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REVIEW

Therapeutic Potential of Silver Nanoparticles in Hepatocellular Carcinoma: From Pathogenesis to Clinical Perspectives

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Abstract: Hepatocellular carcinoma (HCC) is one of the deadliest malignancies worldwide, characterised by late-stage diagnosis, high recurrence rates, and limited responsiveness to conventional therapeutic strategies. Despite advancements in surgical interventions, locoregional therapies, and targeted drugs, survival outcomes remain unsatisfactory due to systemic toxicity, drug resistance, and tumour heterogeneity. In this context, nanotechnology-based therapeutic approaches have attracted considerable interest, particularly silver nanoparticles (AgNPs), owing to their unique physicochemical properties and multifaceted biological activity. AgNPs demonstrate distinct anticancer effects in hepatic cancer models through mechanisms involving reactive oxygen species generation, mitochondrial dysfunction, DNA damage, cell cycle arrest, and activation of apoptosis-related signalling pathways. Additionally, advances in green and biogenic synthesis methods have improved the biocompatibility and safety profile of AgNPs, enhancing their suitability for biomedical applications. Tumour-targeting strategies, including passive accumulation via the enhanced permeability and retention effect and active ligand-mediated targeting, further improve therapeutic selectivity in HCC. The emerging evidence also highlights the potential of AgNP-based systems in combination therapies and stimuli-responsive platforms to overcome therapeutic resistance. However, despite their promising anticancer activity, AgNPs exhibit dose-dependent toxicity profiles characterised by hepatic accumulation, oxidative stress induction, mitochondrial dysfunction, inflammatory cytokine release, and potential off-target organ deposition, particularly in the liver, spleen, and kidneys. Silver ion (Ag^+) release kinetics, particle size, surface chemistry, and repeated exposure significantly influence systemic toxicity. While short-term studies often report tolerable safety margins at therapeutic concentrations, concerns regarding chronic accumulation, redox imbalance, immunotoxicity, and long-term hepatic injury remain incompletely resolved. Therefore, comprehensive pharmacokinetic evaluation and standardised toxicological profiling are essential for safe clinical translation. In this review, silver nanoparticles represent a promising yet safety-dependent nanoplatform for hepatic cancer therapy, warranting further investigation to facilitate their integration into future HCC treatment paradigms.

Keywords: hepatocellular carcinoma, silver nanoparticles, oxidative stress, apoptosis, targeted drug delivery

Introduction

Hepatocellular carcinoma (HCC) is a highly prevalent and deadly form of primary liver cancer. As such, it is one of the top health challenges worldwide due to its rising incidence and poor treatment outcomes.[1–3] The majority of patients with HCC developed their disease as a result of chronic liver injury from hepatitis B and hepatitis C viruses, alcohol-associated liver injury, and metabolic syndromes such as non-alcoholic steatohepatitis, which can lead to progressive inflammation, fibrosis, and cirrhosis (Figure 1).[4–6] In recent years, surgical resection, liver transplantation, locoregional treatments, and molecularly targeted therapy developments have resulted in improved treatment options for HCC patients; however, the survival of HCC patients is dismal. Several factors, including delay in diagnosis, tumour

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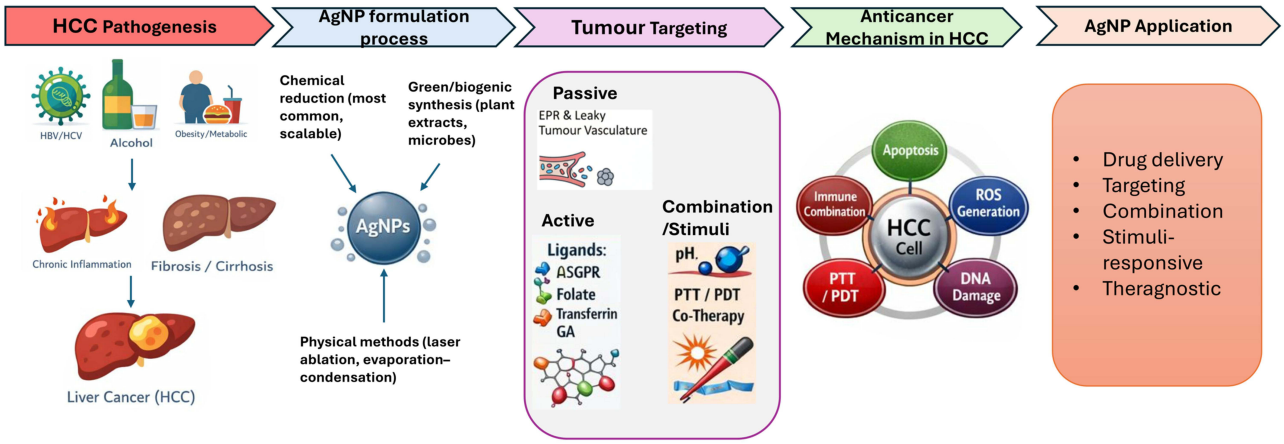
Graphical Abstract



heterogeneity, and frequent recurrence, as well as systemic toxicity and development of drug resistance, significantly limit the effectiveness of conventional treatment modalities.[7–9] Therefore, new therapeutic modalities must be developed to provide innovative treatment options for HCC.

Recent advancements in cancer treatment have led to the development of new methods for delivering drugs into tumours, providing better access for cancer cells to absorb the drug, and allowing for targeted delivery of the drug to the tumour.[10–12] Silver nanoparticles (AgNPs) are extensively studied for their anticancer activity and due to their nanosized properties and ability to target cancer selectively. AgNPs have many unique properties that make them target selective:

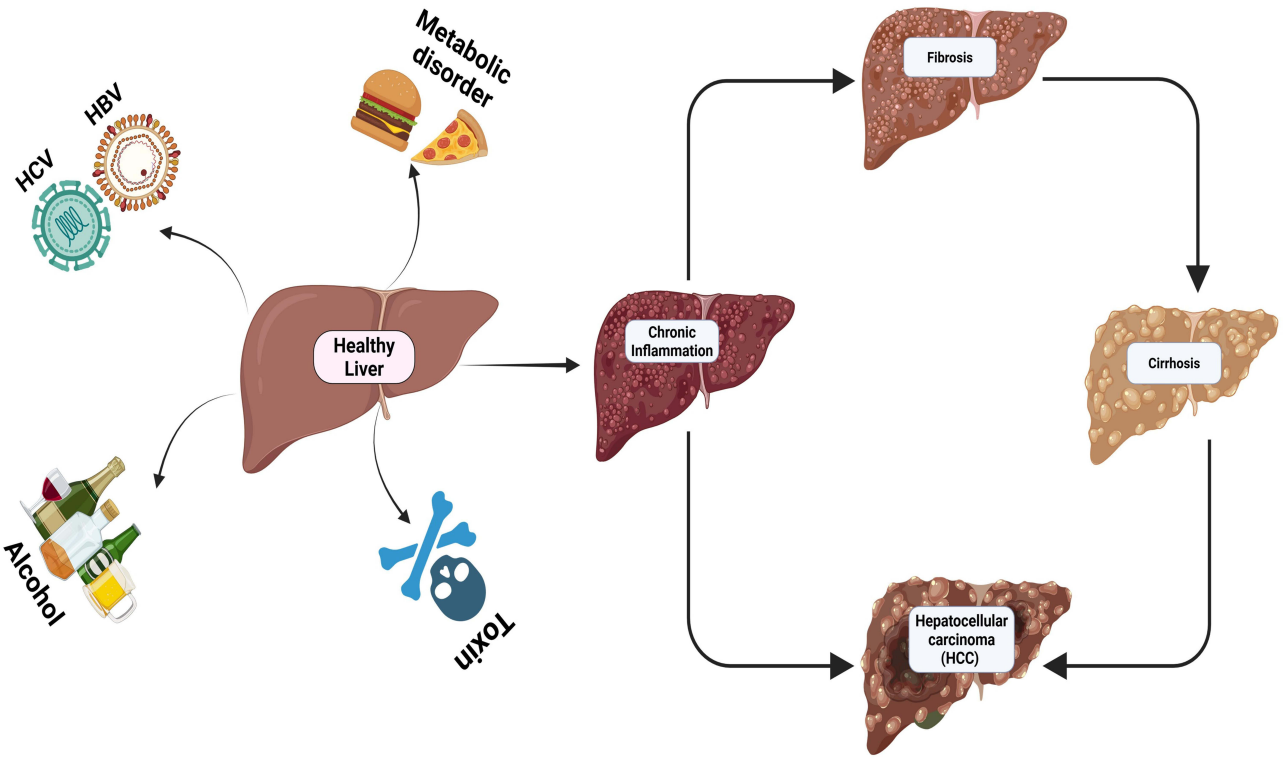


Figure 1 Schematic representation of multi-stage HCC development from common etiological factors.

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they are nanoscale (1 nanometer = 1 billionth of a meter); they have a very large surface area; they are highly reactive; and they can be easily modified[13,14] Although silver has a long history of being used as an antimicrobial agent, AgNPs are also recognised for their ability to kill a variety of cancer cells, including HCC.[15] Research has shown that AgNPs can kill HCC cells through several mechanisms. Examples of those methods include the generation of free radicals that cause oxidative stress, mitochondrial disruption, enzyme inhibition, DNA damage, and activation of the intrinsic apoptosis pathway.[16,17] The ability of AgNPs to have multiple mechanisms of action gives AgNPs the potential to be effective against many of the significant characteristics of cancer cells: uncontrolled growth, the ability to defeat apoptosis, and the ability to spread.[15,18] These characteristics make AgNPs a potentially highly effective treatment for patients with HCC.

The use of biogenic and green synthesis methods, including natural reducing agents and plant extracts, enhances the biocompatibility and safety of AgNPs, making them much better suited for biomedical use. The structure of the liver's unique vascular system and the enhanced permeability and retention (EPR) effect result in enhanced accumulation of AgNPs within the tumour tissue.[19,20] Thus, AgNP therapy has a distinct advantage over many types of cancer therapies for HCC. The ability to functionalize AgNP with phytochemicals, targeting ligands, or chemotherapeutic drugs opens many new avenues for the development of synergistic/targeting anticancer strategies.[21] This review will provide a detailed summary of the pathophysiology of hepatocellular carcinoma, an analytical critique of AgNP anticancer mechanisms, as well as discuss the latest advances from a preclinical perspective, discuss the major strategies to target HCC with AgNP-based products, and provide insights into future clinical use of these products for the treatment of HCC.

This review aims to provide a critical and integrative analysis of the therapeutic potential of silver nanoparticles in hepatocellular carcinoma. Specifically, we evaluate mechanistic evidence underlying AgNP-induced cytotoxicity, compare tumour-targeting strategies, examine biodistribution and hepatic microenvironmental interactions, and discuss translational, regulatory, and long-term safety considerations. Particular emphasis is placed on distinguishing experimental promise from clinically actionable evidence to define realistic pathways toward future application.

