Andrographis Paniculata in the Nanotechnology Era: A Review of Therapeutic Benefits and Novel Formulations

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ABSTRACT

Andrographis paniculata (Burm. f.) Nees, (AP) a traditional medicinal plant, treats various conditions such as microbial infections, diabetes, liver disorders, and cancer. The AP's key diterpenoid, andrographolide (AG), accounts for its major therapeutic properties. The use of AP extracts and their bioactive components in treatments is limited because they have low solubility, are quickly metabolized, and followed by less effective in the body. New ways of delivering drugs, called novel drug delivery systems (NDDS), have been developed. Herbal nanoformulations, comprising natural herbal extracts or bioactive components at a nanoscale size, offer distinct advantages over conventional formulations, such as improved bioavailability and minimized toxicity risk. Some of these systems are nanoparticles, liposomes, phytosomes, niosomes, and lipid nanoparticles. This review summarizes research conducted between 2003 and 2023 on the use of AP extracts and their bioactive components in nanoformulations. It will also discuss recent advances in nanoformulations that make targeting specific therapeutic conditions more precise.

Key Words: Andrographis paniculate, Andrographolides, Novel drug delivery systems, Nanoparticles, Nano formulation, Biopolymers

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INTRODUCTION

Plants contain phytoconstituents that provide therapeutic effects and serve as medication to treat various diseases. Herbs, which are generally less toxic than conventional medications, are becoming more popular as people seek alternative ways to improve their health. According to the recent reports of WHO, herbal medicines are now widely used as nutraceuticals and are the primary source of healthcare for over 80% of the population in developing countries [1-3]. Herbal drugs are considered safer and more affordable than synthetic drugs due to their natural ingredients, which reduce the risk of side effects and adverse reactions. They are also more easily accessible than synthetic drugs [4]. Plant extracts can be challenging to use clinically because of their low solubility, rapid metabolism, elimination from the body, and limited bioavailability. Progress has been achieved by scientists in developing novel drug delivery systems (NDDS), capable of administering medication accurately and gradually. They have a plethora of materials to leverage in formulating these innovative solutions, ranging from polymers and nano/micro-particles to liposomes and lipid-based nanoparticles, as well as gels and emulsions [5]. Nanotechnology is the field of study focused on the development and application of extremely tiny materials and devices, structured at the nanometre level [6]. A nanoformulation consists of nanoparticles (carrier materials) that encapsulate therapeutic compounds. Nanotechnology is used to deliver and transport a large number of promising pharmaceuticals to ensure that they reach their intended recipients' locations. Nano formulations have advantages over conventional formulations, such as better bioavailability, targeting, release control, stability, less toxicity, higher permeability,

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and versatility, making them more effective for different substances and use.

Andrographis paniculata (AP), originating from Southern Asia and belonging to the Acanthaceae family, is frequently referred to as Kalmegh, King of Bitters, or Bhui Neem [7]. There is growing interest among scientists regarding the potential of AP in addressing a variety of health conditions. These include bacterial infections, elevated blood sugar levels, inflammation, oxidative stress, liver impairment, high cholesterol levels, respiratory infections, and neurodegenerative disorders [8]. AP is a key component in many Ayurvedic formulations for liver disorders and is widely used as a home remedy in Ayurvedic, Siddha, and Unani medicine systems [9]. AP is rich in bioactive phytochemicals like diterpene lactones, flavonoids, and polyphenols. Its biological attributes stem from its diverse terpenoid content, with andrographolide being the most crucial [10]. This research aims to contribute a review of the recent advancements in AP and its active compound nanoformulations, pinpointing current trends and recognizing potential avenues for future research and development.

MATERIALS AND METHODS

Extensive research was carried out by exploring articles published from January 2003 until March 2023 using various electronic databases such as PubMed, Taylor & Francis, Wiley Interscience, Springer Link, Science Direct, SCIELO, DOAJ, Science Alert, ResearchGate, Semantic Scholar, and Google Scholar, among others. Based on keywords such as *Andrographis paniculata*, nanoformulation, diabetes, liver disease, and cancer. We identified relevant articles for this review, focusing on peer-reviewed English research articles that discuss AP nanoformulations *in vitro* and/or *in vivo*. Among the 108 publications initially assessed, 73 were included in this study. In this review, duplicate articles, irrelevant content, and publications in other languages have been excluded.

