Nanocarriers in the Enhancement of Therapeutic Efficacy of Natural Drugs

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Abstract
Since time immemorial, plant derived natural products have been used for the treatment of various human diseases before the intervention of modern medicine. The basis of modern medicine is still being inspired from traditional medicine and therapies. However, despite their tremendous therapeutic potential, these natural drugs often have poor bioavailability, metabolic instability, and aqueous insolubility. These factors greatly impede a natural drug’s commercialization potential as a mainstream medicine. Therefore, the development of nanocarrier drug delivery systems is indispensable in overcoming the various constraints of the bottlenecks which occur with natural drugs. Of particular interest in this review are four plant materials endogenous to China with the common names of barrenwort or horny goat weed (Epimedium), Sha Di Huang (Rehmannia glutinosa, RG), ginseng (Panax ginseng), and Dong Quai or female ginseng (Angelica sinensis, AS), each having been scientifically investigated for a wide range of therapeutic uses as has been originally discovered from the long history of traditional usage and anecdotal information by local population groups in Asia. The integration of natural drugs from the East and nanocarrier drug delivery systems developed from the West is paving the way towards further accurate and efficient medicine therapy. We further discuss the potential benefits of these plants and the enhancement of their therapeutic efficacy by nanotechnology intervention.

Keywords
Nanocarriers Intervention, Natural Drugs, Therapeutic Efficacy.

Introduction
Natural products have served as indispensable sources of medicines since the beginning of time. Almost a quarter to a half of all commonly used drugs are originated from the natural products [1]. Because of their better therapeutic activity and because they have less side effects compared with homeopathic chemical medicine, phyto-medicines are attracting increasing interest in clinical trials than was previously the case. Natural products usually contain various drug constituents as crude extracts, they have surprising potential in in-vitro experiments while they have a mediocre performance in-vivo due to their poor solubility and inappropriate size, resulting in low availability and absorption.

A nanocarrier drug delivery system can serve as a novel efficient method to conquer the limits of natural products. Versatile drug delivery systems like polymeric nanoparticles (NPs), magnetic NPs, liposomes, solid lipid NPs, micelles, dendrimers, and carbon nanotubes have been proven to have ability to improve the therapy effect of herbal medicines [2]. Judah Folkman at Harvard first put forward the concept of drug delivery systems in the mid-1960s. The German physicist Gred Bing and the Swiss physicist Heinrich Rohrer invented the scanning tunneling microscope (STM) in a Swiss laboratory in 1981. STM enabled human beings to observe the array status of individual atoms on the surface of a substance and to observe, for the first time, the physical chemical properties related to surface electronic behavior in real time. A few years later, the first nanometer size materials were discovered by Herbert Gleiter in Australia, which initiated research in the field of nanomaterials and contributed to the advances in instrumentation. Thereafter, the NP field began to rapidly develop over the next 30 years in Western countries [3–5]. The novel nanocarrier delivery system have attracted worldwide research. Nanotechnology enhances the bioavailability and bioactivity of phytomedicine by decreasing the size into NPs, modifying surface characteristics, and promoting aqueous solubility and permeability across biological membrane [6].

The combined application of nanotechnology and natural products is a rapidly evolving field. Nanotechnology brings multiple benefits from natural compounds to many chronic human diseases.

Traditional natural tonics consist of four types including: aphrodisiacs, Yin medicine,
Qi boosters, and blood tonics. *Epimedium, Rehmannia glutinosa* (RG), *Panax ginseng*, and *Angelica sinensis* (AS) are representative drugs in these four categories of tonics. *Epimedium* can nourish the kidneys and works as an aphrodisiac, strengthening muscles and bones; *Rehmannia* can nourish yin, enrich fluid production, ease pyrexia, and stem bleeding [7]; *Ginseng* is popular as a strong tonic in strengthening vitality, activating circulation, as well as calming the mind [8–11]; *Angelica* nourishes the blood, promoting good blood circulation, lubricating the intestine, and regulating bowel movement [12–16]. They are commonly used in traditional Chinese medicine in a variety of diseases and are mainly given by oral administration and are sometimes made into drops or given as injections. Natural products have their shortcomings but have been proved to have strong therapeutic effects, similar to nanocarrier drug delivery systems used to treat the same condition. Integrating Eastern and Western medicine may complement their advantages to accomplish targeted efficient treatment as well as decrease side effects, gradually pushing medical discovery [17]. The purpose of this review is to generalize the therapeutic effects of these plants and discuss their further usages using nanotechnology.

