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Neurological Applications of Exosome-Delivered Bioactives from Ancient Chinese Herbal Components

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ABSTRACT

A bioactive substance derived from Chinese herbal medicines with established biological activity and pharmacological effects is known as a traditional Chinese medicine (TCM) monomer. It has drawn a lot of interest for the treatment of neurological disorders. However, the low solubility and poor blood-brain barrier (BBB) crossing of TCM monomers restrict their use. Small extracellular vesicles (EVs) with a diameter of 30 to 150 nm, exosomes may be employed as drug delivery vehicles that target cells or tissues directly. They have special benefits, such as low immunogenicity, low toxicity, high blood stability, and the capacity to pass the blood-brain barrier. The biogenesis, components, stability, surface modification, separation method, benefits, and drawbacks of exosomes as drug carriers are covered in this review, which also contrasts exosomes with other comparable drug delivery systems. Additionally, exosome-encapsulated TCM monomers exhibit neuroprotective properties, such as anti-oxidation, anti-apoptosis, anti-inflammation, and anti-mitophagy, in a variety of neuronal diseases, such as cerebral ischemia and reperfusion (CI/R) injury, Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), and anti-drug resistance, anti-tumorigenesis, and anti-angiogenesis, as well as the promotion of apoptosis in brain tumors. These findings further motivate the development of an exosome-based delivery tool in targeted therapy for neuronal diseases.

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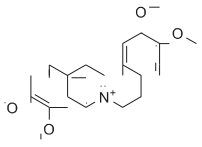
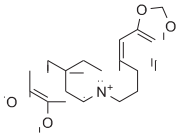
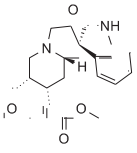
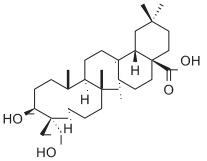
1. Overview

Based on long-term accumulation and ongoing summarization, traditional Chinese medicine (TCM) is a medical system with a distinct theoretical style that is progressively constructed upon medical and life practices and experiences [1]. In contrast to contemporary Western medicine, which is based on ideas, beliefs, and experiences, it is distinguished by indigenous medicine, which helps to maintain health by preventing, diagnosing, and intervening in physical and mental disorders. With a history spanning over 4,000 years, Huangdi Neij-ing, also referred to as the Inner Canon of Huangdi or the Yellow Emperor's Medicine Classic, is the oldest TCM classic. The Chinese philosophy of Yin-Yang and the Five Elements (WuXing) is the foundation of TCM, which combines elements of ancient philosophy, clinical experience, early medical knowledge, regional cultures, and religious beliefs. It is important to note that TCM promotes harmony with the cosmos and is not a comprehensive approach [1,3]. Thus, TCM uses a complete strategy that incorporates several techniques, such as Chinese herbal medicine, herbal enemas, acupuncture, Chinese massage (Tui Na), mind/body exercises, and food treatment, to

achieve its diverse therapeutic benefits [4]. Although the treatment has a clear curative effect, minimal harmful and side effects, and a broad range of indications, the qualities of component variety, ma-trix complexity, and low amounts of active component offer obstacles for TCM research [5]. TCMs primarily consist of natural medicines, including mineral, plant, and animal medicines, as well as preparations that include these natural medicines in different dose forms [6]. TCMs are complex and typically contain a wide range of active ingredients, including flavonoids, alkaloids, lignins, phenols, ter-penoids, amino acid derivatives, organic acids, polyketides, steroids, and sugars, each with unique chemical structures, physico-chemical characteristics, concentrations, and pharmacological and/or toxic activities [7].
In TCM, the ATCM monomer, which is isolated from a single TCM or TCM prescription, is regarded as an active component with a specific molecular formula and spatial structure (Table 1) [8e25] and has shown pharmacological and biological effects.

Table1
ThelistofchemicalstructureoftraditionalChinesemedicine(TCM)monomersintroduced in this article [8e25].