Pharmacological activity

Anti-asthmatic activity

Asthma is a respiratory condition characterized by inflamed, narrowed airways, leading to breathing difficulties due to changes in immune cells and cytokine levels. AG, the primary active component of AP, has shown potential for treating asthma by inhibiting the NF-kB signaling pathway [11]. Liposomal AG dry powder inhalers (LADPIs) demonstrate potential for treating *S. aureus*-induced pneumonia, outperforming AG and penicillin, by effectively delivering to the lungs and reducing pro-inflammatory cytokines while inhibiting the NF-κB pathway [12]. An inhalable AG-β-cyclodextrin inclusion complex (AG-β-CD; diameter: 2.03μm) was developed to treat *S. aureus*-induced pneumonia. Even though the anti-Staphylococcus aureus effect of AG, AG-β-CD, and penicillin *in vitro* was limited, their administration *via* the pulmonary route demonstrated significant beneficial outcomes. AG and AG-β-CD inhibited bacteria and reduced inflammation by regulating immune responses, with AG-β-CD proving more effective due to improved AG dissolution from β-CD inclusion [13]. AG treatment may increase lung HO-1 levels, potentially benefiting lung injury, inflammation, and conditions such as chronic obstructive pulmonary disease (COPD), asthma, and acute lung injury [14]. The AG's potential effectiveness is limited by its low bioavailability and short duration of action in the bloodstream. AG nanoparticles (AG-NP) treatment for asthma demonstrates enhanced bioavailability, increased suppression of NF-κB, reduced inflammatory cytokines like IL-4, IL-5, and IL-13, and greater effectiveness in reducing inflammation and improving therapeutic outcomes when administered through the lungs compared to oral delivery in a mouse model [15].

Anti-microbial activity

Traditional medicine systems have used medicinal plants for centuries to treat and prevent infectious diseases caused by microorganisms. These plants contain bioactive compounds that can act against bacteria, fungi, viruses, and parasites by either killing them or inhibiting their growth and reproduction. Silver nanoparticles offer health benefits such as enhanced antimicrobial activity, improved wound healing, and reduced inflammation. Effective against various bacteria, including *Pseudomonas aeruginosa*, they provide a non-toxic, safe option for treating bacterial infections. Green-synthesized AgNPs using AP (Average size 54 ± 2 nm; Zeta potential -50.7 mV) exhibited potent antifungal activity against *Aspergillus niger* and *Penicillium* sp. at a 10 mg concentration, showing results similar to the reference drug Fluconazole [16]. The silver nanoparticles (AgNPs) synthesized with AP aqueous extract by reducing silver nitrate obtained 40-60 nm protein-capped particles that effectively inhibited seven bacterial strains, with *P. aeruginosa* being the most susceptible. The synthesized AgNP displayed a 21.33 mm zone of inhibition (ZOI) and a 3.125 μg/mL minimum inhibitory concentration (MIC) against *P. aeruginosa*, possibly attributed to the secondary metabolites present in the AP extract [17]. CdO nanoparticles, synthesized from AP plant extract and analyzed using XRD, FESEM, HRTEM, and FT-IR, exhibited a face-cantered cubic structure, a 22 nm average particle size, and a 2.33 eV optical energy bandgap. The biosynthesized CdO nanoparticles demonstrated good antibacterial activity against both gram-(+) and gram-(-) bacterial strains, with a