Epidemiology of HCC

HCC is a significant public health concern due to its increasing incidence and fatality rates globally. In 2022, HCC ranked as the third leading cause of cancer-related mortality and the sixth most common cancer, with 865,269 new liver cancer diagnoses (predominantly HCC) and 757,948 deaths, as illustrated in Figure 2A.[22] Projections further indicate that, if current prevention and management efforts remain unchanged, annual incidence is expected to rise substantially approaching 1.52 million cases by 2050 (Figure 2B) with over 1 million additional cases anticipated by 2025.[23] Geographical disparities are pronounced: because endemic hepatitis B virus (HBV) infection contributes to more than 50% of cases in East Asia and sub-Saharan Africa, these regions continue to show the highest incidence rates.[23] In contrast, in Western countries such as the United States, HCC prevalence has tripled since the 1980s, driven largely by alcohol-related liver disease (ALD), non-alcoholic steatohepatitis (NASH), and metabolic dysfunction-associated steatotic liver disease (MASLD).[24] Asia accounts for over half of global HCC cases and deaths, underscoring substantial regional concentration, which is represented in Figure 2C using the minimum split implied by the text.[25] Moreover, Sex-based differences are also evident, with men affected two to four times more frequently than women, consistent with the male: female ratio scenarios depicted in Figure 2D. Late-stage diagnosis and limited access to curative treatment remain key determinants of the poor 5-year survival rate (15–20%), particularly in low- and middle-income countries with inadequate screening and treatment infrastructure. Addressing this expanding burden requires comprehensive prevention strategies, including antiviral therapy, HBV immunisation, and interventions targeting alcohol use and obesity.[26]

Mechanistic Approach to Anti-Cancer Activity of Silver Nano-Biomaterials Toward Hepatic Cancer Cells

The precise molecular pathways underlying the anticancer effects of biosynthesized silver nanoparticles (AgNPs) against hepatic cancer cells remain incompletely understood. A significant cause of AgNP cytotoxicity is thought to be oxidative stress (OS), or the increase in the body's cellular levels of reactive oxygen species (ROS) due to exposure to AgNPs and the

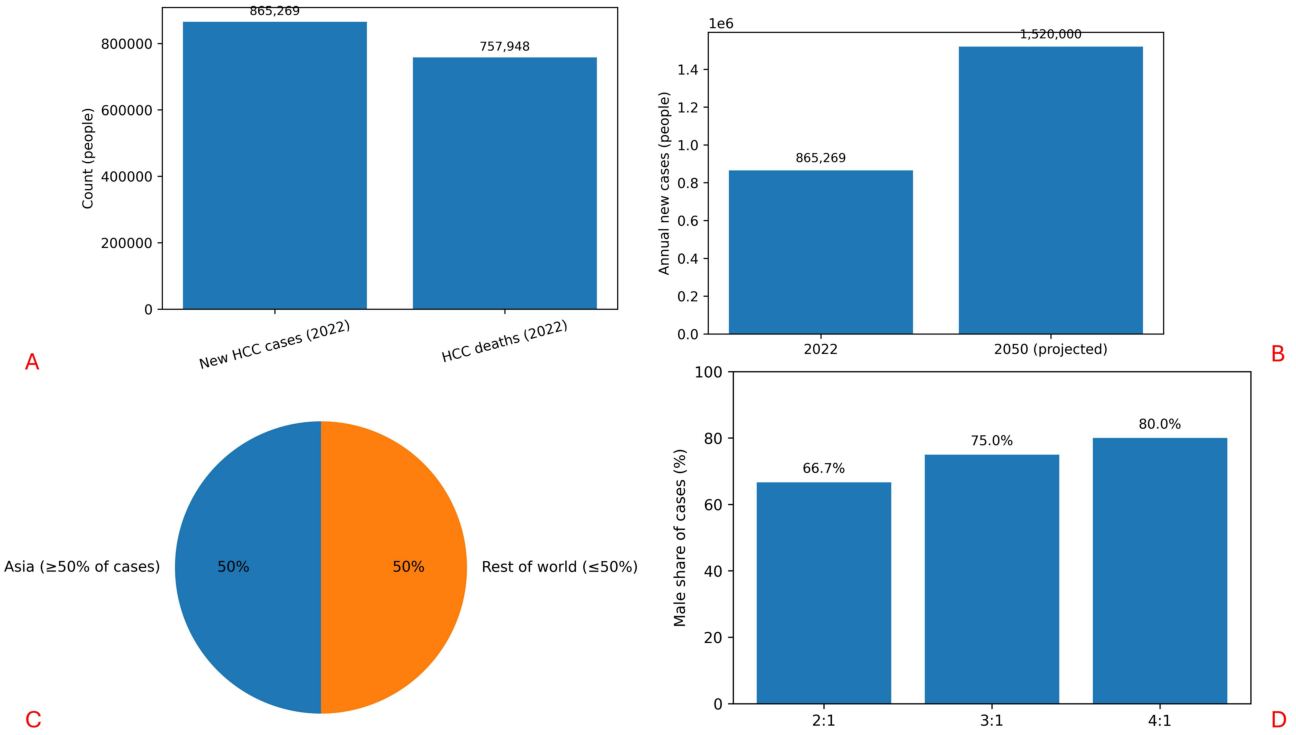

Figure 2 Based summary of key HCC burden and distribution indicators. (**A**) Global HCC burden in 2022, new cases vs deaths. (**B**) Projected increase in annual HCC incidence. (**C**) Regional concentration of HCC cases. (**D**) sex distribution implied by male: female incidence ratios.

reduction in adenosine triphosphate (ATP) production caused by AgNPs. The presence of both ROS and decreased ATP levels will impair mitochondrial function and trigger the intrinsic apoptotic pathway by disrupting the mitochondrial respiratory chain. In addition to mitochondrial dysfunction, AgNP exposure has also been shown to induce the secretion of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α.[27,28] Elevated levels of these cytokines are associated with increased ROS, which can damage cellular DNA through oxidative stress and promote unstable genomes.[29] The effects of AgNPs on biological systems depend on their physicochemical properties, such as size, charge, and shape. These physical characteristics will determine how AgNPs can bind to biomolecules within cells, ultimately creating a variety of consequences within cells, such as enzyme inhibition, ionic imbalance, triggering cell death signalling pathways, as well as being potentially mutagenic. There is evidence suggesting that AgNPs may affect cell membranes, allowing excess calcium to enter the cell and thereby increasing the generation of reactive oxygen species, ultimately leading to apoptotic cell death. This effect on membrane structure is thought to be due to AgNP's ability to strongly interact with different types of thiol-containing groups (cysteines) that are found within membrane phospholipids and proteins and therefore create interactions between AgNPs and the cell membrane, ultimately causing structural damage to both.[30,31] Figure 3 represents anticancer mechanisms of biogenic AgNPs. In a research study, Yassin et al demonstrated a significant increase in intracellular reactive oxygen species (ROS) levels in HepG2 cells following exposure to biogenically synthesized silver nanoparticles. This elevation in ROS was identified as a key event contributing to AgNP-induced cytotoxicity, suggesting that oxidative stress plays a central role in mediating the anticancer effects of biogenic AgNPs in liver cancer cells.[32]

Mechanistic Variability Across Studies

Silver nanoparticle (AgNP)–induced cytotoxicity in hepatocellular carcinoma (HCC) models is most frequently attributed to oxidative stress, mitochondrial dysfunction, DNA damage, and activation of apoptotic signalling cascades.[33–35] However, a critical evaluation of the literature reveals substantial variability in the magnitude and dominant pathways of these effects across studies. This heterogeneity appears to arise from differences in nanoparticle physicochemical properties, experimental design, and cellular context.[36,37]

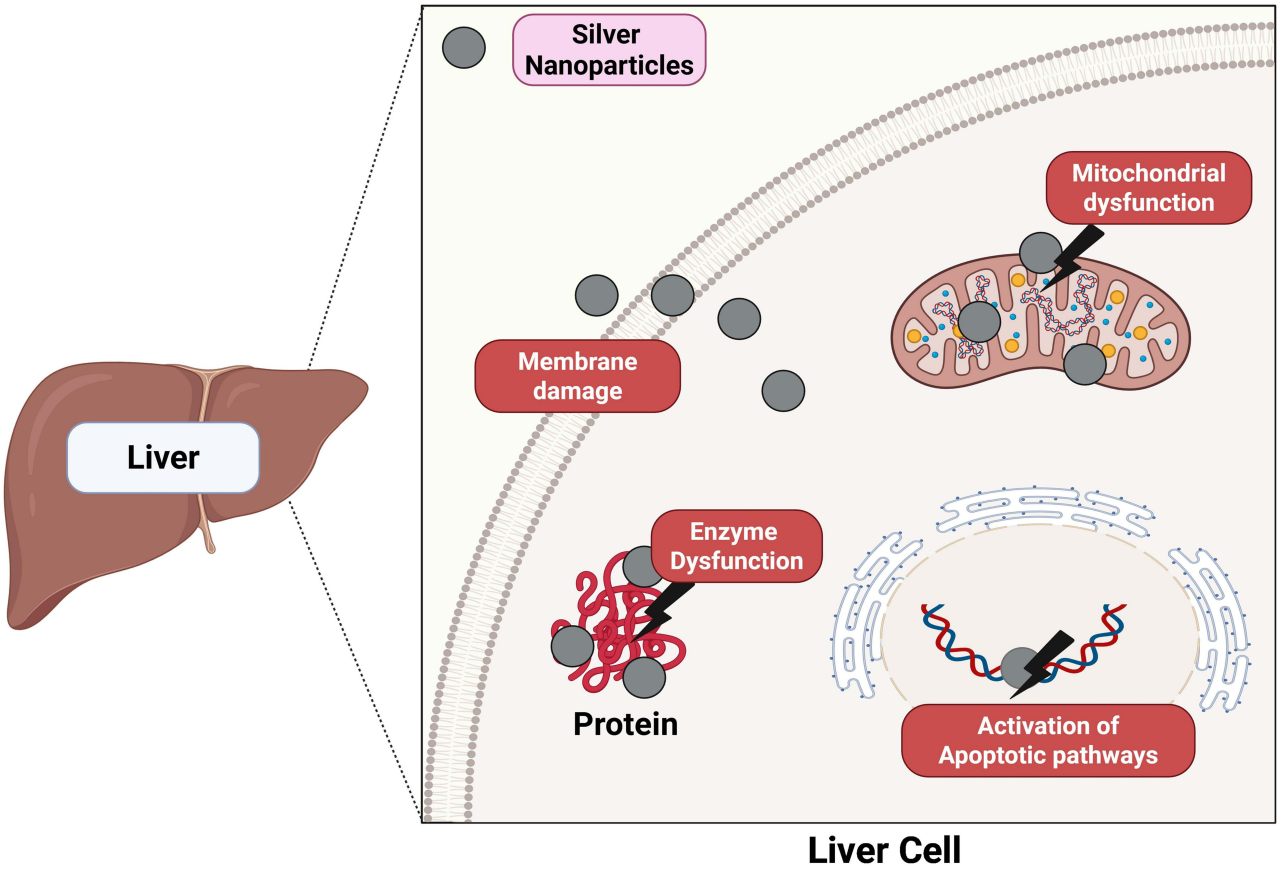

Figure 3 Schematic anticancer mechanisms of biogenic AgNPs.

One major determinant is particle size. Several investigations report enhanced intracellular uptake and greater reactive oxygen species (ROS) production with smaller AgNPs (<20 nm), likely due to increased surface area-to-volume ratios and higher rates of silver ion (Ag^+) dissolution.[35,38,39] In contrast, larger particles may demonstrate reduced cellular penetration but prolonged extracellular interaction, potentially shifting toxicity mechanisms toward membrane-associated damage rather than intracellular mitochondrial disruption 81,106. These size-dependent differences are not consistently controlled or standardised across studies, complicating direct mechanistic comparisons.[37]

Surface chemistry and coating composition further modulate biological responses. Stabilising agents such as polyvinylpyrrolidone (PVP), citrate, polyethylene glycol (PEG), or plant-derived biomolecules influence protein corona formation, cellular internalisation pathways, and immune recognition.[40–42] Biogenic AgNPs are often reported to exhibit enhanced biocompatibility;[43,44] however, comparative mechanistic studies directly contrasting chemically synthesised and green-synthesised nanoparticles under identical conditions remain limited.[38,45] Consequently, whether observed differences in cytotoxicity reflect intrinsic nanoparticle properties or residual phytochemical contributions is not always clearly delineated.