TCMmonomer	Chemicalstructure	
Pal		[8]
Ber		[8,9]
Cory-B		[10]
Hed		[11]

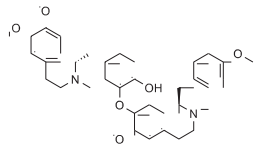
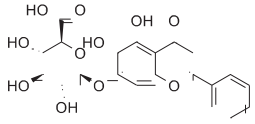
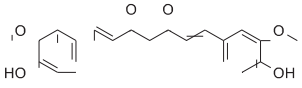
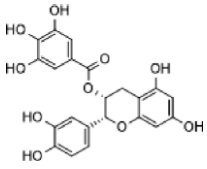
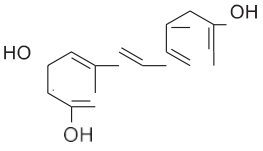
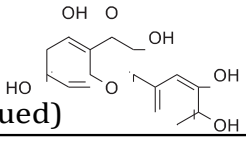
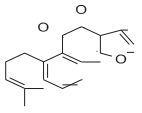
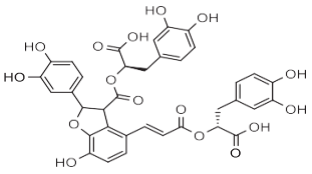
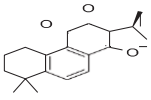
Nef		[11]
Bai		[11,12]
Cur		[13e20]
ECG		[21]
RSV		[22]
Que		[23]

Table1(continued)

TCMmonomer	Chemicalstructure
DHT	[24]
	
SAB	[25]
	
CPT	[25]
	

ECG: epicatechin gallate; RSV: resveratrol; Que: quercetin; DHT: dihydrotanshinone; SAB: salvianolic acid B; CPT: cryptotanshinone; Pal: palmatine; Ber: berberine; Cory-B: corynoxine-B; Hed: hederagenin; Nef:neferine; Bai: baicalin; Cur: curcumin. such as anti-tumor, anti-inflammatory, antibacterial, and anti-diabetic effects [26]. The study of TCM monomers increases the effectiveness of TCM and offers a scientific foundation for its use in contemporary medicine [27]. TCM monomers have special and novel pharmacological advantages that enable them to achieve therapeutic outcomes comparable to those of conventional therapies with less toxicity and side effects.

TCM monomers have also drawn interest because of their ability to cure disorders of the neurons. Nevertheless, there are several difficulties in using TCM monomers to treat neurological conditions. Their clinical efficacy has been limited by their poor solubility and bioavailability in aqueous conditions, high rate of degradation, poor targeting, and low delivery efficiency, particularly their poor ability to cross the blood-brain barrier (BBB) to target the central nervous system (CNS) of TCM [28]. Researchers have started looking at new drug delivery mechanisms, including exosomes, to solve these problems. The mechanisms by which TCM monomers are loaded into exosomes to prevent and cure a variety of neurological disorders are methodically introduced in this study.

1. The introduction of the TCM active component extraction

Low concentrations in TCM, complex structures, multiple types, and unstable properties are characteristics of TCM monomers, which can be further subdivided into alkaloids, flavonoids, terpenoids, saponins, phenols, quinones, and other substances. The separation and purification of a single pure active component from complicated TCM combinations are significantly hampered by these unique properties of TCM monomers. The oldest leaching technique, hot water isolation, is a well-known and conventional strategy. Water separation is facilitated by enzyme-assisted extraction (EAE), which has a high impurity removal and purification efficiency. For TCM extraction, physical techniques like ultrasound and microwave-associated extraction (MAE) are also

often used. Recent technological advancements have led to the creation of novel separation techniques, such as molecular imprinting, supercritical fluid, and semi-bionics. TCM extraction has also made use of technology (MIT)-associated extraction. HWE, or hotwater extraction High temperatures encourage the breakdown of polar and hydrophilic macromolecules from the cell wall, making them more readily soluble in water [29]. Water is the perfect polar solvent for the extraction of polar and hydrophilic molecules. With just one extraction solvent, a straightforward apparatus, and minimal expenses—especially when considering solvent pollution and environmental protection—the HWE process is straightforward [30]. The chemical degrades and its biological activity is reduced as a consequence of the lengthy duration and high temperatures, which make temperature management difficult [31]. Additionally, the target product is difficult to separate from the mixtures using this conventional isolation technique due to the high number of water-soluble contaminants, which results in a poor extraction efficiency [32]. To put it another way, HWE is the most popular and practical way to extract a specific product.

