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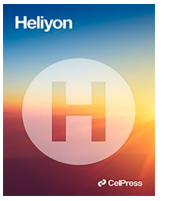

Review article

Plant-derived exosome-like nanovesicles: A novel nanotool for disease therapy☆

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ABSTRACT

Exosomes are extracellular vesicles comprising bilayer phospholipid membranes and are secreted by eukaryotic cells. They are released via cellular exocytosis, contain DNA, RNA, proteins, and other substances, and participate in various cellular communications between tissues and organs. Since the discovery of exosomes in 1983, animal-derived exosomes have become a research focus for small-molecule drug delivery in biology, medicine, and other fields owing to their good biocompatibility and homing effects. Recent studies have found that plant-derived exosome-like nanovesicles (PELNVs) exhibit certain biological effects, such as anti-inflammatory and anti-tumor abilities, and have minimal toxic side effects. Because they are rich in active lipid molecules with certain pharmacological effects, PELNVs could be novel carriers for drug delivery. In this review, the biological formation and effects, isolation, and extraction of PELNVs, as well as characteristics of transporting drugs as carriers are summarized to provide new ideas and methods for future research on plant-derived exosome-like nanovesicles.

1. Introduction

Exosomes are extracellular vesicles secreted by eukaryotic cells after the fusion of multivesicular bodies with the plasma membrane [1]. They have a phospholipid bilayer structure similar to that of liposomes, with a diameter ranging from 50 to 150nm. Their morphology exhibits saucer- or cup-shaped structures under a transmission electron microscope [2]. Eukaryotic cells secrete three subtypes of extracellular vesicles: exosomes produced by the fusion of multivesicular bodies (MVBs) with lipid membranes, microvesicles that emerge directly from lipid membrane sites, and apoptotic vesicles produced during cell death [3]. Almost all living cells secrete extracellular vesicles [4]. In multicellular organisms, the mechanisms controlling exosome production and physiological functions have been investigated, ranging from physiological tissue regulation to pathogenic injury and organ remodeling. Exosomes, which play a significant role in cellular communication, division and development, fertilization, and immune responses, originate biologically from nuclear endosomes, which subsequently interact with other intracellular vesicles and organelles to produce them.

☆ Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

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List of abbreviations

Text	*Abbreviation*
Plant-derived exosome-like nanovesicle	**PELNV**
Multivesicular body	**MVB**
Nuclear erythroid 2-related factor 2	**Nrf2**
Haem oxygenase-1	**HO-1**
Interleukin-10	**IL-10**
Interleukin-16	**IL-16**
Interleukin-1b	**IL-1b**
Influenza A virus	**IAV**
Human umbilical vein endothelial cell	**HUVEC**
Wound healing-related gene	**WHRG**
MicroRNA	**miRNA**

Differences in substances, such as nucleic acids, proteins, lipids, and amino acids, contained in exosomes and their metabolites reflect their different origins. Exosomes have long been recognized as communication mediators that are associated with the activities of normal and diseased organisms, as evidenced by their ability to transfer proteins, DNA, RNA, and other substances to recipient cells [5, 6].

Similar to mammalian cells, which can release exosomes, plant cells also form MVBs as a result of the reaction between the plasma membrane and cell wall by paravesicles. In 1967, researchers used transmission electron microscopy to discover that MVBs are produced during carrot cell culture and fuse with the plasma membrane, generating new vesicles with a membrane structure and releasing them into the extracellular region [7]. Plant-derived exosome-like nanovesicles (PELNVs) were not widely used until 2009, when researchers isolated exosome-like nanovesicles from sunflower hulled liquid using tissue infiltration–centrifugation [8]. To date, researchers have isolated exosome-like nanovesicles of plant origin from ginger, grapefruit, wheat, blueberries, and various Chinese herbs.

Exosomes of animal origin cause damage to organisms because they carry pathogens associated with animal diseases and therefore may deliver them to recipient cells during signaling [9]. Due to their vesicular structural composition and the specificity of their origin, PELNVs may not be recognized by the immune system of the organism, which can increase their circulation cycle and bioavailability efficiency. Owing to their non-immunogenicity, low toxicity, high delivery efficiency, and good biocompatibility, PELNVs can be involved in the delivery and transfer of intracellular substances, such as nucleic acids, active lipids, and cellular proteins. Moreover, they can be used as a new type of carrier to deliver exogenous drugs and active ingredients, such as siRNAs, extracellular proteins, and small-molecule drugs.

Owing to their unique characteristics, PELNVs serve as a medium for intercellular transmission, effectively delivering insoluble drugs or therapeutic compounds. Thus, PELNVs can enhance or weaken the pharmacological activity of the drugs or compounds, achieving precise treatment of diseases. At the same time, the plant lipids contained in PELNVs exhibit natural clinical effects, with broad application prospects in the field of disease treatment [10]. PELNVs have been widely used in areas such as drug delivery, clinical diagnosis, immunotherapy, and regenerative medicine [11–13]. Clinical applications in the treatment of cancer, inflammation, and other diseases, improving treatment effects and prolonging patient survival time, are novel strategies and directions for cancer treatment. Nano-vesicles extracted from camellia and tea leaves can induce apoptosis of 4T1 cells by enhancing ROS levels both in vivo and in vitro [14,15]; ginseng-derived exosome-like nanovesicles stimulate the polarization of macrophages and leads to melanoma apoptosis [16]. Inflammation is caused by physical damage and pathogen infection, leading to immune system abnormalities and the secretion of inflammatory factors. Nano-vesicles extracted from Astragalus membranaceus increases the activity of Th1 cells and cytotoxic T cells, thereby promoting anti-inflammatory responses [17]. Exosome-like nano-vesicles can be extracted from most edible fruits and vegetables. Owing to their edible and safe sources, the treatment of gastrointestinal inflammation with PELNVs has gained considerable attention. Overall, PELNVs serve as potential therapeutic agents in the treatment of various diseases, including cancer, inflammation, and gastrointestinal mucosal injury,.

