysis showed a characteristic surface plasmon resonance peak at 450 nm, confirming nanoparticle formation. FTIR analysis demonstrated the involvement of hydroxyl, amine, and aromatic functional groups of Harmaline in nanoparticle stabilization. XRD studies confirmed the crystalline nature and face-centered cubic structure of silver nanoparticles. SEM analysis revealed predominantly spherical nanoparticles with particle size ranging from 20–60 nm and minimal aggregation. The synthesized H-AgNPs exhibited good stability and controlled morphology. Overall, the study demonstrated that Harmaline-mediated silver nanoparticles may serve as a promising nanocarrier system with potential pharmaceutical and biomedical applications.

Keywords: Harmaline, Silver Nanoparticles, Nanotechnology, Harmala.

How to cite this article: Tyagi H, Pandey HK. Preparation and Characterization of Harmaline-Loaded Silver Nanoparticles. Int J Drug Deliv Technol. 2026;16(52s): 578-584. DOI: 10.25258/ijddt.16.52s.74

Source of support: Nil.

Conflict of interest: None.

Introduction

Nanotechnology has emerged as one of the most promising and rapidly advancing fields in pharmaceutical and biomedical sciences due to its ability to improve drug delivery, therapeutic efficacy, and targeted action of bioactive compounds [1]. Nanoparticles are nanosized materials generally ranging from 1 to 100 nm in size and possess unique physicochemical properties such as increased surface area, enhanced permeability, controlled drug release, and improved bioavailability [2]. Among various metallic nanoparticles, silver nanoparticles (AgNPs) have gained significant scientific attention because of their remarkable antimicrobial, antioxidant, anti-inflammatory, anticancer, and catalytic properties [3]. Due to these multifunctional characteristics, silver nanoparticles are extensively investigated for applications in drug delivery systems, diagnostics, wound healing, biosensing, and nanomedicine [4].

Silver nanoparticles possess unique optical, electrical, and biological properties that are mainly attributed to their nanoscale dimensions and high surface-to-volume ratio [5]. Their small particle size allows efficient interaction with biological membranes, leading to enhanced cellular uptake and improved therapeutic performance [6]. In addition, silver nanoparticles exhibit broad-spectrum antimicrobial activity against bacteria, fungi, and viruses, making them highly valuable in pharmaceutical formulations [7]. Researchers have also explored the use of silver nanoparticles as carriers for natural phytoconstituents and alkaloids to improve their stability, solubility, and bioavailability [8].

Natural bioactive compounds derived from medicinal plants continue to play an important role in the discovery and development of novel therapeutic agents. Among these compounds, alkaloids represent an important class of secondary metabolites with diverse pharmacological activities [9]. Harmaline is a naturally occurring β-carboline alkaloid predominantly isolated

from the seeds of *Peganum harmala* and other medicinal plants [10]. Traditionally, *Peganum harmala* has been widely used in folk medicine for the treatment of various ailments including nervous disorders, microbial infections, inflammation, and gastrointestinal diseases [11]. Harmaline possesses several pharmacological activities such as antioxidant, antimicrobial, anticancer, antidiabetic, neuroprotective, anti-inflammatory, and monoamine oxidase inhibitory effects [12].

The pharmacological activity of harmaline is mainly attributed to its ability to interact with various biological targets, including enzymes, neurotransmitter systems, and free radicals [13]. Studies have demonstrated that harmaline exhibits potent antioxidant activity by scavenging reactive oxygen species and reducing oxidative stress [14]. It has also shown antimicrobial effects against several pathogenic microorganisms, indicating its potential application in infectious diseases [15]. Furthermore, harmaline has gained attention for its neuroprotective and anticancer properties due to its ability to modulate apoptosis, inhibit tumor cell proliferation, and protect neuronal cells from oxidative damage [16].