**Epimedium**

*Epimedium* is a herb in the Berberidaceae family and describes the dried leaves of the *Epimedium* plants: *E. brevicornum* Maxim., *E. sagittatum* Maxim., *E. pubescens* Maxim., or *E. koreanum* Nakai., also known as herba Epimdii, Yinyanghuo, fairy wings, rowdy lamb herb, barrenwort, bishop’s hat, or horny goat weed (Figure 1A, B). It has been used as a medicinal herb in East Asia to increase libido and improve sexual health, cognitive function and brain health, for cardiovascular diseases, to boost testosterone, for osteoporosis and neurasthenia [18]. The main constituents of *Epimedium* are flavonoids, alkaloids, polysaccharides, terpenoids, and lignan compounds [19], which are usually abstracted by conventional Soxhlet extraction, boiling extraction, or using an ultrasonic technique [20]. Flavonoids, the major constituents in *Epimedium*, including icariin and epimedin A–C (Figure 1C), have been shown to implement various clinical efficacies such as promoting cardiovascular health, stimulating sexual function, and as an anti-inflammation, anti-cancer, anti-osteoporosis, and anti-oxidation agents [21, 22]. Alkaloid has also been found to play an important role as antioxidants and anti-glycemic agents [23, 24].

Recent studies have demonstrated that icariin, as the major pharmacologically active flavonoid component of *Epimedium*, induced G2/M arrest of the cell cycle and cell death through the activation of the mitogen-activated protein kinase (MAPK)/extracellular-signal-regulated kinase (ERK) pathway to inhibit the growth of breast cancer [25]. Thus it might be a potent growth inhibitor and trigger apoptosis of cancer cell via nuclear factor kappa B (NF-kB)-mediated cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein (c-FLIP) expression [26] and the mitochondria- and Fas-mediated caspase-dependent pathways [27], or its regulatory mechanism of miR-21 which targets phosphatase and tensin homolog (PTEN), reversion inducing cysteine rich protein with Kazal motifs (RECK) and BCL2 apoptosis regulator (BCL2) [28]. The *Epimedium* herb was further investigated to show that it has anti-angiogenic effects by acting on ERK signaling pathway in human umbilical vein endothelial cells (HUVECs) [29] and overcomes T790M mutation-mediated drug resistance in nonsmall-cell lung cancer (NSCLC) via the combination with gefitinib [30].

In previous studies, the clinical application of icariin has been hindered because of its poor absorption and low bioavailability after intravenous or intra-gastric administration, implying that new efficient carriers should be developed for icariin injection. Yang et al. described that icariin propylene glycol-liposome suspension (ICA-PG-liposomes) had different pharmacokinetic behavior and better tissue distribution compared with ICA-PG-solution [31]. They prepared the ICA-PG-liposome suspension by mixing trehalose solution and ICA-PG solution and collected the tissues and plasma after intraperitoneal administration from mice. In animal experiments, the ICA-PG-liposome with its nanometer size and high encapsulation, could significantly prolong the mean retention time, strengthen icariin absorption, raise the maximum concentration and the area under curve in plasma, while it led to abundant icariin being enriched to the spleen, liver, and other tissues. Thus, liposome-based carriers fulfill the drug therapy effects when combined with icariin than icariin therapy alone. The sudden release and the release rate of drugs are the problems that commonly hamper their application.

Figure 1 (A) The whole plant of *Epimedium*; (B) the medicinal part of *Epimedium* (from the Plant Photo Bank of China); (C) the chemical structures and names of the major compounds isolated from *Epimedium*. 