2. Biogenesis of PELNVs

Compared to mammalian-derived exosomes, PELNVs have been less studied, particularly in terms of their mechanism. The pathway of most PELNVs is the production of MVBs, followed by the formation of extracellular vesicles. When plant cells are attacked by pathogens, they secrete MVBs as a defense response, which forms and functions similar to that of the exosomes of animal origin. Moreover, their morphology and size are similar to those of exosomes of animal origin when observed using electron microscopy, and they have a bilayer phospholipid structure. The fusion of MVBs and the plasma membrane results in the release of vesicles into the extracellular space of fungi and higher plants, and the MVBs are able to participate in cell differentiation, resulting in the thickening of the secondary wall, which confirms that PELNVs are able to participate in the differentiation of cells and tissues [18]. PELNVs can transfer certain antimicrobial compounds to the invading pathogens and stimulate responses in nearby cells. Plant cells attenuate exosome-mediated immune responses in animal cells because of their specialized cell wall structures (Fig. 1) [19]. PELNVs can block

signaling between hypersensitive and normal cells during hypersensitivity reactions, thereby attenuating cell damage [20]. The distinct properties of exosome-like nanovesicles of plant origin have attracted the attention of medical and biological communities and other research fields. The different origins of PELNVs have resulted in the heterogeneity of their isoforms. In addition to the common MVB formation pathway, in *Arabidopsis thaliana* and tobacco cells, an organelle with a bilayer membrane structure can fuse with the lipid membrane, releasing a vesicle with a monolayer membrane structure to bind to the cell wall, producing an extracellular-positive organelle. This is thought to be another pathway for the occurrence of PELNVs secreted by plant cells [21,22].

3. Isolation, purification, and characterization of PELNVs

3.1. Isolation and purification of PELNVs

PELNVs are generally isolated and purified from different parts of various plants according to their physicochemical properties, and the primary methods include ultracentrifugation, sucrose density gradient centrifugation, ultrafiltration centrifugation, polymer precipitation, chromatography, and microfluidic separation technology. These methods have different scopes of application, as well as certain advantages and disadvantages (Table 1).

The ultracentrifugation method uses the difference in density and size between exosomes and other components to separate them via different centrifugal forces. For example, low speeds remove impurities and cellular debris and high speeds separate vesicles and precipitate exosomes. The ultracentrifugation method is simple to operate and extracts and separates more exosomes than other methods. It is suitable for the extraction and separation of most exosomes; however, it is less efficient for analyzing viscous biological fluids [23]. The sucrose gradient centrifugation method is used to form exosome bands using centrifugation at ultra-high-speed using a sucrose concentration gradient. The separation results are easy to observe, but the requirements for centrifugation speed and time are more precise. In addition, repeated centrifugation may cause damage to exosomes, which is suitable for extracting and purifying exosomes from other vesicles and particles [24]. Ultrafiltration centrifugation is a separation technique that uses different particle sizes and has become a new method for extracting exosomes because of its simplicity and high extraction efficiency. It does not alter the activity of the exosomes and does not require other special instruments. Ultrafiltration centrifugation separates exosomes of different molecular weights from biological macromolecules, such as proteins, via fluid pressure. This method does not affect their biological activity but may cause deformation or damage to the exosomes, due to external forces, which may prevent them from maintaining their original form. This method is suitable for the isolation and extraction of exosomes from fractions in which there are significant

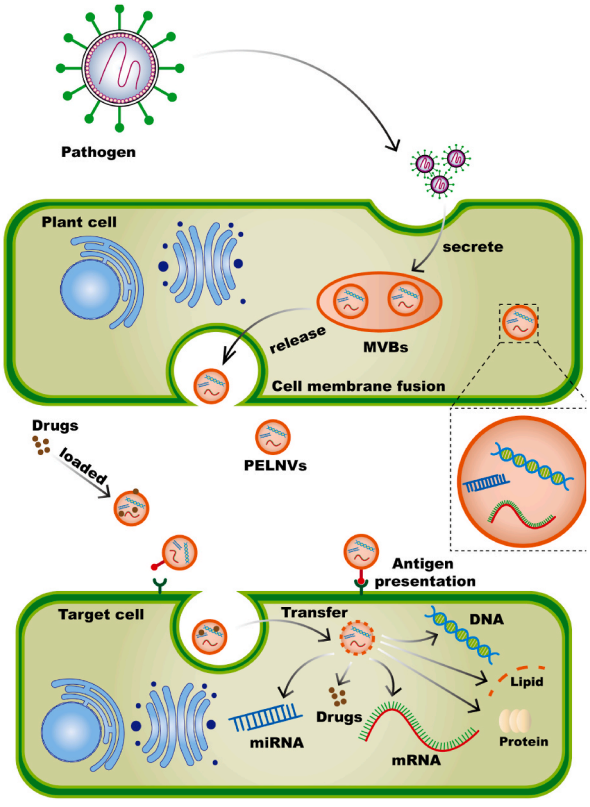

Fig. 1. Biogenesis of PELNVs and their role as vectors for drug loading. Plant cells invaded by pathogens secrete MVBs, which generate PELNVs via exocytosis. These PELNVs can be loaded with drugs and exogenous DNA and RNA and subsequently transferred to the recipient cells for action.