Silver ion release kinetics represent another critical variable. While many studies attribute cytotoxicity primarily to ROS-mediated apoptosis,[33,35,38] the relative contribution of particulate versus ionic silver remains debated.[46,47] Increased Ag^+ dissolution in acidic intracellular compartments (eg, lysosomes) may amplify oxidative stress and mitochondrial membrane depolarisation.[33,46] Yet, few studies quantitatively measure ion release alongside mechanistic endpoints, limiting definitive conclusions regarding causality.[37]

Cellular model differences also contribute to inconsistent findings. Reported IC_{50} values for AgNPs in HCC cell lines such as HepG2 and Huh7 vary substantially, even within similar size ranges.[45,48,49] These discrepancies may reflect differences in metabolic activity, p53 status, antioxidant capacity, and basal redox regulation between cell lines.[50]

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Moreover, many investigations do not include parallel assessment in normal hepatocytes, making it difficult to distinguish cancer-selective cytotoxicity from non-specific oxidative injury.[38,39]

Importantly, while apoptosis is frequently reported as the predominant mode of cell death,[34,35,39] some studies demonstrate concurrent necrosis, autophagy modulation, or cell-cycle arrest.[38,51] The relative dominance of intrinsic (mitochondrial) versus extrinsic (death receptor–mediated) apoptotic pathways is rarely compared systematically.[33,48] In addition, few studies perform time-course analyses to determine whether oxidative stress precedes mitochondrial collapse or represents a secondary consequence of cellular damage.[37]

Collectively, these observations indicate that AgNP-induced anticancer activity in HCC is highly context-dependent and influenced by nanoparticle design, exposure conditions, and cellular phenotype. The lack of standardised experimental frameworks and cross-study comparability currently limits definitive mechanistic generalisation.[36,37] Future investigations would benefit from harmonised reporting of nanoparticle characterisation (size distribution, zeta potential, ion release kinetics), inclusion of appropriate normal-cell controls, and integration of quantitative pathway analyses to enable more robust comparative evaluation.[40,47]

While AgNP-induced ROS generation and apoptosis are widely reported in HCC models,[33–35] it is critical to distinguish tumour-selective cytotoxicity from non-specific oxidative injury. Malignant hepatocytes often exhibit elevated basal ROS levels and dysregulated redox homeostasis, which may increase their susceptibility to further oxidative stress.[16,17] However, several studies demonstrate ROS induction and cytotoxicity in non-malignant cells at higher concentrations, indicating that dose-dependent non-specific toxicity remains a concern.[39,46] Importantly, only a subset of investigations includes parallel assessment in normal liver cell lines or report selectivity indices.[45,52] This limitation complicates definitive claims regarding cancer-specific targeting and underscores the necessity for systematic comparative toxicity profiling using matched malignant and non-malignant hepatocyte models.

Hepatocellular Carcinoma Pathogenesis and Molecular Mechanisms

Hepatocellular carcinoma (HCC) develops through a multistep process driven by chronic liver injury, sustained inflammation, fibrogenesis, and progressive genomic instability.[4–6,53] Repeated hepatocyte damage induced by viral hepatitis (HBV/HCV), alcohol-associated liver disease, and metabolic dysfunction-associated steatotic liver disease promotes compensatory regeneration within a pro-inflammatory and pro-oxidative microenvironment.[4–6,53] Over time, persistent oxidative stress, telomere dysfunction, and impaired DNA repair mechanisms facilitate clonal expansion of genetically altered hepatocytes, ultimately resulting in malignant transformation.[16,17,54]

This chronic inflammatory–oxidative axis is central to HCC biology and has important therapeutic implications, as redox imbalance and mitochondrial vulnerability are also key mechanisms underlying silver nanoparticle (AgNP)–mediated cytotoxicity.[16,17,33,37]

Genetic and Epigenetic Alterations

HCC is characterised by recurrent driver mutations and epigenetic dysregulation that collectively promote proliferation, survival, and immune evasion.[54] Telomerase reverse transcriptase (TERT) promoter mutations represent one of the earliest and most frequent genetic events, enabling replicative immortality.[25,55] Tumour suppressor inactivation—most prominently TP53 mutation—impairs DNA damage response and apoptotic regulation.[25] Activating mutations in CTNNB1 stabilise β-catenin and drive Wnt-dependent transcription of proliferative genes, including CCND1 and MYC.[25] Additional alterations affecting chromatin remodelling (ARID1A/ARID2), DNA damage signalling (ATM/ATR), and Wnt regulation (AXIN1) further contribute to tumour heterogeneity.[25]

Beyond sequence mutations, epigenetic reprogramming plays a critical role in hepatocarcinogenesis.[54] Promoter hypermethylation of tumour suppressor genes (eg, CDKN2A, RASSF1A, APC), global DNA hypomethylation, and dysregulated histone modifications reshape transcriptional landscapes.[54] Non-coding RNAs also exert strong regulatory influence: miR-122 is frequently downregulated, whereas oncogenic miRNAs such as miR-21 and miR-221 are upregulated, promoting proliferation and resistance to apoptosis.[56] Long non-coding RNAs, including HULC, further enhance oncogenic signalling through post-transcriptional mechanisms.[56]

Dysregulated Signalling Pathways

HCC progression is sustained by aberrant activation of interconnected signalling pathways.[53] The Wnt/β-catenin pathway promotes transcription of genes governing cell cycle progression and stemness.[25] The PI3K/AKT/mTOR axis enhances survival, angiogenesis, and metabolic adaptation, frequently activated via PTEN loss or growth factor receptor stimulation.[53,57] Parallel activation of the RAS/RAF/MAPK cascade further drives mitogenic signalling and tumour growth.[58]

Inflammation-associated pathways also play a central role. IL-6–mediated JAK/STAT activation supports tumour proliferation and immune modulation.[1,53] Developmental signalling pathways such as Hedgehog have also been implicated in tumour progression and stem-like phenotypes.[53] In HBV-related HCC, viral integration events and HBx-mediated transactivation of oncogenic pathways, including NF-κB, amplify tumour-promoting transcriptional programs.[2] HCV non-structural proteins promote oxidative stress, endoplasmic reticulum stress, and epigenetic alterations, thereby accelerating genomic instability.[38,56,59] In metabolic liver disease–associated HCC, lipotoxicity and insulin resistance activate NF-κB and JNK signalling, further increasing reactive oxygen species (ROS) production and DNA damage.[53,60]

Collectively, these signalling perturbations reinforce sustained proliferative signalling, apoptotic resistance, metabolic reprogramming, and chronic oxidative stress.[16,17,53]

Tumour Microenvironment Role

HCC arises within a complex and chronically inflamed hepatic microenvironment characterised by immune dysregulation and stromal remodelling.[53] Chronic inflammation remodels hepatic architecture and establishes a tumour microenvironment enriched with tumour-associated macrophages (TAMs), cancer-associated fibroblasts (CAFs), hepatic stellate cells, and myeloid-derived suppressor cells.[61] These stromal populations promote angiogenesis, extracellular matrix remodelling, epithelial–mesenchymal transition (EMT), and immune suppression.[61]

Hypoxia within tumour nodules activates hypoxia-responsive pathways and upregulates pro-angiogenic mediators such as vascular endothelial growth factor (VEGF), thereby sustaining neovascularisation.[53] Concurrently, immunosuppressive cytokines and checkpoint ligand expression attenuate cytotoxic T-cell activity, enabling immune evasion and tumour persistence.[62]

Importantly, the oxidative and inflammatory milieu of the HCC microenvironment may enhance susceptibility of malignant hepatocytes to additional redox stress. However, neighbouring non-malignant hepatocytes exposed to similar conditions may also exhibit heightened sensitivity to oxidative injury, underscoring the importance of therapeutic selectivity.[16,33,46]

Angiogenesis, Metastasis, and Immune Evasion

Advanced HCC is characterised by amplified angiogenesis, driven predominantly by VEGF-mediated signalling.[53,63] Invasive behaviour is facilitated through EMT-associated transcriptional reprogramming and increased matrix metalloproteinase activity, enabling extracellular matrix degradation and metastatic dissemination.[61]

Immune escape mechanisms further sustain tumour progression. Upregulation of immune checkpoint pathways, including PD-L1 expression, recruitment of regulatory T cells, and persistent inflammatory signalling contribute to suppression of antitumour immunity.[62,63] These mechanisms complicate therapeutic responses and contribute to resistance against systemic therapies.[1,62]

Toxicity and Safety Considerations in Normal Hepatic Cells

While AgNPs demonstrate cytotoxic efficacy in HCC models primarily through ROS amplification and mitochondrial disruption, these mechanisms are not intrinsically tumour-selective.[33,37] Non-malignant hepatocytes may also undergo oxidative stress, glutathione depletion, mitochondrial membrane depolarisation, and inflammatory activation following AgNP exposure, particularly at higher concentrations or prolonged exposure durations.[46,47]

Experimental models report dose-dependent elevations in hepatic enzymes (ALT, AST), histopathological alterations, and redox imbalance following systemic silver nanoparticle administration.[64,65] Particle size and surface chemistry

critically influence hepatotoxic potential; smaller nanoparticles with increased Ag^+ dissolution rates exhibit enhanced cellular uptake but may also increase oxidative injury risk.[35,37]

Although malignant hepatocytes often display elevated basal ROS levels and impaired redox buffering capacity, potentially increasing their susceptibility to additional oxidative stress,[16,17] the therapeutic window remains concentration-dependent and requires careful optimisation.[37,46] Chronic hepatic silver accumulation within the reticuloendothelial system further underscores the necessity for long-term safety evaluation.[47,64]

Therefore, precise control of nanoparticle physicochemical properties, dosing regimens, and biodistribution profiling is essential to maximise tumour-selective cytotoxicity while minimising collateral hepatocellular injury.

Tumour Targeting Strategies of Silver Nanoparticles

Passive Targeting

Passive targeting describes the tendency of nanosystems to accumulate in tumours without requiring a specific ligand–receptor interaction. It is commonly explained by the enhanced permeability and retention (EPR) effect, where newly formed tumour vessels are structurally abnormal (more permeable) and lymphatic drainage is inefficient (greater retention). Together, these features can favour the extravasation and prolonged residence of nanoparticles within tumour tissue compared with healthy organs.[66]

In hepatocellular carcinoma (HCC), passive targeting is influenced by the liver's unique biology. Although HCC lesions may present leaky and heterogeneous vasculature that can support EPR-mediated entry, the liver is also a major site of nanoparticle sequestration. Circulating nanoparticles are readily opsonised and taken up by the mononuclear phagocyte system, particularly Kupffer cells, which can reduce the amount of material that remains available to reach tumour nodules. For this reason, "hepatic accumulation" should not be automatically interpreted as "tumour accumulation", and the passive delivery efficiency can vary substantially between models and disease states.[67]

Particle size is a central determinant of passive targeting. In general, nanoscale materials are often designed within a size window where they can remain in circulation long enough to encounter tumour vasculature while still being small enough to extravasate through vascular defects. Very small particles (hydrodynamic diameters around a few nanometres) may be eliminated rapidly via renal filtration, limiting exposure time. Conversely, larger particles can be cleared more aggressively by liver and spleen macrophages, especially when surface properties promote protein adsorption. These competing processes create a practical design trade-off: sizes in the tens of nanometres are frequently used to balance circulation stability with the ability to permeate tumour tissue after extravasation.