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in clinical applications. Chen et al. designed an organic–inorganic hybrid composite microsphere encapsulating icarin with chitosan/nanohydroxyapatite (CS/nHAP) as a drug delivery carrier [32]. Due to the electrostatic interaction, icarin carrying reactive negative hydroxyl (–OH) could combine to the positive amine groups (–NH₂) on the net of CS, and the nHAP inside the CS retained a homogeneously dispersed condition (Figure 2). These two points showed that icarin-loaded microspheres could keep a continuous release in in-vitro studies. As a promising suitable drug delivery system, icarin-loaded CS/nHAP had been proven to do well on up-regulating the bioactivity of osteoblasts. Another study fabricated a CS/gelatin multilayer film coated with icaritin-loaded titanium dioxide (TiO₂) nanotubes that successfully enhance the spreading and adhesion of osteoblasts as well as stimulated the activity of bone-related genes. The composite structure can conquer the shortages (like bio-inertia, passive bone integration, and deficient osteo-induction) of a Ti-implant while controlling the release profile of icarin to achieve better bone repair compared with pure Ti or multilayer coated nanotubes, resulting in the development of implants in the field of orthopedic and dental research [33].

Kedong Tai et al. monitored the characteristic of icarin-loaded liposomes under different surfactants and cholesterol-to-soybean-lecithin (Chol/SL) mass ratios [34] (Figure 3A). Lipophilic icaritin (Figure 3B) is the derivate of icarin as a prenylated flavonoid, used in various fields like cancer intervention, immunosuppressive therapy, and bone repair with the limitation of being sensitive to light, heat, oxidants, and oxygen which makes it to store and limits its application. The study found that the average diameter and encapsulation efficiency exhibited growth when added with cholesterol and icaritin into bilayers, also surfactants such as sucrose esters and Tween-80 can benefit liposomal systems with the highest release levels (75.68 ± 0.25% and 77.23 ± 0.58%) in in vitro digestion experiments and show the best physico-chemical stability. Liposomes could be a candidate vesicle, which can be used to treat the dispersion of icarin and other flavonoid compounds in aqueous solutions because of their better biological activity.

Rehmannia glutinosa

RG, the radix of R. glutinosa Libosch. belongs to the family Scrophulariaceae, is usually harvested in the autumn and processed into ‘Shu-Dihuang’ by stewing with liquor or steaming (Figure 4A–C). The main compounds which are responsible for the pharmacological activity of Rehmannia contain iridoid glycosides, catalpol (Figure 4D) and RG polysaccharides [35]. It is considered that iridoid glycosides have a potential anti-osteoporotic effect, RG polysaccharides can regulate immune response [36] and glucolipid metabolism [37], as well as catalpol which is used in in-vitro experiments in various disease like cancer, diabetes, atherosclerosis, neurovascular diseases, and so on [38–42]. Pharmacological research has shown that RG may act as a potential candidate drug in diabetes, osteoporosis, gynecological, and hematological diseases.

RG might become a prospective medicine to prevent the Alzheimer’s disease via the protein kinase C (PKC) and ERK1/2 pathways by upregulating the expression of the
glial cell line-derived neurotrophic factor gene, which can revive the midbrain embryonic dopaminergic neurons [43]. RG extract could (1) downregulate the expression of transcription factors (NF-κB, c-Fos, NFATc1) and activation of MAPK to defeat against ovariectomy (OVX)-induced bone loss, osteoclast differentiation, and maturation and release of inflammatory cytokine [44], (2) stimulate the IGF-1/PI3K/mTOR pathways to enhance bone formation, resulting in being a hopeful candidate agent to prevent diabetic osteoporosis [45]. Catalpol was found to be able to alleviate atherosclerosis by modulating the PGC-1α/TERT pathway [46].