Table 1
Commonly used techniques for exosome isolation.

Method	Principle	Efficiency	Purity	Applicability
Ultracentrifugation	Separation by different centrifugal forces based on differences in particle density and size	Simple operation and a high number of isolated exosomes	Low purity, repeated centrifugation causes damage to cell structure	Suitable for the extraction and isolation of most exosomes
Sucrose density gradient centrifugation	Exosome strip formation by centrifugation at ultra-high-speed using sucrose concentration gradients	Easy to operate, but prone to causing lipoprotein separation	No labeling required, can purify exosomes	Suitable for extracting and purifying exosomes from most other vesicles and particles
Ultrafiltration centrifugation	Separation of exosomes based on particle size using ultrafiltration membranes	Separate from different sizes without affecting activity	Separate smaller proteins and other substances to further purify exosomes	Suitable for separating and extracting exosomes from components with different particle sizes in compounds
Polymer precipitation	The polymer is used to absorb water molecules, thereby reducing the concentration of exosomes, which are then separated by centrifugation	Separate extracellular vesicles from solution, reduce concentration for separation	Precipitation occurs between lipoproteins and polymers, leading to a decrease in the purity of extracellular vesicles	Suitable for DNA, RNA and other studies of exosomes
Size Exclusion Chromatography	Chromatography for separation based on particle size and dimensions	Processing large volume samples requires concentration treatment	High separation purity and ability to retain the biological activity of extracellular vesicles	Suitable for morphological and structural analysis of exosomes
Microfluidics	Microscale separation of exosomes based on differences in physicochemical properties from other components	Fast processing speed, low cost, and high sensitivity	The purity of the obtained product is relatively low	For biomarker diagnostics only

differences in the particle size of the compound [25]. The polymer precipitation method uses absorbent polymers, such as polyethylene glycol, to reduce the concentration of extracellular vesicles, followed by separation using centrifugation. This can preserve the activity of extracellular vesicles; however, precipitation may occur between lipoproteins and polymers during the extraction process, resulting in a decrease in the purity of extracellular vesicles. This method is suitable for the study of extracellular DNA and RNA [26]. Size exclusion chromatography is a method based on molecular size. The molecules move through a porous fixed-phase filling column, leaving smaller molecules of nucleic acids, proteins, and other substances in the filling column, which are then filtered out. It has a high separation purity and preserves the activity and structural integrity of exosomes; however, the extracted exosomes are diluted, which require specialized instrumentation for concentration. Size exclusion chromatography can be applied to the morphological and structural analyses of exosomes and has been combined with other vesicle separation methods to improve the efficiency of isolation and purification [27]. Based on differences in the biochemical and physical properties of exosomes, microfluidic technology can also be used in their separation. Its advantages include high processing speed, low cost, and high sensitivity, but its disadvantage is producing low yields, thus making it unsuitable for large-scale extraction. Microfluidic technology is only suitable for detecting biomarkers [28].

3.2. Physicochemical characterization of PELNVs

PELNVs have physicochemical properties and characterizations similar to those of animal exosomes, with particle sizes ranging from 10 to 1000 nm. The morphology of the vesicles is saucer- or cup-shaped when observed under a transmission electron microscope. However, the morphology of certain vesicles may be altered due to the operation involving the fixation, dehydration, and staining of the samples. In contrast, cryo-electron microscopy, which requires that the samples be viewed at temperatures of approximately −80 °C, can result in the relative fixation of vesicle morphology. Different plant sources of PELNVs lead to differences in particle sizes and potentials. The average size of the exosome-like nanovesicles of ginger and grapefruit origin is approximately 250 nm, whereas the average size of the exosome-like nanovesicles of wheat origin is between 40 and 100 nm. The surface charge of the PELNVs is typically negative, and the size is generally 0–100 mV, indicating that the nanovesicles are mutually exclusive and do not have aggregation effects. The lipid composition of the PELNVs is divided into phospholipids and glycerol. Proteins are another component of exosomes, and are divided into transmembrane and other lipid membrane proteins. Upon entry into recipient cells, exogenous mRNAs and non-coding miRNAs regulate intracellular RNA and protein levels and influence their gene expression and structural functions [29].

Certain in vitro experiments have confirmed that physicochemical characteristics of PELNVs, such as particle size, morphology, and surface charge, remain stable under the conditions of the simulated gastrointestinal tract, which could indicate that they can be delivered to the body via oral drug delivery systems. Other PELNVs also have gastrointestinal targeting effects. Edible ginger-derived exosome-like nanovesicles have certain targeting effects on colon cells [30]. Grape exosome-like nanovesicles can be absorbed by intestinal stem cells and promote their proliferation, resulting in the induction of intestinal stem cells, thus protecting mice from DSS-induced colitis [31].

3.3. Biochemical characteristics of PELNVs

PELNVs contain numerous biomolecules, including lipids, nucleic acids, and proteins [32]. Biomolecules in PELNVs have shown the same analysis as in animal-derived exosomes, indicating equal plant and animal origins. This also reveals that PELNVs and animal-derived exosomes have certain biological functions [33]; therefore, the active components and functions of PELNVs can also be detected.