Despite its promising therapeutic potential, the clinical application of harmaline is limited due to certain drawbacks such as poor aqueous solubility, instability, low bioavailability, and rapid metabolism [17]. These limitations reduce its therapeutic efficiency and restrict its pharmaceutical applications. Therefore, there is a growing need for advanced drug delivery systems capable of enhancing the stability, solubility, and bioavailability of harmaline while minimizing degradation and improving targeted delivery [18]. Nanotechnology-based delivery systems, particularly silver nanoparticles, offer a promising strategy to overcome these limitations.

Loading bioactive compounds into nanoparticles can significantly improve their pharmacokinetic and pharmacodynamic profiles [19]. Nanoparticle-based systems enhance the dissolution rate of poorly soluble compounds, protect active constituents from degradation, and facilitate controlled and sustained drug release [20]. Silver nanoparticles are considered suitable carriers for alkaloids and phytochemicals because of their ability to adsorb and stabilize bioactive molecules on their surface [21]. The incorporation of harmaline into silver nanoparticles may therefore improve its therapeutic effectiveness, stability, and biological activity.

Several methods have been developed for the synthesis of silver nanoparticles, including physical, chemical, and biological approaches [22]. Chemical reduction methods are among the most widely used techniques because they allow easy control over particle size, shape, and stability [23]. In a typical chemical reduction process, silver nitrate is used as a precursor salt and reducing agents such as sodium borohydride or citrate are employed to reduce silver ions into nanoparticles [24]. Stabilizing agents are often added to prevent aggregation and improve nanoparticle stability [25]. Recently, green synthesis approaches using plant extracts and natural biomolecules have also gained popularity due to their eco-friendly and biocompatible nature [26].

Characterization of nanoparticles is a critical step in evaluating their physicochemical properties and suitability for biomedical applications [27]. Various analytical techniques are employed to characterize nanoparticles, including ultraviolet-visible (UV-Vis) spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), dynamic light scattering (DLS), zeta potential analysis, and X-ray diffraction (XRD) [28]. UV-Vis spectroscopy is commonly used to confirm nanoparticle formation by detecting surface plasmon resonance peaks characteristic of silver nanoparticles [29]. FTIR analysis helps identify functional groups involved in stabilization and drug loading, while SEM and TEM provide information about particle morphology and size distribution [30].

Harmaline-loaded silver nanoparticles may offer several pharmaceutical and biomedical advantages. The synergistic combination of harmaline and silver nanoparticles can potentially enhance antimicrobial and antioxidant activity due to combined effects of the alkaloid and metallic nanoparticles [35]. Additionally, nanoparticle encapsulation may improve the controlled release behavior of harmaline, thereby prolonging therapeutic action and reducing dosing frequency [36]. Such nanoparticle systems may also improve targeted delivery and reduce systemic toxicity associated with free drug administration [37].

In recent years, there has been growing interest in the development of nanoparticle-based herbal formulations because of increasing demand for safer and more effective natural therapeutics [38]. Combining phytoconstituents with nanotechnology can lead to enhanced therapeutic outcomes and novel applications in modern medicine [39]. Harmaline-loaded silver nanoparticles represent an innovative approach for integrating traditional medicinal compounds with advanced nanocarrier systems.

Therefore, the present study aims to prepare and characterize harmaline-loaded silver nanoparticles using suitable synthesis techniques and evaluate their physicochemical properties. The study focuses on analyzing parameters such as particle size, morphology, surface charge, drug loading efficiency, and stability using various characterization methods. The developed nanoparticle formulation may provide improved

therapeutic potential and serve as a promising nanomedicine for future pharmaceutical applications.

Materials and Methods

Chemicals and Reagents

Silver nitrate ($AgNO_3$, analytical grade) was procured from Sigma-Aldrich, USA. Harmaline (≥98% purity) was obtained from a certified chemical supplier. Methanol, dimethyl sulfoxide (DMSO), potassium bromide (KBr), sodium hydroxide (NaOH), hydrochloric acid (HCl), ascorbic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were of analytical grade and procured from standard chemical suppliers. Deionized water (DIW) was used throughout the study for nanoparticle synthesis and characterization procedures.