notable 16 mm zone of inhibition against *E. coli* [18]. Inhalable AG-β-CD demonstrates promise in treating *Staphylococcus aureus* pneumonia, effectively inhibiting *S. aureus* and reducing inflammation through immune response regulation [13]. Biogenic silver nanoparticles (bAgNPs), developed utilizing extracts from the AP stem, demonstrated dose-dependent antibacterial activity against a wide range of both pathogenic and non-pathogenic bacteria. The minimum inhibitory concentration (MIC) for both types of bAgNPs was 0.125µg. Notably, ethanolic-bAgNPs exhibited the greatest antibacterial effectiveness against *S. aureus* at a concentration of 60 µg after 16 hours, leading to an inhibition zone of 28 mm [19]. Nickel-doped cobalt ferrite nanoparticles (Ni-$CoFe_2O_4$), synthesized using AP plant extract through a microwave-assisted method, exhibited a cubic lattice structure (24 - 32 nm; 25 mm ZOI) with a decreasing lattice parameter as nickel content increased. The comparison of the anti-bacterial activity of Ni-doped and undoped Ni nanoparticles showed that Ni-$CoFe_2O_4$ NPs were more effective than undoped $CoFe_2O_4$ NPs [20]. Ionic liquid-assisted samarium oxide nanoparticles were synthesized in an eco-friendly manner using an extract from the leaves of AP (IL-Sm2O3 NPs; 30-50 nm) and were produced through a hydrothermal process. These nanoparticles exhibited antimicrobial attributes, effectively combating *E. coli* and *S. aureus* by discharging metal cations that induced internal toxicity within these microbes. The IL-Sm_2O_3 NPs proved especially effective against *E. coli* because they could penetrate the bacterium's proteins and lipids, resulting in DNA damage [21]. In another study, researchers economically synthesized silver nanoparticles using AP and cetyltrimethylammonium bromide (CTAB) as a capping agent, with characterization revealing high crystallinity and sizes ranging from 0.897 nm to 45 nm. The synthesized silver nanoparticles exhibited antimicrobial properties when compared with streptomycin and ketoconazole [22]. AgNPs arabinoxylan conjugates exhibit dose-dependent antimicrobial activity, showing greater effectiveness against gram (+) bacteria *S. pneumonia* than gram (-) *E. coli*, and reduced sensitivity against *C. albicans* with increased antifungal activity at higher concentrations. The antimicrobial action of AgNPs may occur through various pathways, such as causing cellular leakage, deforming cells, damaging DNA, inactivating enzymes, denaturing proteins, and generating reactive oxygen species [23]. Calcium hydroxide-based AgNPs, synthesized using AP and *Ocimum sanctum* Linn leaves, exhibited potent antifungal and antimicrobial properties with minimal cytotoxicity, suggesting potential use in dental treatments like root canal irrigation or intracanal medicaments [24]. AG-AgNPs exhibit excellent antimicrobial activity against *Burkholderia pseudomallei*, a Gram (-) pathogen causing melioidosis. AG-AgNPs are suggested for fighting *B. pseudomallei* by binding to the surface, neutralizing the membrane charge, and causing biomolecule damage, eventually resulting in bacterial death. They achieve this by inducing structural changes, releasing Ag+ ions, and generating intracellular ROS [25]. Using a hydrothermal method, AG and citric acid were transformed into carbon dots that emit blue fluorescence at a wavelength of 430 nm. These carbon dots showed considerable antibacterial effectiveness against *E. coli* and *S. aureus*, having a minimum inhibitory concentration (MIC) of 1.14 mg/mL. Positively charged carbon quantum dots, with a charge of 16 eV, have the ability to bind to bacterial cell walls and generate singlet oxygen, which carries antibacterial attributes [26]. Orthodontic adhesives were formulated with calcium phosphate monohydrate/Sr-bioactive glass nanoparticles and AG, with the aim to stimulate mineral accumulation and curb bacterial proliferation. The incorporation of Sr/CaP fillers enhanced the discharge of calcium, strontium, and phosphorus ions, promoting the formation of calcium phosphate and boosting the suppression of *S. mutans* by 18%. These adhesives demonstrated enamel bonding capacities comparable to those of commercial materials, coupled with an amplified inhibition of *S. mutans*. This could potentially decrease the incidence of white spot lesions during fixed orthodontic procedures [27].

Anti-cancer activity

Cancer, a leading cause of death globally, can be prevented and treated with the help of phytochemicals from traditional medicinal plants. These plants are essential in cancer drug discovery as they offer bioactive compounds with therapeutic potential. Many contemporary cancer drugs originate from or are inspired by plant-based compounds used in traditional medicine. AG is one of the active compounds in AP and exhibits anti-tumor properties due to its antiangiogenic and antiproliferative effects. However, its clinical application in cancer prevention is limited by challenges such as oral bioavailability, lipophilicity, solubility, taste, protein affinity, and half-life [9, 28]. AG-SLNs, prepared using solvent injection with an average size of 154.9 ± 10.7 nm, demonstrated sustained effects with 77.89% medication dispersal *in vitro* over thirty-six hours and decreased cellular survival in MCF-7 compared to standard AG [29]. A niosomal formulation of AG was developed to enhance its absorption and distribution, using the film hydration/sonication method to achieve a 72.36% entrapment efficiency, a 5.90% drug-loading ratio, and a 206 nm average particle size. Tissue distribution studies revealed improved liver absorption of AG niosomes in mice compared to free AG, while the anti-HCC activity in HepG2 cells demonstrated no significant difference between the two forms of AG [30]. Encapsulating AG in solid lipid nanoparticles (AG-SLNs)