The most important components of PELNVs are lipids, which can be classified as glycerolipids and phospholipids. Animal-derived exosomes also contain lipids; however, PELNVs differ from those derived from animal sources. Exosomes of animal origin are typically high in cholesterol and sphingolipids, whereas PELNVs are rich in phospholipids (primarily phosphatidic acid) and plant lipids, such as digalactosyl diacylglycerol and monogalactosyl diacylglycerol. These substances can be involved in mitotic processes in plants, with phosphatidic acid thought to be the primary substance involved in mitosis. Phosphatidic acid is highly fusogenic when calcium ions are present in the environment and can induce the interfusion of vesicles [34]. Other plant-like lipids in PELNVs may play important roles in signaling between species [35]. In addition to affecting the formation and release of PELNVs, lipids have certain biological roles. For example, phosphatidic acid in ginger-derived exosome-like nanovesicles can inhibit the growth of *Porphyromonas gingivalis* monocytes [36].

The proportion of lipids contained in exosome-like nanovesicles from different plant sources is also different, with different ratios of K-derived exosomes to phosphatidic acid [30,35].

3.4. Safety and toxicological studies of PELNVs

Exosomes, important carriers for intercellular information transmission, possess unique biological activities and functions. In recent years, owing to their natural origin and good biocompatibility, PELNVs have attracted considerable attention. However, the associated safety issues restricts their widespread application. Therefore, a comprehensive safety assessment of PELNVs is of great significance for promoting their development in practical applications. To further understand the toxicological characteristics of plant exosomes, experiments have been conducted to determine their effects on the function of target cells [37]. Molecular and cell biology demonstrated that PELNVs regulate biological processes such as proliferation, differentiation, and apoptosis of target cells, thereby exerting their potential application value in disease treatment and tissue repair. At the same time, a preliminary exploration of the mechanism of action of PELNVs was performed, providing theoretical support for their future applications.

Researchers successfully isolated exosome-like nano-vesicles from medicinal plants and conducted a thorough investigation into their therapeutic effects on triple-negative breast cancer and the potential underlying molecular mechanisms. After a series of animal experiments, the results significantly demonstrated that these medicinal PELNVs possess high biological safety in vivo and do not exhibit significant toxic effects on major organs. This discovery provides important safety evidence for the application of medicinal plant exosomes in the field of cancer treatment [38]. In addition, the application of garlic-derived exosome-like nano-vesicles in the treatment of Staphylococcus aureus-infected wounds have been investigated. Cell experimental results revealed that, compared to free vancomycin, garlic-derived exosome-like nanovesicles exhibit superior effects in wound treatment. Interestingly, the nanovesicles demonstrated excellent biocompatibility and biological safety with minimal toxicity to cells. These findings provide a new therapeutic strategy for wound infections and the broad application prospects of plant-derived exosomes in the field of biomedicine [39].

After a comprehensive evaluation of a series of in vivo and in vitro experiments, we confirmed that PELNVs exhibit good cell and animal safety under various test conditions without significant toxic effects. This discovery provides important safety guarantees for the application of plant exosomes in various fields such as medical treatment, agriculture, and cosmetics. However, given the complex composition and diverse functions of PELNVs, their safety issues still require further exploration.

4. PELNVs have certain pharmacological effects

Exosome-like nanovesicles are secreted by plant cells and tissues for information transfer and signaling. Nanovesicle structures isolated from fresh plant juice extracts contain endogenous substances that can be released into the recipient cells. PELNVs have certain pharmacological effects owing to their natural origin and the plant lipids they contain. Moreover, the good biocompatibility of such nanoscale vesicles renders them biologically efficient for transportation in animals [40].

PELNVs have attracted increasing attention from researchers because of their simple extraction, high yield, and high activity. To investigate the biological effects of plant-secreted nanovesicles, exosome-like vesicles were extracted and isolated from edible fruits and vegetables from various sources. Subsequent studies have demonstrated that exosome-like vesicles isolated from the juice of fresh edible fruits and vegetables exhibit certain biological activities and pharmacological effects.

PELNVs have certain regulatory and ameliorative effects on the immune system and functions in animals. For example, cauliflower-derived exosome-like nanovesicles can inhibit DC cell activation by mediating adenosine phosphate-activated protein kinase [41]. Exosome-like nanovesicles extracted from shiitake mushrooms inhibit the activation of the NLRP3 inflammatory vesicle, thereby reducing D-galactosamine and other induced liver injury in mice [42]. Exosome-like nanovesicles of ginger administered via gavage in mice attenuated alcohol-induced liver injury, where alcohol-derived metabolites stimulated the production of pro-inflammatory factors, tumor necrosis factor (TNF)-α, and reactive oxygen species (ROS), which can lead to liver injury. Ginger-derived exosome--like nanovesicles can inhibit ROS production and mediate the activation of nuclear erythroid 2-related factor 2 (Nrf2) owing to specific active substances inside the nanovesicles [43]. Nrf2 activation leads to the expression of hepatic detoxification and antioxidant genes, thereby reducing liver injury. Ginger-derived exosome-like nanovesicles are readily taken up and absorbed by intestinal flora, and

their embedded miRNA can be directly involved in gene expressions in specific bacteria, thereby affecting gene regulation and enhancing the intestinal barrier system to alleviate colitis in mice [44,45]. The dry decoction of licorice contains high numbers of methylated miRNAs that are not susceptible to degradation and can regulate the expression of protein-coding genes by inhibiting the translation of protein-coding mRNA or promoting mRNA degradation. The results of sRNA and miRNA extracted from the aqueous decoction of licorice, which can be used to isolate healthy human peripheral blood mononuclear cells, indicated that miRNAs from licorice-derived exosome-like nanovesicles can significantly regulate human peripheral blood mononuclear cells by inhibiting the expression of T-cell differentiation, inflammation, and apoptosis-related genes [46]. Lemon-derived exosome-like nanovesicles can target tumor cells and inhibit tumor growth by activating TNF related apoptosis inducing ligands [47].