*Rehmannia glutinosa* polysaccharide (RGP) has been found that struggling in short action time, poor targeting effect and being easily cleared in the body while need to use a large clinical dosage in the clinical use. Huang et al. used the thin film hydration method and ultra-sonication technique to self-assemble the PEGylation nano-RGP (pRL), and they optimized the preparation to fit the drug targeting effect and enhance the immunological function [47]. RGP solution was hydrated with the dry film which was made with lipid and cholesterol. After being roto-evaporated, homogenized, and filtered, the pRL became a homogeneous solution with the size of 31.98 ± 2.6 nm and encapsulation efficiency of 95.81 ± 1.58%. Polymer polyethylene glycol (PEG) is widely used in nanotechnology and drug delivery for its biocompatibility and because it is non-toxic. Huang et al.’s report found that PEGylation of NPs could effectively improve its pharmacokinetic profile, reduce dosing concentration, and promote the targeting effect in the use of RGP. In the in-vitro experiments, pRL promoted the release of pro-inflammatory cytokines, encouraged macrophage proliferation, and enhanced cellular uptake which was better than nano-RGP.

Qin et al. combined the RGP-liposome (RGPL) with antigen to show the role of NP-based vaccine adjuvant [48]. RGPL was known to have the desired immunomodulation enhancement in a low dosage after encapsulating by liposomes. This study further researched how RGPL worked in the immunological environment. When carrying the antigen [OVA-specific immunoglobulin (IgG) or porcine circovirus type 2 (PCV-2)-specific IgG] on the surface, RGPL could stimulate a higher level of antibodies than RGP. In addition, (1) larger production of interferon-gamma (IFN-γ) and interferon (IL)-4, and a strong IgG response were found in *in vivo* experiments which were regarded as the signal of the activation of Th1 and Th2 subsets, and (2) RGPL did a good job in generating immune memory and activating dendritic cell (DC) maturation compared with antigen-carried RGP. Polysaccharide-based liposome adjuvants might be a promising candidate in developing a novel vaccine adjuvant system.

### Panax ginseng

Panax ginseng, the dry radix and rhizome of *P. ginseng* C. A. Mey, in the family Araliaceae (Figure 5A, B), means ‘all-healing’ due to its wide clinical therapeutic effects [49]. Depending on its different planting environments and processing methods, ginseng has different product names like ‘garden ginseng’ (artificial cultivation), ‘Zi-Hai’ (grown naturally in the wild in mountain forests), ‘Shengshai ginseng’ (washed and dried) and ‘red ginseng’ (steamed and dried), etc. [50]. Several clinical reports on ginseng have proved its therapeutic properties such as promoting glucose metabolism, moderating the immune response, regulating hypertension, and strengthening the body [51]. Saponins, polysaccharides, and phenolics are the key compounds of ginseng exact [52–54] (Figure 5C). Saponins in ginseng are usually known as ginsenosides, which have been reported to have anti-cancer and immune-modulatory capacity with polysaccharides [55, 56]. Phenolics are known to have antioxidant activity.

As one of the active metabolites of ginseng saponin, compound K can induce tumor cell apoptosis by reviving epigenetically-silenced genes and suppressing DNA methyltransferase 1 protein expression to have antitumor effects [57]. Polysaccharides were reported to modulate Twist and target its downstream gene expression to accomplish cell apoptosis and cell cycle arrest [58], while ginsenoside Rp1, through regulating CD29-mediated cell adhesion, suppresses the production of IL-1β by the NF-κB pathway [59, 60]. And Rp1 can reduce the proliferation of cancer cells via the insulin-like growth factor 1 receptor/Akt pathway [61].
Crude and major active compounds of ginseng are degraded by intestinal microbiota after oral administration, resulting in low oral bioavailability, limited solubility, and untargeted cytotoxicity. As a result, the development of a drug delivery system and biomolecular conjugation techniques may inspire the clinical application of ginseng extract. Kim et al. explored the new ingredient of gold NPs (AuNPs) synthesized from ginseng berry (GB) extract in the field of cosmetics [62]. They found that the components of GB extract could make the metal salts stabilize and turn into biocompatible functional NPs. GB-AuNPs were synthesized by adding gold (III) chloride trihydrate, used as precursor salt, into aqueous GB extract. After co-cultivating with the human dermal fibroblast (HDF) and murine melanoma B16BL6 (B16) cells, cell viability was still in a stable rate while GB-AuNPs could decrease the damage caused by hydrogen peroxide (H2O2), generally enhance the moisture retention function, and download the expression of cellular tyrosinase and melanin. GB-medicated AuNPs, being multifunctional and biocompatible, could be a promising cosmetic ingredient.