enhances its solubility and bioavailability. Using cetyl alcohol as the lipid, polysorbate 80 as the emulsifier, and ethanol as the solvent, optimized SLNs were produced with small particle size (154 nm), narrow size distribution (PDI of 0.172), high entrapment efficiency (91.4%), and high drug loading (18.6%), leading to a 3.41-fold improvement in oral bioavailability and enhanced antitumor activity in mice compared to drug suspension [31]. Utilizing biodegradable PLGA-PEG-PLGA triblock copolymers, polymeric micelles were developed to encapsulate hydrophobic AG, improving its bioavailability and anticancer effectiveness. The AG-loaded micelles had a stable, nearly spherical shape (124.3 ± 6.4 nm), a narrow size range, high loading (92%), and good encapsulation. They exhibited a sustained release mechanism, enhanced anticancer efficacy on MAD-MB-231 cells, and increased bioavailability in rats compared to free AG [32]. Using the self-emulsification-solvent evaporation method, a successful nanoformulation with particle sizes between 63-111 nm was prepared and a polydispersity value of 0.162. Treating HepG2 cells with AG and betulinic acid individually and in combination inhibited cell proliferation, with the combined treatment showing greater efficacy in reducing cell viability [33]. Eco-friendly and non-toxic silver nanoparticles in terpenoid (Ag-NPs-TAP) showed notable effectiveness against HeLa and Hep-G2 cancer cells, with 59.01% and 48.79% activity at 250μg/ml, respectively [34]. The AG nanoconstruct offers enhanced water solubility, controlled drug release, and superior tumour targeting, utilizing water-soluble NPs formulated using a polymer obtained from phenylboronic acid for systemic administration, demonstrating exceptional *in vitro* and *in vivo* targeting properties and significantly reducing tumour growth [35]. An efficient and secure method was used to develop AG-loaded regenerated silk fibroin (RSF) nanoparticles ranging in size from 200 to 1000 nm. The nanoparticles released AG over three days with a 25.9% drug loading rate and an 87.3% encapsulation efficiency. RSF nanoparticles demonstrated minimal cytotoxicity and were effectively bound to HeLa and MDA-MB-231 cells [36]. AgNPs (18–70 nm, spherical) derived from AP methanol leaf extract exhibited activity against neuroblastoma cells, while demonstrating non-cytotoxicity towards Vero cell lines, with a CC_{50} value of 329.29 μg/ml [37]. Using a microwave-assisted method and AP as a chelating agent, synthesized green nickel oxide nanoparticles (NiO NPs), which showed cytotoxicity against MCF-7 cell lines with an IC_{50} value of 44.91 µg/mL, involving ROS-mediated DNA damage [38]. The aqueous solubility of AGs increased by developing an AG-loaded nanoemulsion (AG-NE) and evaluating its effectiveness against keratinocyte carcinoma cancer cells. Using high-pressure homogenization to prepare AG-NE, the resulting formulation demonstrated nanometre droplet size, low viscosity, and good physical stability. It effectively induced apoptosis in A-431 cancer cells with IC_{50} values of 25.83 µg/ml and 54.80 µg/ml, respectively, without causing toxicity to normal HFF-1 cells at low concentrations [39]. In another study, AG-loaded nanocochleates (AGNCs) demonstrated a more potent effect on MCF-7 cell survival at concentrations as low as 5 µg/mL compared to free AG, while also exhibiting a higher Cmax and extended terminal half-life. According to the study on AGNCs, researchers found that they had a 1.18-amplified uptake *via* oral administration and a decrease in accumulation in other organs [40]. By using reflux extraction and Soxhlet fractionation techniques, a rich extract of AG was obtained and then nano-encapsulated with PLGA and 1% PVA. The nanoparticles that were produced exhibited a size of 163 nm, a PDI of 0.26, and a zeta potential of -57.85 mV. The process managed to achieve an encapsulation efficiency of 80.0%, while the *in vitro* drug release was recorded at 84.2%. These nanoparticles were successful in inhibiting the growth of cervical and neuroblastoma cells without causing any harm to normal human skin cells, primarily because of Bax-induced apoptosis. Additionally, in mice with HeLa tumours, the andrographolide-rich nanoparticles significantly reduced tumour size by 73% [41]. Another report revealed that AgNPs developed from AP extract displayed significantly elevated cytotoxicity, induced programmed cell death, and increased cellular absorption when tested against the free drug in MDA-MB-453 breast cancer cells [42]. In a different study, AG-SLN showed a significantly lower IC_{50} than free AG in multiple cell lines and demonstrated to be more efficient in causing cellular cycle arrest and programmed cell death. Moreover, AG-SLN treatment showed increased intracellular absorption of AG in HN6 cells as opposed to free AG [43]. MTT experiments conducted on ovarian carcinoma PA1 cells revealed that water-soluble Ag NPs-arabinoxylan conjugates displayed dose-dependent cytotoxicity. With an LD_{50} of 10 µg/mL and almost total cell destruction at 50 μg/mL [23]. By utilizing an aqueous extract from AP leaves, the research team synthesized AgNPs and investigated their potential to induce cell death in HeLa and EAC cells in a mouse model as part of their in vitro studies. A dose-dependent cytotoxic effect was found on HeLa cells with an IC50 of 7.285 µg/mL. To evaluate tumor formation, they injected twenty-four male mice intraperitoneally with AgNPs (350 g/kg BW) and plant extract (80 mg/kg BW) for 10 days after tumor induction using a calculated dose of 40 µL. AgNPs effectively eradicated cancer cells by restoring biological markers and hematological indicators to almost normal levels in treated groups. By utilizing an aqueous extract from AP leaves, the research team synthesized AgNPs and investigated their potential to induce cell death in HeLa and EAC cells in a