Exosome-like nanovesicles derived from edible plants exhibit natural targeting effects. For example, exosome-like nanovesicles extracted from grapefruit can target macrophages in the colon, upregulate the expressions of haem oxygenase-1 (HO-1) and interleukin-10 (IL-10), and inhibit the production of interleukin-6 (IL-6), interleukin-1b (IL-1b), and TNF-α, thereby reducing lesions in the colon [48]. Honeysuckle-derived exosome-like nanovesicles, when administered via continuous drinking or gavage, elevated peripheral blood and lung tissue levels in mice and could target various Influenza A viruses (IAVs). miRNA2911 is the first active ingredient in Chinese herbal medicine that has been studied to directly target various IAVs, such as H1N1 and H5N1. miRNA2911 was also able to stabilize honeysuckle decoction, which suggests that the primary component of honeysuckle that can exert antiviral activity is miRNA2911. It inhibits viral replication and provides an idea for future research that can effectively inhibit viral infection [49].

PELNVs can be delivered to targeted sites via oral administration and can promote certain anti-inflammatory effects. For example, ginger-derived exosome-like nanovesicles contain large quantities of active ingredients that are nontoxic, which can reduce acute colitis, promote intestinal repair, and prevent colon cancer. Following gavage treatment, the survival rate of the rat colitis model significantly increased, levels of related inflammatory factors decreased, and expression of anti-inflammatory factors was enhanced, thereby suggesting a healing effect. Ginger-derived exosome-like nanovesicles have been investigated as effective carriers for drug delivery and release into Colon-26 tumor cells in combination with the anti-tumor drug Adriamycin, modified by the targeting ligand folic acid [31]. The results showed that it could enhance the inhibitory effect of Adriamycin on tumor drugs. Oral administration of

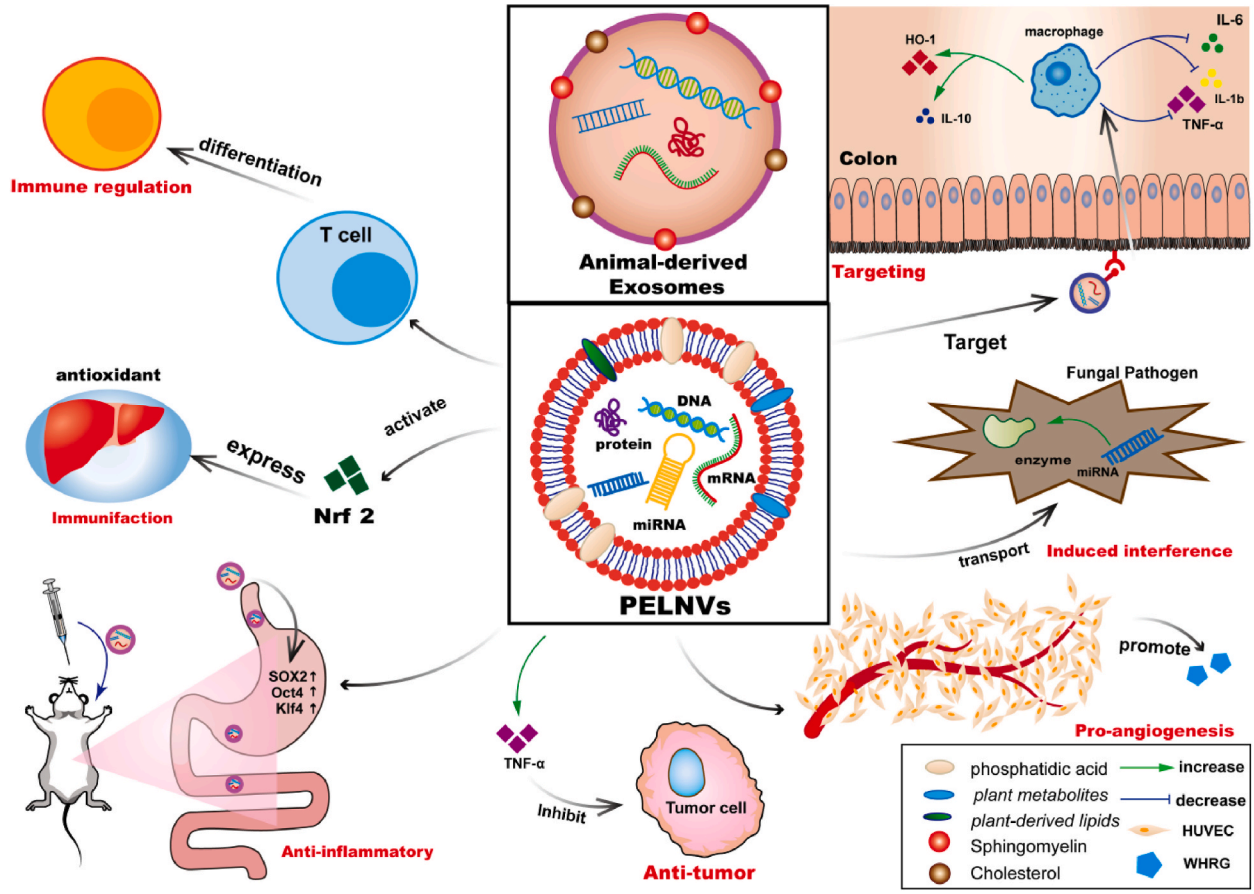

Fig. 2. Pharmacological effects of PELNVs. PELNVs differ from cholesterol sphingomyelin contained in animal-derived exosomes and possess special plant-derived lipids ，PELNVs has the functions of promoting liver detoxification, immune regulation, targeting, stem cell marker expression, promoting angiogenesis, inducing effects, and anti-tumor effects.

ginger-derived exosome-like nanovesicles also improved bone loss caused by *P. gingivalis* [50]. Exosome-like nanoparticles isolated from grape juice were able to reach the intestinal site after oral administration in mice and could diffuse into intestinal stem cells, causing upregulation of the stem cell marker expressions (i.e., SOX2, Oct4, and Klf4), thereby maintaining homeostasis of the gastrointestinal epithelium, accelerating the absorption of epithelial tissue, promoting the recovery of the colon system, and exerting an anti-inflammatory effect [51].