For silver nanoparticles (AgNPs), size-dependent biodistribution is particularly important because silver can localise strongly in clearance organs, and its biological effects depend on particle dimensions, coating, and the extent of silver ion release. From a passive targeting standpoint, the objective is typically to maintain colloidal stability and adequate systemic residence time while enabling tumour entry and intratumoural spread. Accordingly, the selection of an AgNP size range (for example, tens of nanometres) should be justified in relation to the intended administration route, the expected HCC microenvironment, and the broader formulation strategy.[36]

Active Targeting

Active targeting is achieved when silver nanoparticles (AgNPs) are functionalised with ligands that recognise receptors or antigens enriched on hepatocellular carcinoma (HCC) cells (and, in some cases, tumour-associated endothelium). In practice, the ligand first enables selective binding at the tumour cell surface and then promotes receptor-mediated internalisation (commonly via clathrin- or caveolae-associated pathways), thereby increasing intracellular delivery of silver (or silver-linked payloads) compared with non-targeted AgNPs.[68] Because uncoated AgNPs are highly reactive in biological fluids, active targeting is typically implemented on top of a stabilising surface "shell" (eg, citrate, PVP, PEG, protein, silica, or polymer coatings) that maintains colloidal stability and provides functional groups for ligand attachment. Common coupling strategies include Ag–S anchoring of thiolated ligands (eg, HS–PEG–ligand) and amide-bond formation via EDC/NHS chemistry on carboxylated coatings. Importantly, ligand density and spacer length are critical formulation parameters, as they can strongly influence serum protein adsorption, cellular uptake, and cytotoxicity in AgNP systems.[69]

Several ligand classes have been used for HCC-oriented targeting. ASGPR-directed targeting uses terminal galactose or GalNAc motifs to bind the asialoglycoprotein receptor (ASGPR) and promote hepatocyte/HCC uptake through receptor-mediated endocytosis; performance can vary with tumour differentiation and receptor expression.

Folate receptor targeting employs folic acid to enhance uptake in folate receptor-positive tumour cells; it is widely used due to the ligand's small size and straightforward conjugation chemistry, although receptor expression remains tumour-dependent. Transferrin receptor targeting leverages overexpression of TfR/CD71 in rapidly proliferating cells to drive transferrin–TfR binding and endocytosis, thereby increasing nanoparticle internalisation.

Glycyrrhizin/glycyrrhetinic-acid family targeting uses licorice-derived ligands (commonly glycyrrhetinic acid) to bias uptake toward hepatoma cells via receptor-associated pathways described in liver-directed nanoplatforms.[68] Finally, aptamer targeting applies nucleic-acid ligands that bind HCC-associated surface markers (including AFP/HepG2-related targets), enabling constructs that support therapeutic delivery and, where appropriate, diagnostic imaging (theranostics).[70]

Combination and Stimuli-Responsive Approaches

Recent AgNP platforms increasingly employ multifunctional designs to enhance tumour selectivity and therapeutic efficacy in HCC by combining pharmacological synergy with triggered activation in the tumour microenvironment. In combination therapy, AgNPs may be co-delivered or chemically conjugated with chemotherapeutics or biomolecules to produce synergistic cytotoxicity and increase intracellular drug exposure; for instance, AgNP–doxorubicin systems have been reported to improve intracellular accumulation and help counter multidrug resistance (MDR) mechanisms in HCC-relevant models.[71]

Although combination approaches involving AgNPs and chemotherapeutic agents demonstrate enhanced cytotoxicity in HCC models,[71–73] most studies report percentage reductions in cell viability without formal quantitative assessment of drug–nanoparticle interaction. True mechanistic synergy requires mathematical evaluation using established pharmacodynamic models such as the Chou–Talalay combination index or Bliss independence analysis. In the absence of such modelling, observed improvements in cytotoxicity may represent additive effects rather than genuine synergistic interaction.[37] Furthermore, few investigations differentiate between enhanced intracellular drug delivery, ROS amplification, and independent parallel cytotoxic mechanisms when interpreting combination outcomes. Future studies should incorporate rigorous combination modelling frameworks to substantiate claims of synergy and clarify the mechanistic basis of combined AgNP-based therapies.

To minimise off-target effects, AgNP formulations can also be engineered as internal stimuli-responsive systems that preferentially activate under tumour conditions; because tumours and intracellular vesicles (endosomes/lysosomes) are relatively acidic, pH-responsive AgNPs may accelerate Ag^{+} release and/or payload liberation in these environments, thereby improving treatment specificity compared with physiological pH.[74] In addition, AgNPs can be incorporated into external stimuli-responsive systems by pairing them with photoactive agents for photothermal therapy (PTT) and/or photodynamic therapy (PDT). Upon light exposure, these platforms generate localised heat (PTT) and/or reactive oxygen species (ROS) (PDT), enabling spatially controlled tumour damage and, in some cases, image-guided and minimally invasive intervention.[37]

The principal active and passive targeting strategies explored for AgNP delivery in hepatocellular carcinoma are summarised in Table 1.

While both passive and active targeting strategies are proposed to enhance AgNP accumulation in HCC, their relative contributions remain difficult to isolate.[68] The enhanced permeability and retention (EPR) effect may facilitate tumour deposition in murine models;[66] however, EPR variability in human tumours is significantly higher and often less pronounced than in preclinical systems.[66] Moreover, hepatic sequestration by the reticuloendothelial system (RES), particularly Kupffer cells, can dominate nanoparticle uptake regardless of targeting ligand presence.[37,64,65] Active targeting strategies—such as ligand-mediated ASGPR or transferrin receptor engagement—may enhance cellular internalisation following tumour exposure[68,70] 50–52, yet they do not fully overcome systemic clearance mechanisms or macrophage-mediated sequestration.[37,47] Therefore, the translational impact of ligand-mediated targeting in human HCC requires cautious interpretation and further validation in clinically relevant model.

Table 1 Targeting Strategies for Silver Nanoparticles in Hepatocellular Carcinoma: Design Principles, Mechanisms, Advantages, and Translational Limitations

Strategy	Mechanistic Basis in HCC	Engineering Parameters	Therapeutic Advantage	Translational Challenges	Refs
Passive targeting (EPR-mediated)	Accumulation via enhanced permeability and retention (EPR) arising from aberrant tumour angiogenesis, sinusoidal fenestrations, and impaired lymphatic drainage. No ligand–receptor interaction required.	Size optimisation (typically tens of nm); colloidal stability; surface coating to modulate Ag^+ release; control of protein corona formation.	Simple design; prolonged circulation may enable tumour deposition when EPR is permissive.	Clinical EPR heterogeneity in HCC; dominant uptake by RES/MPS (Kupffer cells); fibrosis and cirrhosis alter biodistribution; size/coating-dependent off-target sequestration.	[36,67,75]
Active targeting (ligand-mediated)	Ligand-functionalised AgNPs bind receptors overexpressed on HCC cells or tumour endothelium, enabling receptor-mediated endocytosis and enhanced intracellular delivery.	Surface stabilisation (citrate, PVP, PEG, silica, polymers); Ag–S anchoring; EDC/NHS coupling; optimisation of ligand density, spacer length, and orientation. Common ligand–receptor pairs: Gal/GalNAc→ASGPR; folic acid→FR; transferrin→TfR/CD71; glycyrrhizin→hepatocyte affinity; aptamers→AFP/HepG2 markers.	Improved cellular selectivity and internalisation; reduced non-specific exposure; compatible with theranostic platforms.	Receptor heterogeneity; ligand masking by protein corona; altered pharmacokinetics; density-dependent cytotoxicity; scalability and reproducibility issues.	[68–70]
Combination & stimuli-responsive systems	Multifunctional AgNP platforms combine cytotoxic synergy (eg, ROS amplification, mitochondrial disruption) with internal or external activation triggers to enhance tumour specificity.	Co-loading with chemotherapeutics (eg, AgNP–doxorubicin); pH-responsive coatings (acidic tumour/endosomal release of Ag^+ or payload); external triggers (PTT/PDT → heat/ROS generation).	Synergistic efficacy; spatial and temporal control; potential MDR circumvention; image-guided or minimally invasive therapy.	Trigger dependence; risk of uncontrolled Ag^+ release; off-target oxidative stress in normal hepatocytes; engineering complexity and regulatory hurdles.	[37,71,74]
Immunomodulatory targeting (microenvironment-directed)	AgNP-induced modulation of hepatic immune microenvironment, including Kupffer cell activation, macrophage polarisation (M1/M2 shift), and cytokine signalling (eg, TNF-α, IL-6), potentially influencing antitumour immunity.	Size- and surface-dependent immune interaction; corona engineering; surface chemistry tuning to modulate macrophage uptake and inflammatory signalling.	Potential enhancement of antitumour immune responses; synergistic integration with immunotherapy strategies.	Risk of inflammatory hepatotoxicity; uncontrolled cytokine release; limited mechanistic clarity; safety–efficacy balance remains incompletely defined.	[74]

Abbreviations: PTT, Photothermal Therapy; PDT, Photodynamic Therapy; MDR, Multidrug Resistance; ROS, Reactive Oxygen Species; RES, Reticuloendothelial System; MPS, Mononuclear Phagocyte System; EDC, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; NHS, N-Hydroxysuccinimide; EDC/NHS, Standard Amide Bond Coupling Chemistry; Gal/GalNAc→ASGPR, folic acid→FR, transferrin→TfR/CD71, glycyrrhizin/GA motifs, aptamers (AFP/HepG2-associated markers); ASGPR, Asialoglycoprotein Receptor; HepG2, Human Hepatocellular Carcinoma Cell Line (G2); AFP, Alpha-Fetoprotein.

Blood-Brain and Liver Microenvironmental Interactions

The distinct liver tumour microenvironment in the case of HCC has been identified to have a pivotal role in the biodistribution and therapeutic efficacy of silver nanoparticles (AgNPs). The HCC liver microenvironment is distinguished by the presence of chronic inflammation, hypoxia, highly fibrotic tissue, and the immunosuppressive tumour microenvironment.

Liver Microenvironment and AgNP Dynamics

HCC TME is characterized and enriched with TAMs, CAFs, HSCs, and MDSCs, and all these cells support immunosuppression, angiogenesis, and ECM remodelling.[33] The liver offered a natural reticuloendothelial system (RES) seclusion to a major fraction of extravasated nanoparticles, leading to natural hepatic tropism of AgNPs. It was supplemented with a prominent change associated with a permeable vascularity found within HCC, because of which there was a prominent accumulation of NPs at HCC nodules.[76]

AgNPs can modulate the tumour microenvironment (TME) through multiple complementary mechanisms, including reprogramming tumour-associated macrophages (TAMs) from an immunosuppressive M2 phenotype toward a pro-inflammatory M1 phenotype, which is associated with reduced vascular endothelial growth factor (VEGF) signalling and inhibition of tumour neoangiogenesis.[64] They may also inhibit hepatic stellate cells (HSCs) and cancer-associated fibroblasts (CAFs), downregulate matrix metalloproteinases (MMP-2 and MMP-9), and thereby reduce extracellular matrix (ECM) stiffness, epithelial–mesenchymal transition (EMT), and metastatic potential.[61,76]

In addition, AgNPs can promote selective oxidative stress within hypoxic tumour regions, leveraging the heightened susceptibility of cancer cells to reactive oxygen species (ROS). Collectively, these immunoregulatory and stromal-modulating effects support the potential use of AgNPs as an adjunct strategy to promote immunogenic conversion of otherwise immunosuppressive HCC.[64]