mouse model as part of their in vitro studies. A dose-dependent cytotoxic effect was found on HeLa cells with an IC50 of 7.285 µg/mL. To evaluate tumor formation, they injected twenty-four male mice intraperitoneally with AgNPs (350 g/kg BW) and plant extract (80 mg/kg BW) for 10 days after tumor induction using a calculated dose of 40 µL. AgNPs effectively eradicated cancer cells by restoring biological markers and hematological indicators to almost normal levels in treated groups [44]. The envelopment of AG in PLGA NPs (particle size of 100–150 nm) provided sustained release over 48 hours and significantly improved in vitro anticancer efficacy in opposition to the LM2 cell line, compared to the unbound drug (IC50 of 27.68 µM for the free drug vs. 16.80 µM for nanoformulation). At the concentrations used, neither DMSO solvent nor PLGA polymer exhibited cell damaging effects on LM2 cells. The nanoformulation maintained sustained cell damaging effects on cell survivability for twelve days, whereas the free drug's effects were temporary, persisting for only six days. This formulation, compared to the free drug, demonstrated enhanced and prolonged suppression of triple-negative LM2 cell growth. The analysis of the cell cycle showed that the drug and nanoformulation caused cell accumulation in the G2/M phase. The nanoformulation showed a stronger therapeutic effect because it led to more cells in this phase [45]. Due to its low solubility, AG faces challenges in product development despite its potential skin benefits. An improved AG-loaded nanoemulsion (AG-NE) was established to overcome this issue, which shows promising cytotoxicity against human malignant melanoma and non-melanoma cells and inhibits intracellular tyrosinase activity. AG-NE effectively reduces skin coloration and harm in rats, without toxicity to normal skin fibroblasts like HFF-1 cells, suggesting its potential as a treatment for skin cancers and UVB-induced skin disorders [46]. AG interacts with host transcription factors to inhibit EBV lytic reactivation, partially due to HDAC6's involvement with these factors, and induces apoptosis in Epstein–Barr virus-associated gastric cancer cells (EBVaGC) [47]. The ionic gelation method was employed to produce chitosan nanoparticles incorporating extracts from *Berberis aristata*, AP, and *Thevetia peruviana*. The combination of these extracts in different ratios (2:1:1) containing berberine (2.188%), AG (0.472%), and rosmarinic acid (0.264%) demonstrated cytotoxic and anticancer properties. The combination treatments (2:1:1, 1:1:1, 1:1:2, and 1:2:1) showed IC_{50} values ranging from 13.96 to 16.47µg/ml against HepG2 cell lines [48]. A molecular complex of AG with soya-L-α-phosphatidyl choline (AGSPC) formed self-formed soft nanoparticles, which displayed significantly lower IC_{50} values and better endocytosis in Neuro2a cells compared to free AG. *In-vitro* assays demonstrated that the self-formed soft AGSPC-NPs induced programmed cell death in Neuro2a cells by decreasing mitochondrial membrane potential and increasing ROS generation [49]. L-α-phosphatidylcholine and sonication were used to obtain optimal particle size of 243.7 ± 7.3 nm, PDI of 0.310, and EE of 72.20 ± 4.53 in AG-loaded phytosomes. The controlled release of AG in this improved formulation successfully impeded the proliferation of HepG2 liver cancer cells over 24 hours. AG-loaded phytosomes (AG-PTMs) amplified the uptake of AG in HepG2 cells, resulting in a G2-M cell cycle phase arrest and induction of apoptosis in the pre-G1 phase. These effects were attributed to oxidative stress, mitochondrial dysfunction, and altered expression of genes related to apoptosis. The AG delivered through phytosomes effectively halted the proliferation of HepG2 cells by intensifying cellular uptake, pausing the cell cycle, and inducing apoptotic effects in the mitochondria [50].

Anti-diabetic activity

Diabetes is a common metabolism-related illness, that affects millions worldwide, with cases expected to surpass 600 million by 2045. Modern antidiabetic drugs, though effective, have side effects, leading to growing interest in alternative therapies like plant extracts and bioactive compounds. Herbal drugs can potentially become more effective in treating diabetes when combined with nanocarriers. This is due to the nanocarriers' ability to boost solubility and bioavailability while offering controlled and targeted drug delivery, ultimately resulting in safer and more efficient usage of herbal-based antidiabetic treatments [51]. ZnO NPs were developed using AP leaf extract, which exhibited efficient reducing and capping properties. Analytical evidence supports the contribution of phytochemicals present in the extract to the process of nucleation and stability. Both spherical and hexagonal shapes were observed in the ZnO NPs, which had sizes ranging from 57 ± 0.3 nm to 96-115 nm. The developed ZnO NPs demonstrated modest α-amylase inhibition activity, with lower IC_{50} values (121.42 µg/mL) compared to AP leaf extract and $ZnNO_3$ 149.65 and 178.84 µg/mL, respectively [52]. The solvent evaporation method was used to develop Nano-DDA by adding 14-deoxy, 11, and 12-didehydro andrographolide to polycaprolactone nanoparticles. The Nano-DDA had a spherical shape, measured 252.9 nm in size, had a zeta potential of -38.9 mV, and had a 91.98 ± 0.13% encapsulation efficiency with 15.09 ± 0.18% loading efficiency. Nanoencapsulation led to enhanced glucose absorption in L6 myotubes because of the prolonged and steady release of the drug. *In vitro*, the drug discharge demonstrated a biphasic trend, with a quick burst within the first 24 hours, and a consistent release lasting for eleven days [53]. Casein micelles were used to encapsulate the herbal anti-diabetic drug, AP extract. The active ingredients, which are responsible for the