PELNVs also exhibit pro-healing and antifibrotic effects. For example, wheat-derived exosome-like nanovesicles stimulate wound healing by promoting growth and healing effects on endothelial and epithelial cells and fibroblasts in epidermal tissues [52]. In addition, wheat-derived nanovesicles exhibit a pro-angiogenic effect by promoting the formation of tubular structures in human umbilical vein endothelial cells (HUVECs), which enhance the expression of wound healing-related genes, suggesting that wheat-derived nanovesicles promote wound healing**.** Extracellular vesicles isolated from the extracellular fluid of sunflower seedlings were characterized using electron microscopy and proteomic analysis, which revealed that their morphological structure and biological effects were similar to those of animal cellular exosomes. Moreover, these vesicles could inhibit fungi and participate in intercellular signaling [53].

Active substances, such as polysaccharides and saponins, contained in edible herbs have anti-inflammatory and anti-tumor effects. Some studies have extracted exosome-like nanovesicles from these edible herbs and investigated them, finding that they also have certain pharmacological effects [54]. As an edible Chinese herbal medicine, *Rhodiola* has been widely used because of its ability to nourish qi, moisten the lungs, and nourish the heart. Exosome-like nanovesicles isolated and extracted from *Rhodiola rosea* contain phosphatidylcholine, which mediates anti-fibrotic small RNA (HJT-sRNA-m7) and can effectively reduce the expression of fibrosis marker genes and proteins [55]. *Rhodiola*-derived exosome-like nanovesicles have positive effects in mice, and that PELNVs are composed of lipids, proteins, and RNA. Exosome-like nanovesicles of *Rhodiola* and dandelion can significantly ameliorate bleomycin-induced pulmonary fibrosis in mice, suggesting that exosome-like nanovesicles of herbal origin could be used as a new route for oral drug delivery [56]. Plant-secreted exosome-like nanovesicles can serve as ideal carriers for transporting proteins and sRNAs for signaling between cells. For example, exosome-like nanovesicles were isolated from *Arabidopsis thaliana* leaf extraplastidial cells and analyzed using proteomics to identify the proteins associated with their expression [57]. The host *Arabidopsis* can inhibit fungal infection by secreting exosome-like nanovesicles that deliver miRNA into fungal pathogens and induce the expression of active cleavage enzymes. This phenomenon suggests that *Arabidopsis* can induce RNA interference via the transmembrane transport of miRNA, thus modulating immune responses [58]. Plants infected with *Pseudomonas syringae* and salicylic acid-treated plants secrete large quantities of exosome-like nanovesicles, thus demonstrating the involvement of exosome-like nanovesicles in the immune regulation of the plant. Researchers have studied PELNVs not only in food and Chinese medicinal herbs but also in the phloem and xylem of woody plants. They found that secreted exosome-like nanovesicles participate in the delivery of endo-1,4-glucanase, an enzyme involved in the formation of xylose fibers. Therefore, exosomes may also be produced in angiosperms and gymnosperms and can participate in the storage and transport of internal 1,4-glucanase in the secondary phloem and xylem [59] (Fig. 2).

Not only do PELNVs have certain pharmacological effects, but their vesicle-like morphology and the proteins, lipids, and nucleic acids they contain make them non-immunogenic, which plays an important role in intercellular material exchange and information transfer [60]. Exosomes not only protect miRNAs from maintaining their integrity under physical conditions, such as ultrasound, but also maintain their stability under acidic conditions. They also play a role in regulating the expression of certain specific human genes [61,62]. PELNVs contain plant-specific active derivatives because of their distinctive origins. For example, broccoli-derived exosome-like nanovesicles are rich in the active substance raphanin, and ginger-derived exosome-like nanovesicles contain 6-gingerol and 6-gingerenol, two active substances with anti-inflammatory effects [63].

5. PELNVs as novel drug carriers

5.1. Comparison of PELNVs with animal-derived exosomes

As biological vesicle-like structures between 50 and 150 nm in size, exosomes are capable of intercellular substance delivery and signaling. They are biocompatible, highly functional, and can carry drugs and target genes for further drug delivery studies. Exosomes can cross the blood–brain barrier in organisms, but not the placental barrier. They can signal and transmit substances in tissues and regions with low blood irrigation, such as joints and stroma. The surfaces of exosomes carry adhesion proteins, such as tetraspanins and integrins, which facilitate the entry of exosomes into cells and membrane fusion [64]. The morphology of the vesicles and surface substances they carry enable them to be modified on the membrane surface to obtain functions that exosomes do not possess on their own. For example, the modification of cells and tissues, or targeting titanium with targeting functions on the surface of vesicles, enables them to be targeted to specific cells or tissues. This results in the targeted delivery of substances and drugs encapsulated in the body, reducing the degree of drug aggregation in the liver and other parts of the body, thereby reducing hepatotoxicity. Therefore, exosomes and exosome-derived structures have been considered good carriers for the in vivo transportation of substances in numerous studies.