Cross-Talk Between Liver and Systemic Barriers

Despite their substantial hepatocellular accumulation, nanoparticles smaller than 20 nm may still cross the blood–brain barrier (BBB), potentially via receptor-mediated transcytosis or transient disruption of tight junctions; this penetration can trigger neuroinflammation through elevated pro-inflammatory cytokines/mediators and may induce oxidative stress in neural tissues.[40] In the context of targeted HCC therapy, such BBB permeability is a safety concern—particularly with repeated or frequent administrations—because it may increase the risk of neurotoxicity. To mitigate these risks, liver-specific targeting ligands (eg, glycyrrhizin or asialoglycoprotein receptor ligands) and related guidance strategies are employed to preferentially confine AgNP circulation and uptake to the hepatoportal system.[26]

Immunological Responses

The interactions between AgNP and immune cells present in the liver are dose-dependent:

Additionally, the activation of Kupffer cells has been shown to improve the phagocytosis of tumour debris and antigen presentation, hence stimulating anti-tumour immunity. Overstimulation can lead to overproduction of pro-inflammatory cytokines (TNF-α Inhibition of hepatic stellate cells reduces fibrogenesis and increases nanoparticle access to the fibrotic areas.[25,77]

Immunological and Oxidative Responses

The toxicity of AgNPs is closely related to the disruption of the redox balance through the Nrf2/Keap1 pathway. Keap1 binds Nrf2 in the cytoplasm under physiological conditions. The generated ROS from AgNPs triggers the release and translocation of Nrf2 to the nucleus to initiate the transcription of antioxidant proteins (HO1 and NQO1). This mechanism is overpowered in HCC cells at therapeutic levels of AgNPs and results in apoptosis through the mitochondrial route; however, normal cells are less affected and have the capability to balance redox. The complex interplay between AgNPs and the liver microenvironment in HCC reveals their potential in acting as both cytotoxic agents and modulators of the TME. Surface engineering and combination therapy are critical in exploiting their hepatic specificity while countering systemic challenges associated with immunotoxicity.[33,46]

Beyond direct cytotoxicity, AgNP exposure influences immunological dynamics within the liver microenvironment.[37,64] Activation of Kupffer cells and modulation of macrophage polarisation (M1 vs M2 phenotypes) have been reported following nanoparticle uptake, where M1 macrophages are generally associated with pro-inflammatory, antitumour immune responses, while M2 phenotypes are linked to anti-inflammatory and tissue-repair functions, contributing to both antitumour immune stimulation and inflammatory injury.[37,46] Cytokine release, including TNF-α and IL-6, may further amplify oxidative stress signalling and tumour–stroma interactions.[46] In addition, nanoparticle–protein corona formation influences complement activation, immune recognition, and macrophage-mediated clearance.[41] Recent immunological analyses highlight the dualistic nature of nanoparticle–immune interactions in hepatic disease, emphasising the need to balance immune activation with potential immunotoxicity.[78] The complex interplay between AgNP-mediated redox stress and immune modulation in HCC remains incompletely characterised and warrants systematic investigation in clinically relevant models.

Pharmacokinetics and Biodistribution of Silver Nanoparticles

Therapeutics based on nanotechnology, especially silver nanoparticles (AgNPs), have become viable options for treating HCC. AgNPs have special physicochemical and biological characteristics that allow for targeted administration and increased anticancer action, such as strong surface reactivity, regulated release, and preferential accumulation in tumour tissues.[36] Determining the pharmacokinetics, tumour targeting strategies, and interactions in the hepatic milieu is crucial for creating AgNP-based treatments for HCC. The ensuing segments emphasise these facets, connecting basic biopharmaceutical principles with prospective applications in liver cancer.

Absorption, Distribution, Metabolism and Excretion (ADME)

The absorption, distribution, metabolism/biotransformation, and excretion (ADME) fate of AgNPs in HCC therapy is summarised schematically in Figure 4.

Absorption

AgNP bioavailability and hepatic deposition in HCC therapy are strongly dependent on the administration route. Parenteral delivery (particularly intravenous and intraperitoneal administration) generally provides the most reliable systemic exposure by bypassing gastrointestinal degradation and first-pass absorption barriers. Following injection, nanoparticles can reach the liver through hepatic blood supply; intraperitoneal dosing may additionally favour hepatic exposure via peritoneal absorption pathways that drain into portal circulation, whereas intravenous dosing distributes through the systemic circulation with subsequent hepatic uptake.[79] In contrast, oral administration typically yields low AgNP absorption because of gastric/intestinal instability, mucus barriers, limited epithelial transport, and efflux mechanisms. Nevertheless, appropriately engineered nanoformulations—such as lipid-coated systems or chitosan-functionalized AgNPs—may enhance gastrointestinal stability and mucosal interaction, thereby improving uptake relative to unmodified AgNPs.[41] Once in the bloodstream, AgNPs rapidly adsorb plasma proteins to form a protein corona, which effectively defines their "biological identity" and plays a major role in opsonization, macrophage recognition, and downstream hepatic sequestration. Size is also a critical determinant of cellular uptake: smaller AgNPs (eg, <20 nm) often display higher apparent internalization in hepatocytes and HCC cells, consistent with more favorable endocytic processing and intracellular trafficking compared with larger particles.[65] Finally, surface engineering—such as PEGylation or coating with natural polysaccharides—is commonly used to improve colloidal stability, prolong circulation, and reduce premature clearance by the mononuclear phagocyte system, thereby increasing the probability of tumour exposure.[41]

Distribution

In HCC, the reticuloendothelial/mononuclear phagocyte system (RES/MPS)—particularly Kupffer cells—is a dominant determinant of AgNP biodistribution after systemic dosing. Once in blood, AgNPs are rapidly opsonised and cleared by macrophage-rich organs, which explains why in vivo studies repeatedly report major deposition in liver and spleen, with measurable uptake also observed in the lungs depending on particle size, coating, and agglomeration state. This RES-driven sequestration is a key "delivery barrier" because it can limit the fraction of the injected dose that remains available to reach tumour nodules.[37] At the same time, tumour-bearing livers can display vascular abnormalities that support

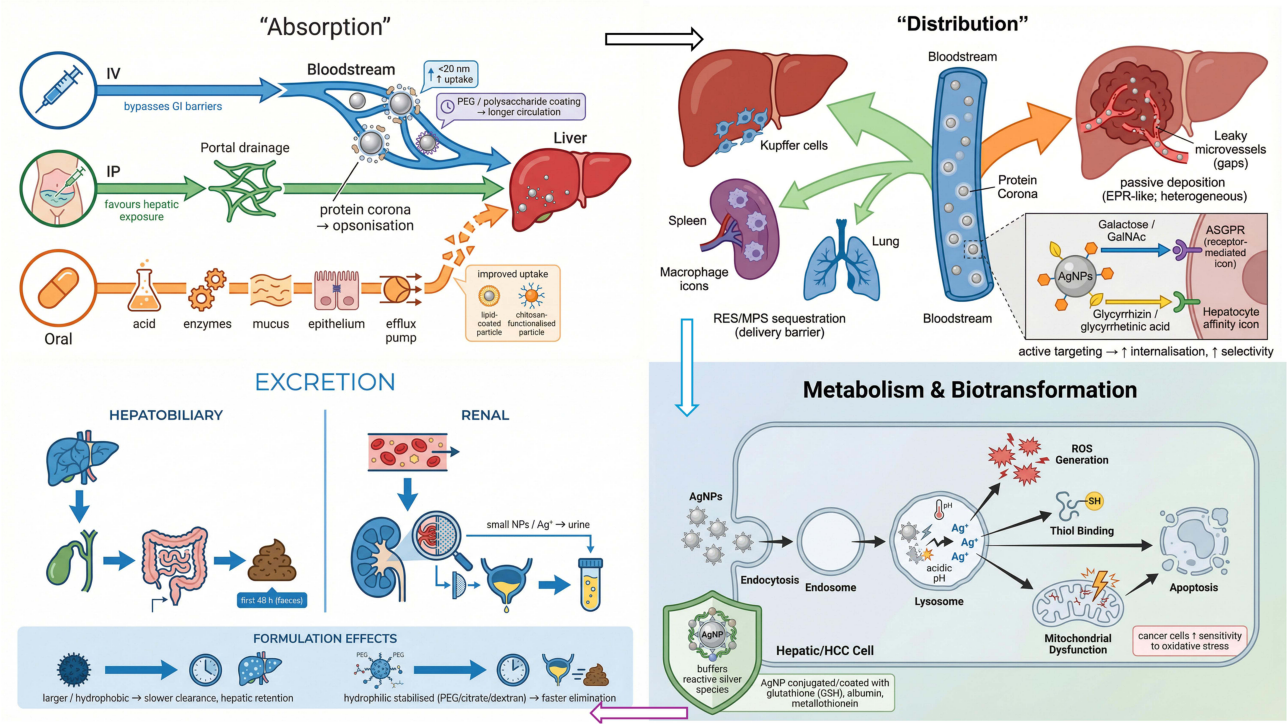

Figure 4 Schematic overview of the absorption, distribution, metabolism/biotransformation, and excretion (ADME) of silver nanoparticles (AgNPs) in hepatocellular carcinoma (HCC).

passive tumour deposition. Analogous to the enhanced permeability and retention (EPR) effect, HCC-associated angiogenesis and altered sinusoidal/tumour microvasculature can increase endothelial permeability and reduce effective clearance from tumour interstitium, thereby promoting nanoparticle accumulation within tumour regions (noting that this effect is heterogeneous across models and tumour differentiation).[75] To shift uptake from non-specific RES capture toward cell-selective internalisation, AgNPs can be surface-engineered with hepatocyte/HCC-targeting ligands. Examples include galactose (or GalNAc-like motifs) that bind the asialoglycoprotein receptor (ASGPR) and promote receptor-mediated endocytosis, as well as glycyrrhizin/glycyrrhetinic-acid–related ligands reported to increase affinity toward hepatocyte/hepatoma-associated recognition pathways. By leveraging receptor overexpression (or receptor accessibility) on malignant hepatocytes, these approaches can enhance intracellular delivery at the tumour site and, in principle, reduce systemic toxicity by improving therapeutic selectivity.[75]

Long-term accumulation of silver within hepatic tissue represents a significant safety consideration.[37,64] Experimental models have demonstrated persistence of silver deposits in the liver and spleen following systemic administration, reflecting reticuloendothelial system (RES) sequestration and limited clearance.[46,47] Such accumulation may promote chronic oxidative stress, mitochondrial dysfunction, and sustained inflammatory activation in hepatic tissue.[33,46] Kupffer cell engagement and prolonged redox imbalance may further contribute to hepatocellular injury at higher or repeated dosing.[37,64] Recent pharmacokinetic evaluations emphasise the importance of longitudinal biodistribution assessment and organ-specific toxicity profiling to clarify accumulation patterns and safety margins.[64] While short-term studies often report tolerable toxicity profiles at therapeutic concentrations, comprehensive chronic exposure investigations, particularly in cirrhotic or inflamed hepatic microenvironments, remain limited.[78]

Metabolism and Biotransformation

Reactive oxygen species (ROS) can be generated when AgNPs undergo oxidative dissolution within hepatic cells, releasing Ag^+ ions that bind to intracellular thiol-containing proteins, disrupt redox homeostasis, and trigger apoptotic pathways.[54] This redox-mediated cytotoxicity may provide a degree of tumour selectivity because malignant cells are often more vulnerable to oxidative stress than normal hepatocytes. The lysosomal compartment is considered a primary

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site for AgNP biotransformation, where the acidic environment promotes Ag^+ release and can facilitate secondary nanoparticle formation.[79] To moderate toxicity while maintaining anticancer activity, AgNPs may be conjugated with biomolecules such as glutathione, metallothioneins, or albumin, which can buffer reactive silver species. Overall, leveraging these regulated intracellular processes can support safer and more selective AgNP-based approaches for HCC therapy.[54]