Preparation of 1 mM Silver Nitrate Solution

A 1 mM aqueous silver nitrate solution was prepared by dissolving 0.017 g of silver nitrate ($AgNO_3$) in 100 mL of deionized water. The prepared solution was stored in an amber-colored bottle at room temperature to protect it from photodegradation and maintain stability [40].

Preparation of Harmaline Solution

Harmaline solution was prepared by dissolving an appropriate quantity of harmaline in deionized water. In cases where complete solubility was not achieved, a minimal quantity of ethanol was added prior to dilution with deionized water. The final concentration of the prepared harmaline solution was maintained at approximately 1 mg/mL. Freshly prepared solution was used for nanoparticle synthesis [41].

Green Synthesis of Harmaline-Loaded Silver Nanoparticles (H-AgNPs)

Harmaline-mediated silver nanoparticles were synthesized using a green synthesis approach. Briefly, 10 mL of harmaline solution was added dropwise into 20 mL of 1 mM silver nitrate solution under continuous magnetic stirring at room temperature. The reaction mixture was subsequently diluted with 170 mL of deionized water and incubated under dark conditions to prevent photo-oxidation.

The formation of silver nanoparticles was indicated by a visible colour change from colourless to yellowish-brown due to the reduction of silver ions (Ag^+) into metallic silver nanoparticles (Ag^0). The reaction mixture was incubated for 24 h to ensure complete reduction and stabilization of the nanoparticles [42].

Characterization of Synthesized H-AgNPs

UV–Visible Spectroscopy

The formation and stability of synthesized harmaline-loaded silver nanoparticles were confirmed using UV–Visible spectroscopy. The absorbance spectra were recorded using a Systronics double beam spectrophotometer (2202, India) within the wavelength range of 300–600 nm. The characteristic surface plasmon resonance (SPR) peak corresponding to silver nanoparticles was observed and recorded [43].

Scanning Electron Microscopy (SEM)

The morphology and surface characteristics of synthesized nanoparticles were evaluated using scanning electron microscopy (ZEISS EVO MA 15). For analysis, a thin film of nanoparticle suspension was prepared on a carbon-coated grid and allowed to air dry prior to imaging. SEM analysis provided information regarding particle shape, surface morphology, and aggregation pattern [44].

X-ray Diffraction (XRD)

The crystalline nature of the synthesized nanoparticles was determined using X-ray diffraction analysis [45]. Diffraction patterns were recorded, and the average particle size was calculated using the Debye–Scherrer equation:

$$D = \frac{K\lambda}{\beta \cos\theta}$$

Where:

- D= Crystallite size
- K= Shape factor
- λ= Wavelength of X-ray radiation
- β= Full width at half maximum (FWHM)
- θ= Bragg diffraction angle

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was carried out to identify the functional groups present in harmaline responsible for the reduction and stabilization of silver nanoparticles. The FTIR spectra were recorded using potassium bromide pellet technique over an appropriate scanning range. The obtained spectra were analyzed to determine possible interactions between harmaline and silver nanoparticles [46].

Results and Discussion

Synthesis of Silver Nanoparticles Using Harmaline

Visual Observation

The synthesis of silver nanoparticles using Harmaline was initially confirmed through visual observation. Upon addition of silver nitrate solution to the Harmaline solution under continuous stirring, the reaction mixture gradually changed from colorless or faint yellow to yellowish-brown and finally dark brown within 30–60 minutes. This color transformation indicated the successful reduction of silver ions (Ag^+) into metallic silver nanoparticles (Ag^0). The characteristic brown coloration is attributed to the surface plasmon resonance (SPR) phenomenon exhibited by silver nanoparticles. Unlike plant extracts containing multiple phytoconstituents, Harmaline acted as both a reducing and stabilizing agent during nanoparticle synthesis. No visible precipitation or aggregation was observed, indicating effective stabilization and dispersion of nanoparticles. The synthesized Harmaline-loaded silver nanoparticles (H-

AgNPs) remained stable for several days under ambient conditions, suggesting efficient capping action by Harmaline.