In another report, Ahn et al. used the leaf extract of P. ginseng Meyer (PG) to complex with AuNPs and discovered that PG-AuNPs could exert an anti-inflammatory effect via the p38 MAPK pathway to block lipopolysaccharide (LPS)-mediated NF-κB activation in macrophages, downregulate the expression of nitric oxide (NO), prostaglandin E2 (PG2), IL-6, and tumor necrosis factor-alpha (TNF-α) [63]. The research on PG-AuNPs may provide a novel therapy method to mediate inflammation. The silver NP (AgNP) synthesized from PG could also stimulate the MAPK pathway to control cell apoptosis, and reduce the viability and migration of cancer cells in anti-cancer therapy [64]. Singh et al. first found that fresh root extract of P. ginseng was suitable to composite with gold and silver NPs and manufactured a rapid and convenient method for the biosynthesis [65]. In brief, ginseng root stock filtrate was mixed with sterile water, silver nitrate was then added and the reaction mixture was kept at 80°C for 2 h. Using the same methodology for AuNPs, gold (III) chloride trihydrate was added instead at 80°C for 5 min. The change in color of the mixture indicated the end of reaction, the synthesis of AgNPs and AuNPs was done when the color turned from being white to being brown or pink. Ginsenosides and polysaccharides, the phytochemicals of P. ginseng, can influence the production of NPs due to its efficient reduction action. The saponin glycosides can stabilize the NPs by providing a coating on the surface of the NPs.

Ramya et al. designed a self-assembled conjugated spherical NP, using hydrophobic ginsenoside compound K (CK) and hydrophilic glycol chitosan (GC), to enhance water solubility and the targeted delivery of ginsenosides [66]. Through an acid-labile linkage, CK was covalently conjugated to the frame of GC and had two particles sizes (255 nm and 296 nm) according to the degree of CK substitution (Figure 6). CK-GC NPs gradually degrades in acidic conditions (pH 5.0) and remains stable in a physiological buffer (pH 7.4), this made it possible for CK targeted intracellular or tumor tissue delivery for suitable pH conditions. Compared with the free drug, utilizing nanosized drug carriers to encapsulate the drugs could improve its pharmacological efficacy. Due to its targeted delivery and the higher cytotoxicity of cancer cells, CK-GC conjugate could promote the anti-tumor activity of CK as a tumor-specific delivery carrier. In addition, using polyglutamic acid (PGA) or fucoidan (Fu), which are antithrombotic materials, to
encapsulate the red ginseng, extract loaded CS NPs could enhance ginsenoside solubility and support its release in an acidic environment [67].

**Angelica sinensis**

AS, the radix of the *A. sinensis* (Oliv.) Diels in the Umbelliferae family (Figure 7A–C), is a traditional herbal medicine known as ‘Danggui’ in Chinese for its ‘blood nourishing’ quality [68]. It is commonly used to animate and replenish blood, alleviate pain, and lubricate the intestines as a medicinal and edible plant. Recent pharmacological studies showed that AS extract has wide bioactive effects including: anti-inflammatory, anti-cancer, anti-cardiovascular, memory amelioration, neuroprotective, immunomodulatory, anti-oxidative, radioprotective, and anti-hepatotoxic, etc. [69]. AS has been used therapeutically in nephrotic syndrome, gynecologic diseases, nervous system diseases, and cardiovascular disease. Phthalides, ferulic acid, and polysaccharides are considered as the active components of AS [70] (Figure 7D). Phthalides are reported in diastolic vascular smooth muscle and fight against oxidative stress [71]; ferulic acid exhibits estrogenic activity, and polysaccharides contribute to anti-cancer therapy and immunomodulation [72].

Cancer studies have reported that two polysaccharides from AS can promote the proliferation of the splenocytes, advance the mRNA expression of IL-2/6, IFN-γ in splenocytes, and impel the release of TNF-α and NO in peritoneal macrophages to display anti-tumor activities [72]. Other studies suggested that AS can activate the Nrf2 pathway, down-regulate IL-2β and cause TNF-α secretion of LPS induced to protect against oxidative stress [73]; as well as demethylate the Nrf2 promoter CpGs, re-express Nrf2 and its target genes to prevent the development of prostate cancer [74].