antidiabetic activity, were rapidly released as the micelles degraded in simulated gastric fluid [54]. Using a modified liquid dispersion technique, a nano-phytovesicular formulation with semi-purified AG extracts and soya phosphatidylcholine was developed to enhance antihyperglycemic activity and oral absorption in rats. The nano-phytovesicles were spherical, unilamellar, and globular with a particle size of 395.5 ± 5.80 nm. In rats, the semi-purified AP extract at 25 mg/kg provided significant protection against hyperglycemia, surpassing the results of 50 mg/kg free AG. This resulted in improvements in body weight, glucose tolerance, and blood glucose control. Both *in vitro* and *in vivo* tests demonstrated enhanced intestinal uptake, systemic availability, and blood sugar-lowering effects [55].

Anti-hyperlipidaemic activity

Hyperlipidemia is defined as an abnormal increase in the bloodstream levels of one or more lipoproteins, including triglycerides, cholesterol, LDL, or VLDL. It is the most significant risk factor for coronary heart attack and stroke. AG-packed SLNPs (AG-SLNPs) were synthesized *via* high-pressure homogenization, with a spherical shape, an average diameter of 286.1 nm, and a zeta potential of -20.8 mV. Hyperlipidaemic mice were induced using a 75% yolk emulsion, and the model was successfully established. Treatment with simvastatin significantly reduced serum lipid levels, including TC, TG, and LDL. AG-SLNs also reduced TC levels at various dosages, with a high dosage (40 mg/kg) significantly decreasing TG and LDL levels, and a medium dosage (20 mg/kg) significantly reducing LDL levels. However, AG alone only had modest lipid-lowering effects. AG-SLNs increased AG's bioavailability and antihyperlipidemic activity by enhancing its solubility and stability, and modifying its delivering method in Caco-2 cells, culminating in a 241% enhancement in intestinal uptake contrasted to AG suspension [56].

Anti-inflammatory activity

Inflammation is an inherent reaction of the immune system in the body, triggered by harmful stimuli like injury, contaminants, allergens, or infections. This response is linked to several diseases, including rheumatoid arthritis, atherosclerosis, obesity, and cancer. Nanoparticles of AG were produced using wet media milling and freeze-drying techniques, with the help of stabilizers such as D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) and sodium lauryl sulfate. These optimized nanosuspensions had a particle size of 244.6 ± 3.0 nm, showed rapid dissolution *in vitro*, and had a re-dispersibility index of 113 ± 1.14%. The anti-inflammatory properties of AG-NS were demonstrated by its ability to decrease paw swelling, increase NO, IL-1, and TNF-α levels in serum, and enhance superoxide dismutase activity [57]. According to the results of the protein denaturation assay, the highest inhibition activity was achieved through the bio-mediated fabrication of ZnO NPs using AP leaf extract, with an IC_{50} value of 66.78 µg/mL. This was found to be more effective than using AP leaf extract alone (75.42 µg/mL) or $ZnNO_3$ (91.33 µg/mL). However, the reference compound, diclofenac, had a slightly lower IC_{50} value of 62.55 µg/mL [52]. Samarium oxide nanoparticles were synthesized through the hydrothermal method with AP leaf extract. These nanoparticles displayed antibacterial, antioxidant, and anti-inflammatory properties. They had a cubic structure and measured 30-50 nm on average. In anti-inflammatory evaluation, Ionic liquid-Sm_2O_3 NPs exhibited notable inhibition in albumin denaturation assays, comparable to standard diclofenac sodium across all concentrations [21].