Characterization of Synthesized Silver Nanoparticles

UV–Visible Absorption Spectrum

The UV–visible absorption spectrum of Harmaline exhibited a characteristic absorption peak around 280 nm, corresponding to π–π* electronic transitions associated with the aromatic β-carboline structure and conjugated functional groups of Harmaline. These electron-rich systems facilitate reduction of silver ions into metallic nanoparticles.

The UV–visible spectrum of the synthesized silver nanoparticles demonstrated a distinct surface plasmon resonance (SPR) band centered at 450 nm, confirming successful formation of silver nanoparticles. The SPR band results from collective oscillation of free surface electrons upon interaction with visible light, which is a characteristic feature of metallic silver nanoparticles. The presence of a single and well-defined SPR peak suggested that the synthesized nanoparticles were predominantly spherical in shape. Slight peak broadening indicated a moderate degree of polydispersity and variation in particle size distribution. Harmaline effectively acted as both reducing and stabilizing agent, producing stable nanoparticles with controlled morphology.

FTIR Analysis

FTIR analysis was carried out to identify functional groups involved in the reduction and stabilization of silver nanoparticles. The FTIR spectra of Harmaline and Harmaline-mediated silver nanoparticles showed several significant peak shifts, confirming interaction between Harmaline and silver nanoparticles.

A broad absorption peak observed at 3314.06 cm^{-1} in Harmaline shifted to 3317.95 cm^{-1} in H-AgNPs, corresponding to O–H and N–H stretching vibrations. This shift suggested involvement of hydroxyl and amine groups in nanoparticle formation and stabilization. New peaks observed at 2923.10 cm^{-1} and 2854.04 cm^{-1} corresponded to C–H stretching vibrations of aliphatic groups, indicating structural stabilization of nanoparticles.

The peak at 1639.72 cm^{-1} assigned to C=C and C=N stretching vibrations shifted to 1600.26 cm^{-1} after nanoparticle synthesis, confirming interaction of aromatic and nitrogen-containing groups with silver ions. Additional peaks at 1541.44 cm^{-1} and 1319.62 cm^{-1} corresponded to N–H bending and C–N stretching vibrations, respectively, further supporting the role of nitrogen-containing functional groups in nanoparticle stabilization. Overall, FTIR analysis confirmed that Harmaline acted as both reducing and capping agent during synthesis of silver nanoparticles.

Table 1. FTIR Peak Modification of Harmaline and H-AgNPs

Wave Number (Harmaline)	Wave Number (H-AgNPs)	Bond Assignment	Functional Group	Observation
3314.06 cm^{-1}	3317.95 cm^{-1}	O–H / N–H stretching	Hydroxyl / Amine	Peak shift observed
—	2923.10 cm^{-1}	C–H stretching	Aliphatic group	New peak formed
—	2854.04 cm^{-1}	C–H stretching	Aliphatic group	New peak formed
1639.72 cm^{-1}	1600.26 cm^{-1}	C=C / C=N stretching	Aromatic ring / Imine	Shift and broadening observed
—	1541.44 cm^{-1}	N–H bending	Amine	New peak appeared
1400.59 cm^{-1}	1405.59 cm^{-1}	C–C stretching	Aromatic ring	Slight shift observed
—	1319.62 cm^{-1}	C–N stretching	Amine	New peak observed
—	1077.89 cm^{-1}	C–N stretching	Aliphatic amine	Broad peak observed

XRD Analysis

The crystalline nature and phase structure of synthesized Harmaline-mediated silver nanoparticles were analyzed using X-ray diffraction (XRD). The XRD pattern exhibited characteristic diffraction peaks at 2θ values of 27.84°, 32.26°, 38.14°, 44.36°, 46.24°, 57.48°, 64.51°, and 76.74°, corresponding to the lattice planes of (210), (113), (111), (200), (124), (240), (220), and (311), respectively.