Excretion

The principal routes for AgNP elimination are hepatobiliary excretion and renal clearance. Experimental detection of silver in animal models supports that a substantial fraction of hepatic elimination occurs through biliary secretion, with silver subsequently recovered in faeces, including within the first 48 hours after dosing.[56] In parallel, the kidneys can contribute to elimination, particularly for smaller nanoparticles and ionic silver species that are sufficiently small (or have been transformed into filterable forms) to pass through the glomerular filtration barrier and be excreted in urine.[50] Clearance kinetics are strongly influenced by surface chemistry and colloidal properties. Coatings such as PEG, citrate, or dextran can alter protein adsorption and aggregation behaviour, thereby shifting organ sequestration and excretion. In general, larger and more hydrophobic AgNP formulations tend to show slower clearance and prolonged hepatic retention, whereas more hydrophilic, stabilised surfaces are associated with relatively improved renal handling and faster systemic elimination.[47] These pharmacokinetic considerations are critical for HCC-directed applications, because they help guide formulation choices that achieve sufficient tumour silver exposure while minimising the risk of long-term liver accumulation and related safety concerns.[66]

Toxicity Profile of Silver Nanoparticles in HCC Context

Silver nanoparticles exhibit a complex toxicity profile that is strongly influenced by physicochemical parameters including particle size, morphology, surface charge, coating composition, and silver ion (Ag^+) dissolution kinetics.[33,37,47] In hepatic systems, AgNPs preferentially accumulate within the liver due to reticuloendothelial system (RES) sequestration, particularly by Kupffer cells.[37,64] This accumulation may induce oxidative stress, mitochondrial membrane depolarisation, DNA damage, and activation of pro-inflammatory pathways, including TNF-α and IL-6 signalling.[33,46]

Dose-dependent hepatotoxicity has been reported in animal models, characterised by elevated liver enzymes (ALT, AST), histopathological alterations, and redox imbalance.[47,65] Smaller nanoparticles (<20 nm) generally demonstrate increased cellular uptake and higher Ag^+ release rates, which may enhance anticancer activity but also increase cytotoxic risk.[35,37] Surface modifications such as PEGylation or biomolecule capping can partially mitigate rapid clearance and excessive inflammatory activation, yet long-term silver persistence in hepatic and splenic tissues remains a concern.[47,64]

Importantly, while malignant hepatocytes often exhibit heightened susceptibility to ROS-mediated apoptosis, non-malignant hepatocytes may also experience oxidative injury at higher exposure levels.[46,52] Current evidence suggests that therapeutic windows exist; however, chronic exposure studies and cirrhotic-liver models remain limited. Therefore, careful dose optimisation, longitudinal biodistribution analysis, and standardised toxicological frameworks are essential prerequisites for clinical development of AgNP-based HCC therapeutics.

Silver Nanoparticles as a Nanotechnological Platform in HCC Therapy

Hepatocellular carcinoma (HCC) remains a leading cause of cancer-related mortality worldwide, largely due to late diagnosis, tumour heterogeneity, and limited responsiveness to systemic therapies.[60,80] Conventional chemotherapeutics are frequently constrained by systemic toxicity, suboptimal tumour selectivity, and acquired resistance.[7–9] These limitations have driven increasing interest in nanotechnology-based strategies capable of enhancing tumour-specific cytotoxicity while reducing off-target injury.[10–12]

Silver nanoparticles (AgNPs) have emerged as multifunctional nanomaterials with intrinsic anticancer properties, attributable to their high surface reactivity, tunable physicochemical characteristics, and ability to induce oxidative stress–mediated apoptosis.[36,37,81,82] In HCC models, AgNPs consistently demonstrate cytotoxic effects through reactive

oxygen species (ROS) generation, mitochondrial membrane depolarisation, DNA damage, cell cycle arrest, and activation of intrinsic apoptotic pathways involving Bax, caspases, and cytochrome c.[33,35,48]

Green and Chemically Synthesised AgNPs: Comparative Evidence

Green-synthesised AgNPs using plant or microbial extracts are frequently reported to exhibit enhanced biocompatibility due to phytochemical capping agents that improve colloidal stability and modulate protein corona formation.[43,44] Reported IC_{50} values for plant-mediated AgNPs in HepG2 models range widely—from approximately 3–80 μg/mL depending on synthesis source, particle size, and experimental design.[59,83–86] A representative example of green-synthesised AgNP characterisation and corresponding cytotoxic evaluation in HepG2 cells is illustrated in Figure 5. A comparative summary of representative AgNP-based therapeutic systems investigated in hepatocellular carcinoma models is presented in Table 2.

However, chemically synthesised AgNPs demonstrate overlapping cytotoxic ranges in comparable HCC models.[35,48] Because particle size, zeta potential, and silver ion release kinetics are rarely standardised across comparative studies, it remains unclear whether observed efficacy differences are attributable to synthesis methodology or physicochemical variability.[37,46] Rigorous head-to-head comparisons under controlled conditions are required to determine whether green synthesis confers measurable therapeutic advantage beyond improved formulation stability.

Drug-Conjugated and Hybrid AgNP Systems

To enhance therapeutic specificity and potency, AgNPs have been conjugated with chemotherapeutic agents and bioactive compounds. AgNP–gemcitabine systems demonstrated enhanced apoptosis and tumour regression in chemically induced HCC rat models compared with gemcitabine alone, associated with Bax upregulation and Bcl-2 downregulation.[72] The corresponding cytotoxicity profile and IC_{50} determination are illustrated in Figure 6. Similarly, Raptinal-loaded AgNPs showed increased caspase-3 and cytochrome c expression in murine HCC models relative to free drug administration.[111]

In vitro studies further indicate that AgNP–doxorubicin constructs may improve intracellular accumulation and partially overcome multidrug resistance through modulation of ABC transporter activity.[74] However, most combination studies report enhanced cytotoxicity without formal quantitative synergy modelling (eg, Chou–Talalay combination index), limiting definitive mechanistic interpretation. Distinguishing additive effects from true pharmacodynamic synergy remains an important methodological priority.

Hybrid nanoplatforms integrating AgNPs with liposomal, polymeric, or mesoporous carriers have also demonstrated improved tumour penetration and controlled silver ion release in preclinical systems.[67,69] These multifunctional systems offer improved pharmacokinetic stability while preserving ROS-mediated cytotoxic mechanisms.

In vivo Evidence and Translational Constraints

Several animal studies report reduced tumour burden, improved histopathology, and modulation of inflammatory mediators following AgNP administration in chemically induced HCC models.[72,112] Nevertheless, heterogeneity in dosing regimens, nanoparticle size distribution, and exposure duration complicates cross-study comparison.

Importantly, the majority of available data derive from in vitro HepG2 systems or small rodent models. Differences in tumour vasculature, immune microenvironment, and nanoparticle clearance between murine and human HCC limit direct translational extrapolation.[47,66] Moreover, chronic silver accumulation within hepatic tissue and the reticuloendothelial system remains insufficiently characterised in long-term tumour-bearing models.[64,65]

Collectively, current evidence supports the mechanistic plausibility of AgNP-based HCC therapy but underscores the need for standardised nanoparticle characterisation, quantitative synergy assessment, longitudinal biodistribution analysis, and clinically relevant disease modelling prior to therapeutic generalisation.

Future Perspectives and Research Directions

The field of developing therapies based on silver nanoparticles (AgNPs) for hepatocellular carcinoma (HCC) is advancing rapidly; however, many obstacles remain before broad clinical use. Translation of AgNPs is hampered by low tumour selectivity, safety issues, and unpredictability in synthesis processes, despite their vigorous anticancer

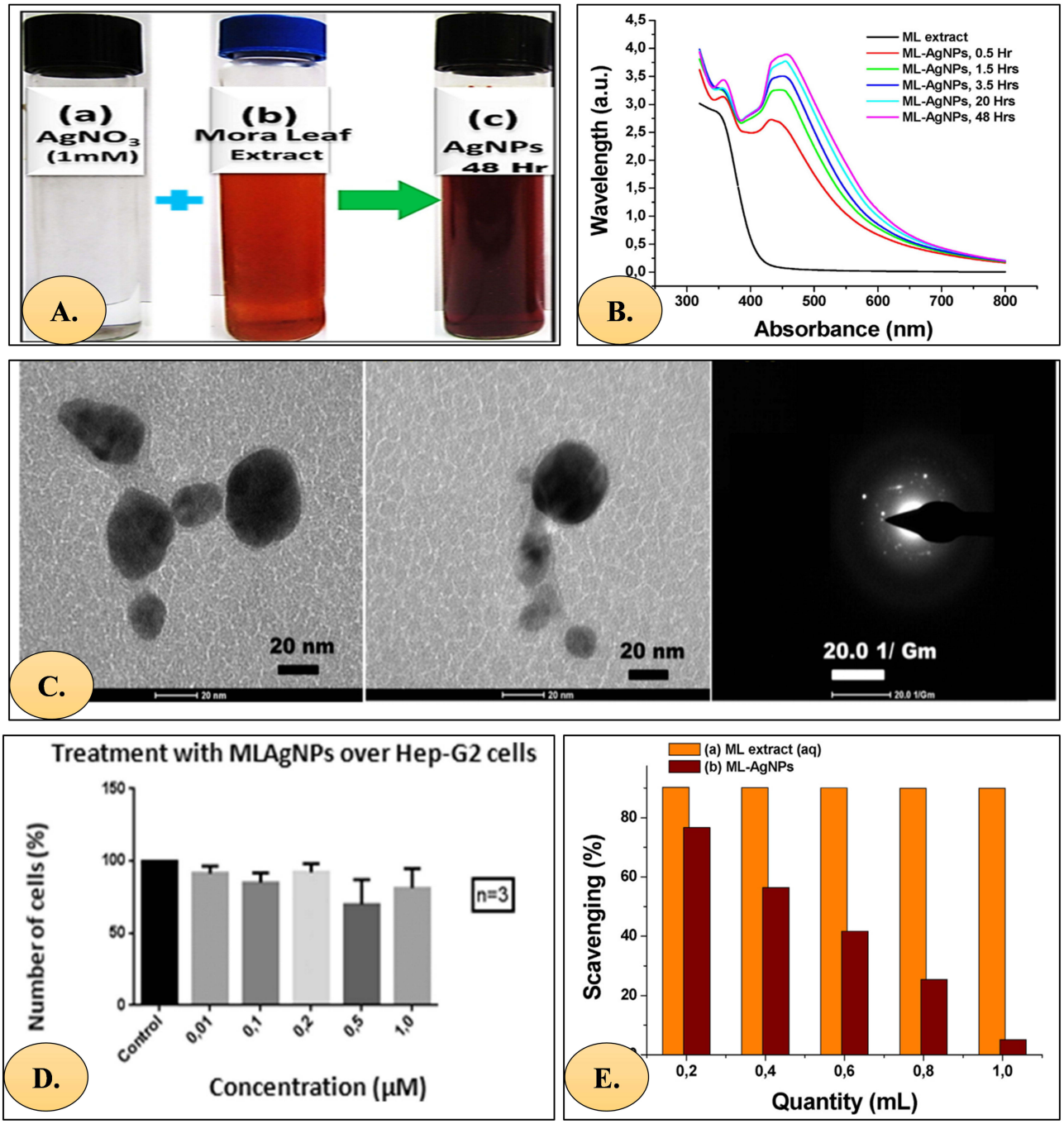

Figure 5 (**A**) 1 mM AgNO3, Andean ML extract and ML-AgNP solution after addition of extract to AgNO3 solution (**B**) UV–vis absorption spectra of ML-AgNPs. (**C**) TEM micrograph of synthesized ML-AgNPs. (**D**) Histogram showing cellular densities of Hep-G2 cells after exposure to different concentrations of ML-AgNPs. A control (no ML-AgNPs) is included. (**E**) Antioxidant activity of (a) ML extract and (b) ML-AgNPs. Reuse with permission.[87]

activity and distinctive physicochemical characteristics. Advanced targeting and intelligent carrier systems, tailored nanomedicine, integration with new biological therapies, and strong long-term clinical validation should be the main focuses of future research.[113]