In mammals, exosomes are extramembrane vesicles released by several cell types, including T, B, and dendritic cells. Both facilitate intercellular active substance transport and signaling communication and participate in RNA processing in the nucleus and cytoplasm [65–67]. The cavity vesicle-like structure of exosomes renders them ideal carriers for transporting drugs and molecules that can be targeted to target cells and organs after modification [68–70]. Exosomes from animal sources have a good ability to transport and conduct signaling pathways, but exosome vesicles from certain sources are easily recognized and killed by lysosomes. Synthetic liposomes, as well as modified exosomes, produce adverse effects, such as cellular stress and apoptosis of inflammatory vesicles.

Plants also secrete exosome-like nanovesicles, which not only exhibit similar carrier effects and biosafety as exosomes from animal sources, but also have certain pharmacological and targeting effects owing to their special origin. Moreover, they are not recognized by organisms in the animal kingdom. Although research on the use of animal cell exosomes as carriers to transport drugs to the target site is more mature, reports on exosomes from edible plants, such as medicinal plants, have gradually become the recent focus of research. Because of their edible nature, extracted nanovesicles can be orally administered to achieve cross-species targeting and the regulation of gene expression. They have also become new and safe carriers for transportation, providing a new research direction for drug delivery. PELNVs have been widely studied as drug carriers because they have been found to have the following characteristics: 1) good biocompatibility; 2) non-toxicity and low immunogenicity; 3) specific targeting ability; 4) ability to prolong the duration of action of the drug and extend the metabolic cycle of the drug; 5) simple extraction method and mass production; and 6) can cross the blood–brain barrier, but cannot cross the placental barrier [71].

5.2. Plant derived exosome-like nanocapsules can serve as drug delivery carriers

Exosomal vesicles can carry specific RNAs and proteins from the host cell and efficiently deliver bioactive molecules to the recipient cell for release via multiple active transport pathways [72–76]. Internalization pathways are typically different for different cell types. In addition to carrying exosomes and their own natural components, exosomes can be loaded with exogenous therapeutic molecules, such as proteins, expression vectors, siRNAs, and DNA [77,78].

Currently, documented methods for loading drugs into plant vesicles are categorized as passive or active loadings. Both methods require the extraction of PELNVs, which are then co-encapsulated with the drug. Passive loading occurs from the interdiffusion between the drug molecules and phospholipid bilayer of the PELNVs, which diffuse into the interior of the vesicles after incubation at a certain temperature [79]. Owing to the negative electronegativity of the vesicle surface, it can bind to positively charged drugs, such as adriamycin, via adsorption and can be sonicated into the interior of the vesicle. Negatively charged and neutral surfaces can also be encapsulated into PELNVs using vesicle membrane fusion modification, because the lipophilic effect of the compound surface is stronger than the electrostatic effect associated with the vesicle surface. Strongly negatively charged molecules, such as siRNA and DNA, that are loaded into the vesicles can exhibit good biological activity [80]. Active loading techniques enable the drug to enter inside the vesicle via physical or diffusion methods by altering the morphology and structure of the cell membrane. The vesicle is then restored to its biological activity and cellular morphology. The commonly used active loading techniques are physical squeezing and pushing and electroporation techniques.

PELNVs can be used as carriers for novel drug delivery owing to the specificity of their sources. For example, grapefruit-derived exosome-like nanovesicles can deliver drugs, RNA, and DNA into receptor cells and enable the drug-targeted delivery of CT-26 and SW620 cells to inhibit tumor growth by modifying the target receptor folate. Compared with synthetic liposomes, PELNVs are unable to pass the placental barrier when injected intravenously into mice because of their low toxicity, thus proving that they can be used for substance transportation and drug delivery with a certain degree of safety. Ginger-derived exosome-like nanovesicles can encapsulate anti-tumor drugs, such as adriamycin, and the surface of the vesicles can be modified by the colonic cell-targeting ligand folic acid, which can target Colon-26 and HT-29 cells. This can effectively release chemotherapeutic drugs to inhibit tumor growth. When stained with DiL fluorescent dye, the uptake mode was observed, and the mechanism of internalization may be conducted via the phagocytosis pathway [30]. Owing to their stability and antioxidant properties, pomegranate membrane extracts can improve nanomedicines, exerting a certain protective effect on the skin [81]. The encapsulation of sulforaphane with broccoli membrane vesicles enhances the antioxidant and antitumor effects of sulforaphane, as well as increase its anti-inflammatory effects on macrophages in vitro [82,83]. Red cabbage extracts and nanocapsules of cabbage plasma membrane vesicles improves the gastrointestinal microbiota of obese patients [84]. Nanoencapsulation with membrane vesicles derived from cauliflower improves the stability of isothiocyanates (ITCs) extracted from Bimi®, addressing the low stability of ITCs in aqueous solutions [85]. In summary, nanovesicles extracted from plant membranes possess the characteristics of PELNVs. As drug carriers, the nanovesicles can be combined with drugs to enhance their therapeutic effects and reduce side effects.

5.3. Modification and transformation of PELNVs

PELNVs contain lipids and active components that can be used to deliver active substances such as DNA and RNA to recipient cells, mediating intercellular communication. However, due to their lack in targeting ability and poor homologous effects, this delivery method is not ideal. Therefore, modification and transformation is required to effectively load drugs, nucleic acids, and other substances into exosomes and enhance their binding to target cells. Currently, the modification methods for PELNVs mainly include surface modification, membrane fusion, bionic composite nanotechnology, and artificial modification, which allows for wider range of functions and treatment efficiency improvement.