The intense diffraction peak at 38.14° corresponding to the (111) plane confirmed the formation of face-centered cubic (fcc) crystalline silver nanoparticles.

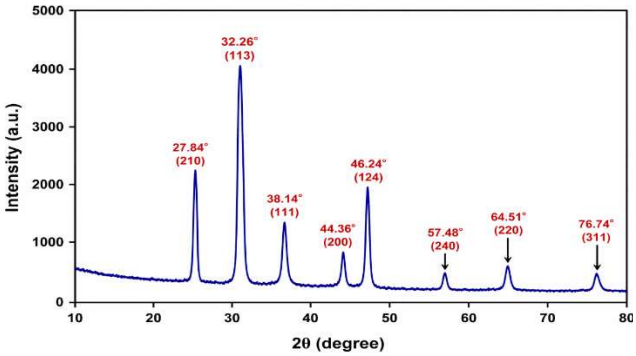

Figure 1. XRD spectrum of Harmaline-mediated synthesized silver nanoparticles (H-AgNPs).

Scanning Electron Microscopy (SEM) Analysis

Scanning electron microscopy (SEM) was performed to evaluate the morphology and particle size of synthesized H-AgNPs. SEM images revealed that the nanoparticles were predominantly spherical to quasi-spherical in shape with relatively uniform distribution. The particle size of synthesized nanoparticles ranged approximately between 20–60 nm. The nanoparticles appeared well dispersed with minimal aggregation, indicating effective stabilization by Harmaline. Presence of a few larger clusters along with smaller particles suggested slight polydispersity, which correlated with the broadening observed in the UV–visible SPR band.

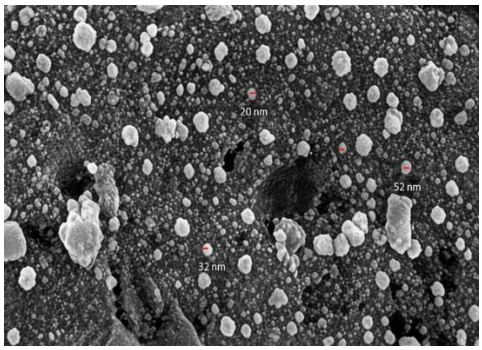

Figure 2. SEM image of silver nanoparticles

The smooth surface morphology and distinct particle boundaries confirmed successful formation of silver nanoparticles. The minimal agglomeration observed may be due to strong capping action of Harmaline, where nitrogen-containing functional groups and aromatic π-electron systems interacted with the nanoparticle surface and prevented excessive aggregation and particle growth. Overall, SEM analysis confirmed successful synthesis of nanosized, stable, and predominantly spherical Harmaline-loaded silver nanoparticles.

Conclusion

The present study successfully demonstrated the green synthesis and characterization of Harmaline-loaded silver nanoparticles using Harmaline as both reducing and stabilizing agent. The synthesized nanoparticles showed characteristic surface plasmon resonance, confirming successful formation of silver nanoparticles. FTIR analysis revealed the involvement of hydroxyl, amine, and aromatic functional groups of Harmaline in nanoparticle reduction and stabilization. XRD studies confirmed the crystalline face-centered cubic structure of the synthesized nanoparticles, while SEM analysis revealed predominantly spherical nanoparticles with particle sizes ranging between 20–60 nm and minimal aggregation. The nanoparticles exhibited good stability and controlled morphology due to effective capping by Harmaline. The combination of Harmaline and silver nanoparticles may provide enhanced therapeutic potential because of their synergistic biological activities. Overall, the developed Harmaline-loaded silver nanoparticles represent a promising nanotechnology-based formulation with potential applications in drug delivery, antimicrobial therapy, antioxidant therapy, and other biomedical fields.

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