EAE

In order to liberate intracellular active components from cells, the EAE approach leverages the catalytic capacity of enzymes to break down complicated biological structures [32]. This method is well regarded for its moderate reaction conditions, which make it economical in terms of energy and investment needs, efficient, simple to use, and ecologically benign [33]. The specificity and selectivity of enzymes are essential character-istics that might impact the extraction process. Enzymes such asproteases, cellulases, amylases, glucanases, and endoproteases areused in EAE to catalyze cell wall destruction. However, because asingle enzyme may not be sufficient to meet the extraction objec-tives, a cocktail of enzymes with a broad spectrum of activities isoftenusedtodisruptthecellwallmoreeffectively[33,34].EAEcanimprovethe level of hydrolysis as a resultof the synergistic effectsbetween different enzymes. Optimizing conditions for enzymaticactivity is critical for EAE. Each enzyme has an ideal set of re-action parameters, including enzyme concentration, temperature,time, and pH, which together determine the extraction efficiency[35]. The ideal conditions for a combination of enzymes may differfrom those for a single enzyme. The presence of enzyme inhibitors, substrate types, and synergistic effects must thus be taken into account. EAE is not often employed in isolation; instead, it is coupled with other extraction techniques to boost the yield of chemical components. EAE enhances the extraction activity by helping to remove and purify impurities in addition to acting as an auxiliary process for water extraction.

UAE stands for ultrasound-associated extraction. UAE is a contemporary method that makes use of the physical effects of ultrasonic waves to improve the release of medicinal ingredients from plant materials. It works on the basis of cavitation, which is the process by which ultrasonic vibrations create tiny bubbles in a liquid medium. These bubbles burst and burst violently, creating microstreams and shock waves that break down cell walls and spill their contents [36]. Although the application of UAE has some challenges, the extraction efficiency is affected by the limited rate of conversion of sound energy to mechanical energy, insufficient ultrasonic power can lead to low extraction rates, and prolonged sonication can cause thermal effects that can degrade heat-sensitive ingredients [38]. UAE operates at lower temperatures, has a higher yield and efficiency, shorter extraction time, is more versatile, and is more suitable for various plant materials and target compounds [37]. A variety of variables, including ultrasonic frequency and power, the number of ultrasonic cycles, the ratio of plant material to solvent, and duration, might influence the yield and purity of the target chemical during extraction procedures [39]. In conclusion, UAE is a promising method for extracting pharmaceutical chemicals and has benefits including efficiency, rapidity, and the ability to preserve components that are sensitive to heat. However, careful study of opera-tional factors and equipment features is important tooptimize the extraction process and produce best outcomes.

MAE

Bioactive chemicals may be extracted from natural sources using the MAE separation process. It is based on the thermal effect of microwaves,whichcauses polarsubstances,particularlywatermoleculesin thecellsofthematerial,toabsorbmicrowaveenergyandproduceheat.Thisrapidincreaseinintracellulartemperaturel eadstothevapor-ization of water, which in turn generates pressure that breaks cellwalls and membranes, creates pores, and facilitates the release ofactive ingredients into the solvent [40]. MAE is often used to separate substances such alkaloids, flavonoids, volatile oils, and polysaccharides. However, MAE is not applicable to active ingredients with poor water absorption and heat-sensitive components, such as proteins and peptides [32]. In other words, the MAE is regarded as an auxiliary tool that is limited by the transformation rate of heat power. MAE offers benefits, such as a high extraction rate, short extraction time, and reduced solvent consumption.