Through the precise operation of chemical coupling, we have successfully integrated peptides, proteins, or other compounds with the vesicular membrane, maintaining the integrity of the vesicles while endowing them with superior properties. Specifically, the targeting ability of the vesicles have been significantly enhanced, enabling drugs to reach the desired location more accurately. In addition, their bioavailability was also improved, leading to enhanced therapeutic effects. Additionally, through techniques such as fluorescent labeling, we can precisely locate the vesicles, providing powerful tools for disease diagnosis and treatment. For instance, azide compounds are loaded onto the surface of vesicles via chemical binding to enhancing stability and significantly improve bioavailability, overall improving drug efficacy. Furthermore, through incubation coupling, folic acid can tightly bind to the surface of vesicles, enabling precise targeting of colon receptor cells. This targeting reduces drug decomposition in non-target areas and increases

its concentration in the target area, resulting in more effective treatment outcomes. In summary, surface modification techniques can protect the integrity of vesicles and enhance their targeting ability and bioavailability, providing new strategies and directions for drug delivery and disease treatment [86–88].

PELNVs, due to their origin from specific organisms, have a surface rich in unique components such as plant proteins and plant lipids, which to some extent limits their internal space and loading capacity. To overcome this limitation, we have adopted a strategy of using artificially synthesized liposomes. These synthetic liposomes significantly expand the internal cavity structure of the vesicles, providing a larger loading space for more drugs or biomolecules. Through physical methods such as extrusion and electroporation, synthetic liposomes can be tightly integrated with PELNVs without compromising their inherent functions. This integration retains the original biological characteristics of PELNVs and greatly enhances their drug-loading capacity [89,90].

Certain animal-derived cells possess a unique homing effect, enabling precise location and adherence to specific tissues or cells. To more effectively deliver active substances such as nucleic acids and DNA, to otherwise inaccessible target cells, we have employed cell membrane fusion technology. Using this technology, active substances are encapsulated within the vesicles of animal-derived cells, leveraging their homing effect to achieve precise targeted delivery [91]. A common issue in the transportation of drugs and nucleic acids is the aggregation of biological macromolecules, which significantly reduces delivery efficiency. To address this issue, we have introduced organic-metal bioframeworks. These frameworks effectively inhibit the aggregation of biological macromolecules, ensuring the stability and activity of drugs and nucleic acids during transportation. The introduction of bioframeworks significantly improves delivery efficiency, allowing more drugs and nucleic acids to successfully reach target cells and exert their therapeutic effects [92].

In clinical disease treatment, the loading of drugs and nucleic acids into exosomes significantly enhance their biological effects, thereby improving the outcome of disease treatment. Specifically, in gene therapy, by precisely loading therapeutic genes into exosomes, we can achieve targeted and efficient treatment for genetic diseases. This approach improves treatment precision and reduces potential risks, providing new strategies for the treatment of genetic diseases [93]. In tumor immunotherapy, exosomes loaded with antigens or immunostimulatory molecules activate the immune system of the body, suppressing tumor growth and metastasis [94]. In regenerative medicine, exosomes loaded with growth factors or transcription factors promote tissue repair and regeneration [95].

6. Conclusion and future perspectives

Synthetic liposomes have been widely studied as carriers for loading drugs, such as DNA, siRNA, proteins, and other molecules; however, their use as drug transportation carriers must be considered in terms of biosafety, cost of extraction, and whether they can be produced on a large scale. Natural plant-derived exosomes can overcome the disadvantages of synthetic liposomes, such as biotoxicity, and PELNVs have been widely used in drug carrier research owing to their low biotoxicity, high loading efficiency, low extraction cost, and large-scale extraction.

PELNVs do not carry any drugs by themselves and can be modified by fusing other vesicles or liposomes with vesicle indications using membrane fusion technology [96]. This improves the performance and drug-carrying capacity of the vesicles and can result in a significant increase in their transportation and loading efficiency owing to their small size and ability to cross various biological barriers. PELNVs contain numerous natural active ingredients, and modification of the vesicles using membrane fusion technology increases the killing effect of the nanovesicles, resulting in an interaction between the natural active ingredients they carry and the loaded exogenous drug. Because PELNVs can mediate targeted cellular communication, piggyback drug delivery to receptor cells has recently become a new therapeutic modality.

PELNVs enable cross-species intercellular signaling. They are changed by cell membrane surface modifications to make them derivatives of PELNVs and by modifying the targeting ligand and drug aptamer to enable targeting of the cell for the release of the loaded drug.

PELNVs have a liposome-like bilayer membrane structure and the ability to penetrate some skin cells; therefore, transdermal drug delivery is a possible mode of drug delivery for PELNVs. Moreover, owing to the natural origin of vesicles and their edible nature, they can be used for oral drug delivery, and their retention effect in the body can be studied using pharmacokinetics, rendering them safe drug carriers. Recently, several studies have indicated that edible plant-derived exosome-like nanovesicles, as well as Chinese herbal medicines, not only have certain pharmacological effects but can also participate in intercellular signaling. Edible plant-derived exosome-like nanovesicles and their derivatives have recently become popular research topics, providing a new research direction for safe nanodrug delivery carriers.

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Ze Jin: Writing – original draft. **Jintong Na:** Investigation. **Xia Lin:** Investigation. **Rong Jiao:** Investigation. **Xiyu Liu:** Writing – review & editing. **Yong Huang:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing interests.

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