Antileishmanial activity

Leishmaniasis is a global public health concern with limited treatment options. The search for effective and safe antileishmanial molecules is crucial, as current chemotherapy faces challenges like toxicity and drug resistance. There is evidence that medicinal plants, including organic extracts and natural compounds, may have antileishmanial properties. AG exhibits potent antileishmanial properties but faces chemotherapy limitations due to limited absorption, rapid plasma clearance, and insufficient target site accumulation. AGNPs were developed using PLGA to deliver AG to monocyte-macrophage cells containing leishmanial parasites. By applying PVA as a stabilizer, they refined AGNP characteristics, producing spherical particles with an average size of 173 nm and a zeta potential of -34.8 mV. The anti-leishmanial activity of AGNPs with 4% PVA was significant (IC_{50} - 34 µM) and required only reduced to a quarter of the dosage of pure AG (IC_{50} - 160 μM) [58]. AGNPs combined with vitamin E TPGS, a P-gp efflux inhibitor, to specifically target drug-resistant Leishmania strains. These spherical, smooth-surfaced nanoparticles had an average size of 179.6 nm, a PDI of 0.245, and a zeta potential of 237.6 mV. AGNPs with TPGS proved effective against both pharmaceuticals and environmentally sourced Leishmania strains, requiring lower doses and causing less cellular damage compared to standard drugs. Macrophages absorbed these nanoparticles almost completely within an hour [59]. Similarly, another study found that AG-AUNPs were effective against both wild and drug-resistant forms of leishmaniasis, had low toxicity, and were quickly taken up by macrophages [60].

Antimalarial activity

Malaria, caused by *Plasmodium* spp., leads to fever, anemia, and an enlarged spleen. Drug resistance to chloroquine and sulfadoxine-pyrimethamine worsens

illness and fatalities. Therefore, antimalarial drugs must be delivered in effective ways. AP leave extracts were deployed as diminishing and capping agents to produce AgNPs, which exhibited an average particle size of 35-55 nm and a cubic structure. The AgNPs showed anti-plasmodial activity against *Plasmodium falciparum*, with IC_{50} values of 26 ± 0.2% at 25 µg/ml and 83 ± 0.5% at 100 μg/ml [61]. Compared to andrographolide alone, AG-carboxymethyl chitosan nanoparticles reduced melting points and boosted drug dissolution by 6.5 times, leading to 1.65 times increase in *in vivo* antimalarial activity [62].

Hepatoprotective activity

Liver problems are a worldwide concern, making it crucial to safeguard liver health since the organ faces various threats, such as pathogens, toxins, drugs, and alcohol. Medicinal plants provide a valuable resource for hepatoprotective agents with reduced side effects. AG's clinical use is restricted because of inadequate water solubility. The Eudragit® EPO based NPs suspension of AG (particle size 255.9 nm) displayed enhanced hepatoprotection as contrasted to pure AG, with half the dose providing comparable results. Improved hepatoprotective activity may be attributed to submicron drug particles and the interaction between polycationic polymers and negatively charged gastrointestinal mucous, with the NPs efficacy further supported by reduced severity of histopathological hepatic lesions [63]. At doses of 25 and 50 mg/kg, the AG herbosome boosts bioavailability and enhances hepatoprotective properties compared to the same doses of pure AG. The complex-derived AG plasma concentration (Cmax - 9.64 µg/mL) exceeds that of the pure AG (Cmax - 6.79 µg/mL), maintaining effective levels for a more extended period. Administering AG herbosomes to rats before CCl_4-induced liver injury effectively shields their livers and restores normal enzyme activity [64]. In another study, a film hydration/sonication approach was used to develop an AG niosomal formulation that exhibited a 72.36% entrapment efficiency, 5.90% drug-to-carrier ratio, and an average particle size of 206 nm, enhancing its bioavailability and tissue distribution. Although the tissue distribution results demonstrated increased AG niosome absorption in the HEPG2 cells compared to free AG, no notable distinction in anti-hepatocellular carcinoma activity was observed between the two [30]. In both normal and N-acetyl-p-aminophenol (APAP)- induced liver damage, AG-loaded PLGA-NPs rapidly localized to the liver cells. Mice with APAP overdose experienced efficient hepatoprotection from AG-NPs, which quickly regenerated antioxidant capacity and restored hepatic GSH stores [65]. Nanoencapsulated AG was developed, which has an average diameter of 65.8 nm and a 64% encapsulation efficiency. Both AG and its nanoencapsulated form were able to lower arsenic-induced liver markers, arsenic deposition, and ROS levels while increasing antioxidant enzyme levels in the liver. Notably, nanoencapsulated AG was five times better than AG at protecting the liver from damage caused by arsenic [66]. The AG-loaded NE with chitosan and alginate resulted in enhanced transparency and stability, with particle sizes ranging from 90.8 to 167.8 nm and zeta potential values indicating high stability. In mice afflicted with Galactosamine-LPS toxicity, the regulated release of NE in simulated digestive fluids enhanced liver function and lowered serum cytokine levels. Owing to its greater solubility, stability, and bioavailability, NE demonstrated improved hepatoprotection in *in vivo* studies [67]. AG nanocrystals were developed using solvent diffusion and homogenization using Tween 80 (1%) as a stabilizer, resulting in a 630 ± 12 nm average particle size and modified crystallinity, leading to a nearly fivefold increase in aqueous solubility. AGNC demonstrated a notable hepatoprotective effect against paracetamol-induced liver injury at lower doses compared to crude AG [68].