Personalized Nanomedicine Approaches

According to the patient's genetic, molecular, and metabolic characteristics, personalised nanomedicine seeks to customise nanoparticle-based treatment. Individualised targeting strategies are required in HCC due to molecular

Table 2 Therapeutic Potential of Silver Nanoparticles on the Hepatocellular Carcinoma

Drug/Nanomaterial	Method Used	Particle Size (nm)	Zeta Potential (mV)	Model Used	Main Limitations/Notes	Conclusion	Ref
Genistein–AgNPs	Chemical reduction	123.47 ± 3.76	29.37 ± 1.83	HepG2	In vitro only, no in vivo validation. Pharmacokinetics and long-term safety not assessed.	Enhanced apoptosis and improved genistein efficacy in HepG2 cells.	[88]
Doxorubicin–AgNPs	Electrostatic adsorption	–	–	HepG2 + in silico	Synergy not quantified using combination index modelling; no in vivo safety evaluation.	Improved intracellular accumulation and cytotoxicity.	[73]
Cymodocea serrulata–AgNPs	Biogenic synthesis	30.5 ± 2.5	–	A549	Evaluated in non-HCC mo no in vivo tumour validation; synthesis reproducibility not addressed.	Demonstrated anticancer potential via green synthesis.	[89]
Chuanminshen–AgNPs	Green synthesis	89.56 ± 0.64	−22.08 ± 2.12	Multiple cancer lines + HepG2	Mechanistic work focused on non-HCC models; no animal validation.	Dose-dependent antiproliferative and pro-apoptotic activity.	[63]
Punica granatum–AgNPs	Chemical reduction	35–60	−26.6	HepG2	Anticancer specificity vs normal hepatocytes incompletely characterised; no in vivo validation.	Multifunctional bioactivity with cytotoxic potential.	[90]
Ananas comosus–AgNPs	Green synthesis	–	–	HepG2	Primarily exploratory multifunctional study; limited mechanistic depth; no in vivo validation.	Dose-dependent cytotoxicity and antioxidant activity.	[91]
Kaempferol–AgNPs	Green synthesis	200	−20.2	HepG2	Single HCC cell line; no normal hepatocyte comparison or in vivo validation.	Inhibited migration and induced ROS-mediated apoptosis.	[52]
Alnus nitida–AgNPs	Green synthesis	150.25 ± 5.30	−19.85 ± 2.35	Multiple cancer lines incl. HepG2	In vitro only, no tumour-bearing animal mo Ag^+ release kinetics did not standardise.	Selective cytotoxicity toward cancer cells.	[86]
Silver nitrate–AgNPs	Chemical synthesis	2	−15	HepG2	Single cell line mo no in vivo safety profiling.	ROS-mediated apoptosis induction.	[35]
Schinus molle–AgNPs	Green synthesis	35.8	−24.9	HepG2	In vitro evidence only; autophagy/apoptosis pathways not validated in vivo.	Induced oxidative stress and apoptosis.	[38]
Nigella sativa–AgNPs	Green synthesis	10–20	18.8 ± 0.372	HepG2	Limited mechanistic investigation; no animal validation.	Promising alternative anticancer approach.	[92]
Dragon fruit peel–AgNPs	Green synthesis	10–50	–	HepG2	Short-term (24 h) cytotoxicity only; no pharmacokinetic or in vivo assessment.	Dose-dependent apoptosis induction.	[93]
Zea mays–AgNPs	Green synthesis	100	–	HepG2	Focus on antioxidant + cytotoxic activity; tumour selectivity not fully evaluated.	Significant cytotoxic and antioxidant effects.	[94]
Reynoutria japonica–AgNPs	Green synthesis	35–100	−28.5	Multiple cancer lines incl. HepG2	No in vivo tumour mo systemic toxicity not evaluated.	Selective cytotoxicity toward cancer cells.	[95]
Fusarium oxysporum–AgNPs	Biogenic synthesis	56.43 ± 19.18	−26.8 ± 7.55	Huh7	Limited anticancer efficacy; no animal validation.	Moderate anticancer activity.	[49]

(*Continued*)

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Table 2 (Continued).

Drug/Nanomaterial	Method Used	Particle Size (nm)	Zeta Potential (mV)	Model Used	Main Limitations/Notes	Conclusion	Ref
Limosilactobacillus fermentum–AgNPs	Probiotic biosynthesis	6–23	−7.11	Huh7	High IC_{50} values; moderate potency; no in vivo validation.	Dose-dependent cytotoxicity.	[96]
Anabaena variabilis–AgNPs	Green synthesis	17–35	−19.5	Multiple cancer lines incl. HepG2	In vitro only; no long-term safety assessment.	Selective anticancer activity.	[97]
Sargassum vulgare–AgNPs	Phyco-synthesis	6.9–16.97	−29.6	Multiple cancer lines incl. HepG2	Mixed hepatoprotective and cytotoxic findings; mechanistic clarity limited.	Anticancer and antioxidant activity.	[98]
Senna sophera–AgNPs	Biogenic synthesis	30.6	−12	HepG2	Relatively high IC_{50}; no animal tumour model validation.	Cytotoxic and antioxidant effects.	[99]
Conocarpus lancifolius–AgNPs	Green synthesis	5–30	–	Breast cancer cells	Evaluated in non-HCC mo limited hepatic relevance.	ROS-mediated cytotoxicity.	[100]
Morus alba–AgNPs	Biogenic synthesis	10–50	−11.3	HepG2 + rat model	Dual hepatoprotective and cytotoxic effects; mechanistic separation unclear.	Restored redox balance in liver model.	[101]
Rumex hastatus–AgNPs	Green synthesis	20.34–54.67	–	HepG2 + mouse model	Concurrent hepatoprotective and cytotoxic roles require clarification; long-term safety not addressed.	Cytotoxic and hepatoprotective potential.	[102]
Clerodendron phlomoides–AgNPs	Green synthesis	125	−6.02	Rat HCC model	Rodent model only; biodistribution and chronic toxicity not evaluated.	Demonstrated anticancer efficacy in vivo.	[103]
Colloidal AgNPs	Chemical synthesis	~13 ± 1	–	HepG2 + MCF-7	Non-targeted system; tumour selectivity and systemic safety not assessed in vivo.	Dose-dependent ROS-mediated cytotoxicity.	[39]
Achillea fragrantissima–AgNPs	Green synthesis	16–22	–	HepG2 + MCF-7	In vitro-only evidence; no pharmacokinetic evaluation.	Strong cytotoxicity and apoptosis induction.	[104]
Artemisia carvifolia–AgNPs	Biogenic synthesis	80 ± 6	–	HepG2	Mechanistic focus on Rap2A; requires validation in animal tumour models.	Downregulated Rap2A and induced apoptosis.	[105]
Catharanthus roseus–AgNPs	Green synthesis	–	–	HepG2 + THLE-3	Transcriptomics limited to in vitro mo no in vivo safety profiling.	Selective cytotoxicity and pathway modulation.	[45]
Berberine–AgNPs	Green synthesis	171.5 ± 4.2	−12.4	HepG2 + normal cells	No tumour-bearing animal validation; biodistribution not studied.	Enhanced apoptosis and anticancer activity.	[106]
Prodigiosin–AgNPs	Biosynthesis	9.98	–	HepG2	Mechanistic support partly in silico; no animal validation.	MEK pathway inhibition and cytotoxicity.	[107]
Taraxaci extract–AgNPs	Green synthesis	218.7	−39.54	HepG2 + HUVEC	In vitro co-treatment only; immune and hepatic microenvironment not evaluated.	Strong cytotoxicity and improved drug tolerance.	[108]
Pyracantha crenulata–AgNPs	Biogenic synthesis	1340.8	−18.7	Hep-2	Reported size (>1 μm) raises nanoparticle classification concerns; no in vivo validation.	Suppressed liver cancer cell proliferation.	[109]
DFPAE–AgNPs	Green synthesis	10–50	–	HepG2	Short-term cytotoxicity only; no long-term safety or pharmacokinetic evaluation.	Induced apoptosis with IC_{50} 37.98 μg/mL.	[110]

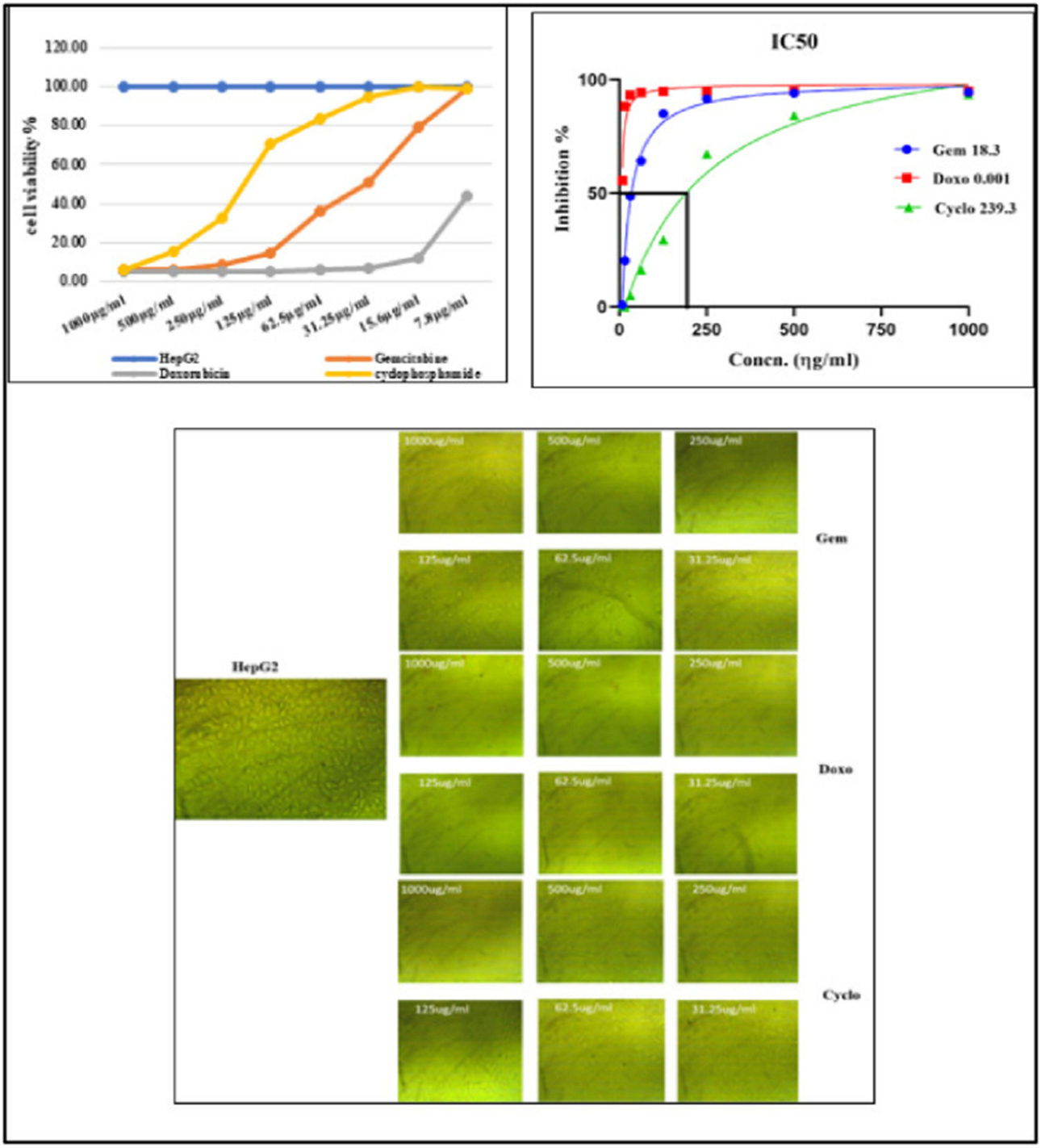

Figure 6 MTT cytotoxicity assay (% of cell viability) and IC50 of different chemotherapeutic drugs on HepG2 cells (the value displayed as means ± SE (n = 3) of two independent experiments.[72]

heterogeneity and varied receptor expression (eg, ASGPR, EGFR, or glypican-3).[50] To increase selectivity and decrease off-target effects, silver nanoparticles can be engineered with ligand combinations or surface chemistries that correspond to the receptor profile of a particular tumour subtype.[23] Furthermore, including biomarker-guided nanoparticle dose, such as by employing circulating AFP or microRNA signatures, can assist in tracking treatment response and making dynamic therapeutic adjustments.[24] By monitoring biodistribution and treatment results in real time, emerging "theranostic" AgNP

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systems that combine diagnostic imaging and therapy further assist individualized management.[50] The changing paradigm of personalized cancer care is in line with these precision-based approaches.