Deng et al. provided the first report showing that *A. sinensis* polysaccharide (ASP) had been tested extensively as an efficient gene delivery material for its advantages...
including biodegradability, biocompatibility, and reduced cytotoxicity. They designed a gene vectorcationized ASP (cASP) which was modified with a branched low-molecular-weight poly-ethylenimine (LMW PEI) (1200 Da) through the linkage of the –NHCO– group to deliver the plasmid DNA encoding transforming growth factor β1 (pTGF-β1) into mesenchymal stem cells [75] (Figure 8). cASP has a positive charge on the surface so that it can combine and reverse the charge of plasmid DNA from negative to positive while ASP without modification could not really retain DNA. Polysaccharides could be transported into cells through the known biological process, resulting in the better transfection. More importantly, the combination with low molecular weight polyethylenimine (LMW PEI), which has low cytotoxicity, and biodegradable natural polysaccharide cASP might help shape cASP/pTGF-β1 NPs to have reduced toxicity and efficient transfection. The cationized ASP (cASP) was combined with the plasmid encoding transforming factor-beta 1 (pTGF-β1) to form a spherical nano-scaled particle (cASP/pTGF-β1 nanoparticle). It suggested that cASP/pTGF-β1 NPs could advance the delivery of genes into cells faster and more efficiently than the traditional transfection method Lipofectamine 2000 group which was treated with pTGF-β1 plus Lipofectamine 2000 (200 ng pTGF-β1 complexed with 0.5 μL Lipofectamine 2000 for each well).

In another example, Gu et al. used a water/oil/water (w1/o/w2) double emulsion technique to encapsulate ASP into the poly (lactic-co-glycolic acid) (PLGA), resulting in constituting the ASP-loaded PLGA NP system which is able to impel the proliferation of lymphocytes and T cells compared with blank PLGA and free ASP [76]. Due to some characteristics like fast metabolism, low biocompatibility, and non-concentrated action scope, the application of ASP is limited in clinical use. As a widely used drug delivery system, PLGA can control the secretion of the encapsulated drugs to reach the drug target and have better bioavailability. An ASP-PLGA conjugate may combine the immunological activity of ASP with PLGA targeted delivery, with an emerging synergistic effect compared with ASP alone.

Hsu et al. fabricated a complex mixing AS extract with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) (Figure 9) to overcome the insolubility of AS extract [77]. Using the freeze-drying method, AS-HP-β-CD complex was prepared and gradually tested to have better aqueous solubility and homogeneous dispersibility, improving the uptake and cytotoxicity of hepatocellular carcinoma compared with the AS extract.

![Figure 8](image)

**Figure 8** Schematic drawing for the preparation of *A. sinensis* polysaccharide (cASP).

![Figure 9](image)

**Figure 9** Chemical structures of HP-β-CD.
Conclusion and future outlook

Traditional herbal medicines have potential effects which can inspire the development of modern medicines. Mean active ingredients in botanical herbs are gradually being extracted for clinical use as pharmaceutical formulas. Natural products have great biological activity in vitro, however, they suffer the challenges of poor solubility, large molecular size that would cause them to be trapped by the membrane barrier, degradation in the gastrointestinal environment, and the need of taking larger doses to reach effective concentrations. Nanocarrier drug delivery systems can break free from the limits of natural drugs. By changing the NP sizes and characteristics of its surface, using different polymers of nanomaterials to encapsulate active pharmaceutical compounds or to attach to the surface of the drugs to modify its surface properties, nanotechnology can enhance targeted delivery, sustain and control the release of drugs, and change pharmacokinetics to accomplish better clinical outcomes [78]. Epimedium, RG, Panax ginseng, and AS are commonly used in tonics, and their effects have been amplified by the intervention of nanocarrier delivery systems. Integrating natural products and nanocarriers is a major leap in clinical trials, and it gives several inspiring avenues to pharmaceutical development and other areas. The plant-derived drugs combined with nanocarriers will become a potential candidates in clinical therapy.

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