Antiulcer activity

Traditional medicine has long utilized medicinal plants to treat various health conditions, including ulcers, which are lesions in the gastrointestinal tract lining often caused by factors such as spicy food, obesity, stress, and excessive NSAID use. Ulcer treatment aims to alleviate pain, heal the ulcer, and minimize recurrence, with 75% of cases linked to *Helicobacter pylori* infection. Gellan gum and calcium carbonate were mixed to make an AG-loaded floating in-situ gel that was created to treat gastric ulcers. The gel rapidly formed and floated for over 24 hours in simulated gastric fluid, initially releasing a burst of the drug, followed by a continuous and prolonged release. *In vivo* evaluation demonstrated reduced acid and protein levels, increased hemoglobin levels, and a minimal ulcer index while preserving gastric mucosa integrity. The improved anti-ulcer effect was ascribed to AG's prolonged retention in the stomach, leading to enhancements in MPO activity, LPO levels, mucin content, and GPx activity [69]. The AG-packed NE (AG-NE) was developed to improve AG's oral absorption rate and effectiveness contrary to inflammatory bowel disease, featuring an optimized, stable formulation (4 and 25°C for 90 days) with a droplet size of 122 ± 11 nm and a viscosity of 28 cps. By improving the AG-NE formula, researchers found that the jejunum absorbs AG better than AG suspension (8.21 times) and AG ethanol solution (1.40 times). Pharmacokinetic analysis revealed a substantial enhancement in AG absorption with AG-NE compared to AG suspension, resulting in a relative bioavailability of 594.3%. Moreover, pre-treatment with AG-NE effectively reduced the ulcer index and

histological damage scores in mice afflicted with indomethacin-induced intestinal lesions [70].

Wound healing activity

Skin is an important barrier that protects the body from external threats. Wounds can cause physical disabilities by damaging the skin. Plants and plant-derived substances have been used for a long time to treat incisions, burns, and other injuries. AG-containing LNPs were formulated, refined, and effectively integrated into a chitosan-hyaluronic acid scaffold, exhibiting a spherical shape, 253 nm size, 83.04% encapsulation capacity, and extended drug discharge up to 48 hours; their application to second-degree burn wounds significantly diminished scar formation and promoted healing, potentially attributable to the anti-inflammatory and antioxidant effects of chitosan, hyaluronic acid, and nanoparticles [71]. PLGA incorporating AG-mesoporous silica nanoparticles into the wound dressing improved hydrophilicity, mechanical strength, porosity, and pH, while exhibiting strong antibacterial properties and promoting epidermal cell adhesion and growth, ultimately accelerating wound healing in *in vivo* tests [72]. Niosomal gel containing AP ethanol extracts (Size 128.3 ± 1.31 and EE 97.75%) demonstrated remarkable wound healing in Sprague Dawley rats by promoting re-epithelialization activity, while also potentially enhancing topical application through increased drug penetration into skin layers, thereby effectively supporting wound healing and protecting tissue from oxidative stress [73].

CONCLUSION

Medicinal plants play a vital role in preventing and treating diseases effectively, offering significant therapeutic benefits, and promoting a healthy lifestyle. Scientists employ various techniques to convert plant extracts and isolated compounds into nanoparticles using materials such as silver, gold, zinc oxide, and natural or synthetic polymers. These nanoformulations strive to deliver beneficial substances to the body in a targeted and controlled manner while providing enhanced stability and resistance to degradation by gastric fluids. Typically, bioactivity assessments involve *in vitro* studies utilizing cellular and non-cellular models, as well as short-term *in vivo* evaluations using animal models. Many studies have confirmed the remarkable properties of medicinal plant extracts and individual compounds that have been transformed into nanoparticles at the nanoscale. Nanoformulations have demonstrated exceptional advantages compared to their non-nanoformulated counterparts. AP has many health benefits, such as fighting against asthma, microbes, cancer, diabetes, and high cholesterol. It reduces inflammation, heals the liver, and can treat diseases caused by parasites such as leishmaniasis and malaria. AG is a key component of AP, which can enhance a healthy lifestyle. Traditional AG dosage forms have low oral bioavailability for several reasons. Newer methods for making better delivery systems focus on reducing solubility and toxicity issues to improve AG's bioavailability. Traditional AG dosage forms have low oral bioavailability for several reasons. Newer methods for making better delivery systems focus on reducing solubility and toxicity issues to improve AG's bioavailability. Nevertheless, nanoformulations are reported to augment and refine the effects of APs. Toxicological studies are crucial for the safety of herbal and nanoformulated drugs, and while some *in vivo* studies have confirmed safety, further research is needed. This review highlights recent advancements in APs and their active constituents' nanoformulation development, aiming to unlock their full therapeutic potential as an alternative treatment option.

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