Advances in Targeted Delivery and Smart Nanocarriers

RES activity naturally causes AgNPs to accumulate in the liver; however, it remains challenging to deliver AgNPs specifically to tumours in HCC. Smart nanocarriers that respond to internal or external stimuli, such as pH, enzymes, redox gradients, or temperature, are the subject of current research.[57] In acidic tumour settings, for example, pH-sensitive AgNPs preferentially release silver ions while preserving healthy hepatocytes. Drug release from enzyme-triggered coatings that use matrix metalloproteinase or hyaluronidase substrates has been developed in response to tumour-specific enzyme exposure.[58] Furthermore, chemotherapeutic drugs, siRNA, or immunomodulators can be co-delivered using dual-targeting nanocarriers that combine AgNPs with polymeric or lipidic matrices. AgNP–liposome or AgNP–mesoporous silica composites are examples of hybrid nanoplatforms that have demonstrated enhanced tumour penetration, circulation time, and biostability.[67] Spatiotemporal control of drug release is enabled by externally stimulus-responsive devices, such as photoactivated and magnetically guided AgNPs, which improve accuracy and reduce systemic toxicity.[58,114] On-demand medication release and real-time monitoring will probably be combined in the upcoming generation of intelligent AgNP-based nanocarriers, bringing adaptive nanotherapy one step closer.

Integration with Immunotherapy and Gene Therapy

The immunosuppressive milieu in HCC limits the efficacy of genetic therapies and immune checkpoint inhibitors. When used with various techniques, silver nanoparticles may have synergistic effects. AgNPs have intrinsic immunomodulatory qualities that can boost cytotoxic T-cell activity, improve dendritic cell maturation, and improve antigen presentation.[50] AgNPs and immune checkpoint inhibitors (anti-PD-1 or anti-CTLA-4) have been shown to improve tumour regression in preclinical models when administered together.[57] AgNPs can also be used as gene delivery vehicles, delivering CRISPR-Cas9 components, microRNA mimics, or small interfering RNA (siRNA) straight into HCC cells.[115] These platforms enhance treatment effectiveness by enabling silver-mediated apoptosis and gene silencing (eg, VEGF, Bcl-2, or MMP9) at the same time.[115] AgNP integration into multimodal regimens, such as chemo-immunotherapy or immuno-gene therapy, can take advantage of complementary pathways to overcome resistance and foster long-lasting antitumour immunity.[114] To prevent recurrence or progression in high-risk groups, future research should include investigating AgNP-based vaccine adjuvants that target HCC-associated antigens.[53]

Long-Term Safety Studies and Clinical Validation

Despite encouraging preclinical and in vitro results, AgNPs' long-term safety and clinical validation remain incomplete. The main difficulties are chronic oxidative stress in non-tumour tissues, biodistribution heterogeneity, and possible hepatic and renal accumulation.[100] To ensure reproducibility, standardization of nanoparticle manufacturing and thorough pharmacokinetic–toxicodynamic correlation studies are necessary for regulatory acceptability.[64] Potential immunogenicity, chronic exposure concerns, and dose-response correlations should all be assessed in future clinical trials, including a variety of patient populations. Safer prediction of nanoparticle activity before human trials is now possible because to advance in silico toxicological models and organoid-based liver systems.[40] Additionally, translation can be accelerated by synthesis that complies with Good Manufacturing Practice (GMP), biodistribution tracking using real-time imaging, and the use of safety indicators, including hepatic enzymes, oxidative stress markers, and serum Ag levels.[40] Establishing standardized procedures for clinical-grade AgNP formulations will require cooperative frameworks between regulatory bodies, hepatologists, and nanotechnologists. AgNP-based treatments cannot obtain clinical integration and regulatory approval for the treatment of HCC until such thorough validation is completed.[116,117]

Translational Limitations of Preclinical Evidence

The majority of evidence supporting AgNP anticancer activity in HCC derives from in vitro cell culture systems or small animal models.[66] However, extrapolation to human disease is constrained by interspecies differences in tumour vasculature, immune architecture, nanoparticle clearance kinetics, and hepatic microanatomy.[37,64] Murine models often

exhibit more pronounced and homogeneous enhanced permeability and retention (EPR) effects than human tumours, potentially leading to overestimation of nanoparticle accumulation and therapeutic efficacy.[66] Furthermore, controlled laboratory dosing conditions do not fully recapitulate the complexity of chronic liver disease, viral hepatitis, and cirrhotic microenvironments that characterise clinical HCC.[118] Chronic inflammation, fibrosis, and altered immune signalling may significantly influence nanoparticle biodistribution and therapeutic response.[78] These translational discrepancies necessitate cautious interpretation of preclinical efficacy data and underscore the importance of clinically relevant modelling frameworks before therapeutic conclusions can be generalised to human patients.

Challenges and Innovations

Despite their potential, NP-based treatments face many obstacles. With as much as 50–70% of injected NPs accumulating in non-target liver tissue, high liver absorption by the reticuloendothelial system (RES), especially Kupffer cells, prevents tumour-specific delivery.[71] Intratumoural distribution is restricted by the fibrotic HCC stroma and elevated interstitial fluid pressure, which also hinders NP penetration.[119] Because of toxicity issues, including AgNP-induced hepatotoxicity through ROS production, careful dose adjustment is required.[120] PEGylation, which decreases opsonisation and increases circulation half-life, and zwitterionic coatings, which reduce the development of protein corona, are examples of innovations to overcome these difficulties.[42] NPs that respond to stimuli, such as redox-triggered micelles or pH-sensitive liposomes, release payloads selectively in the hypoxic or acidic (pH ~6.5) HCC milieu, increasing their effectiveness.[121] In preclinical models, biomimetic coatings, such as those seen on erythrocytes or cancer cell membranes, improve tumour homing and immune evasion, improving delivery efficiency by two to three times.[122] Synergistic benefits are demonstrated by nanotechnology-enabled combination therapies, such as NP co-delivery of TKIs and checkpoint inhibitors; for example, PLGA NPs co-loaded with sorafenib and anti-PD-1 antibodies decreased tumour development in HCC xenografts by 60% in comparison to monotherapies.[123]

Batch-to-Batch Reproducibility in Green-Synthesised AgNPs

While green and biogenic synthesis approaches are widely promoted for improved biocompatibility and reduced use of toxic reagents, reproducibility remains a significant translational challenge. Plant-mediated or microorganism-mediated reduction processes inherently depend on biological variability, including differences in phytochemical composition, seasonal variation, extraction conditions, and storage stability.[43,44,82] These factors can influence nucleation kinetics, particle growth rate, final size distribution, morphology, surface chemistry, and silver ion release behaviour.[13,37]

Batch-to-batch heterogeneity may result in variations in zeta potential, protein corona formation, dissolution rate, and ultimately biological activity.[40,47] Since AgNP cytotoxicity is highly size- and surface-dependent, even modest physicochemical deviations may significantly alter therapeutic index and toxicity profile.[33,37] To improve reproducibility, several strategies have been proposed, including:

- Standardisation of extract preparation protocols and phytochemical quantification.[44]
- Real-time monitoring of nanoparticle nucleation using spectroscopic methods.[13]
- Rigorous physicochemical characterisation (eg, DLS, TEM, ICP-MS for Ag^+ release).[36,37]
- And in accordance with ICH Q8–Q10 principles synthesis workflows and controlled manufacturing systems.[64,66]

Integration of process analytical technologies and computational optimisation approaches may further enhance batch consistency. However, until robust manufacturing standardisation is achieved, green-synthesised AgNP systems remain at an early translational stage compared with chemically controlled nanoparticle platforms.[36,37]

Conclusion

Hepatocellular carcinoma remains a formidable global health challenge due to its complex pathogenesis, late clinical presentation, and limited effectiveness of current therapeutic options. The growing experimental evidence discussed in this article highlights silver nanoparticles as a versatile nanotechnological platform with significant potential in hepatic cancer management. Through multiple anticancer mechanisms, while exhibiting dose-dependent redox-mediated toxicity

that requires careful optimisation, silver nanoparticles effectively target key hallmarks of hepatocellular carcinoma. Advances in green and biogenic synthesis approaches have further improved their biocompatibility, reducing concerns associated with conventional chemically synthesised nanoparticles.

In addition to their intrinsic cytotoxic properties, silver nanoparticles offer substantial advantages in tumour targeting through both passive accumulation and ligand-mediated active delivery strategies. Their ability to be functionalised with chemotherapeutic agents, phytoconstituents, or stimuli-responsive coatings provides opportunities to enhance therapeutic efficacy while minimising off-target toxicity. Nevertheless, challenges related to nanoparticle stability, biodistribution, long-term safety, and reproducibility remain critical barriers to clinical translation.

Clinical translation of AgNP-based therapeutics requires rigorous compliance with Good Manufacturing Practice (GMP) standards, reproducible nanoparticle characterisation protocols, and validated long-term toxicity frameworks.[37,47] Regulatory approval pathways for nanomedicine demand harmonised physicochemical characterisation, batch-to-batch consistency, and comprehensive pharmacokinetic evaluation to ensure safety and efficacy. Variability in synthesis methods—particularly between chemically reduced and green-mediated systems—currently represents a significant barrier to standardisation and regulatory approval.[36,43] Emerging translational analyses emphasise the importance of integrating pharmacovigilance, immune safety assessment, and disease-specific modelling in chronic liver conditions.[118] Addressing these regulatory and manufacturing challenges is essential before AgNP-based platforms can progress toward clinical implementation in HCC.

Future investigations should prioritise standardised synthesis protocols, comprehensive toxicological profiling, and well-designed in vivo and clinical studies to establish safety and therapeutic relevance. With continued interdisciplinary efforts integrating nanotechnology, molecular oncology, and translational research, silver nanoparticle–based systems may emerge as a valuable component of next-generation therapeutic strategies for hepatocellular carcinoma.

Data Sharing Statement

Data availability does not apply to this article as no new data were created or analyzed in this study.

Ethics Declarations

This article does not contain any studies with human participants performed by any of the authors.

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Disclosure

The authors declare that they have no competing financial interests, affiliations, or personal relationships that could have influenced the work reported in this manuscript.

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