Semi-bionicextraction(SBE)

SBEtechnologysimulatesthetransportprocessoforalmedicinein the gastrointestinal tract using pH-optimized artificialgastricand intestinal juices for successive elution and isolation, based onthe principle of a biopharmacological perspective, combining ho-listic drug research with molecular drug research [41]. SBE can lower expenses, shorten the production cycle, and increase extraction efficiency. However, it is unnecessarily polished and condensed, and it adds incorrect elements [38]. SBE is commonly employed inpharmacological research in the laboratory; nevertheless, its practicallarge-scale applications have not yet been accomplished.

SFE, or supercritical fluid extraction In order to selectively isolate and purify components based on their molecular mass, boiling point, and polarity, SFE is a sophisticated green separation technique that uses a su-percritical fluid as an extracting agent to control the critical temperature and pressure of a component [42]. SFE combines the properties of distillation and liquid-liquid extraction and uses a variety of solvents, including CO2, which have low toxicity, non-flammability, cost-effectiveness, and high purity [43]. Additionally, supercritical fluids' low temperature may shield heat-sensitive materials and is appropriate for separating TCM's bioactive ingredients. Long-term processing, however, may result in solvent waste and bioactive substance degradation. The implementation of SFE is further complicated by issues with huge equipment size, high equipment expenses, and high operating demand [38]. SFE is an extraction technological breakthrough that offers a number of advantages that may improve the effectiveness and caliber of research on natural products. Its implementation challenges must be taken into account, though, and additional optimization and technological advancements might be required to get past these obstacles.

MIT

The principle of selective recognition and binding to certain target molecules that imitate the recognition capabilities of biological antibodies or receptors is the foundation of MIT, a molecular recognition technology [44]. Three processes are used to prepare molecularly imprinted polymers (MIPs): i) imprinting, in which functional monomers are grouped around a template molecule to create complexes through covalent or non-covalent interactions; ii) polymerization, in which the complexes are cross-linked to create a stable network that is subsequently solidified; and iii) template removal, in which the template is removed, leaving behind particular binding sites that are capable of selectively identifying the target molecule [45]. Structure predictability, identification specificity, and application extensiveness are the three primary attributes of the created MIPs [46]. MIPs have high selectivity, high affinity, strong mechanical and chemical stabilities, and are simple to synthesize

in large quantities [38]. MIPs do, however, have certain drawbacks, including ineffective adsorption and desorption, difficult preparation procedures, low batch-to-batch reproducibility, and possible contamination. Despite these chal-lenges, the potential applications of MIPs are universal and theyare used in manyfields, such as sample pre-concentration for theextraction and concentration of analytes from complex samples,as stationary phases in chromatography, as bioassays and bio-sensors, and as enzyme and receptor mimics [47]. As technology advances, new techniques will be created to overcome the present drawbacks of MIPs, such increasing their repeatability and simplifying their production. This will increase the number of scientific and commercial domains in which MIT technology may be used.

2.Exosomes

All cell types emit exosomes, which are tiny extracellular vesicles (EVs) produced in the endosomes of eukaryotic cells. They typically have a diameter of 30 to 150 nm [48]. are present in stomach acid, blood, urine, and other substances [49]. Exosomes perform key functions in cell communication by transferringvarious proteins, metabolites, and nucleic acids to recipient cells,thus influencing numerous life processes and are also related with theoccurrence of many illnesses [50]. Exosomes may also be employed as medication delivery vehicles to treat a number of illnesses, including neurological conditions. Low toxicity, low immunogenicity, and high engineering capabilities are benefits of exosome-mediated drug delivery that can enhance drug targeting and delivery effectiveness. By encapsulating medications in exosomes, drugscontaining TCM monomers may be directed to cells more efficiently, enhancing therapeutic effectiveness and decreasing negative effects[51].

Exosome biogenesisThe primary process for producing exosomes is the fusion of nuclear endosomes and plasma membranes [48]. Early endosomes' limiting membrane protrudes inward to create exosomes [49]. Subsequently, bioactive chemicals begin to concentrate inthe early sorting endosomes. When other related proteins needed for transport and endocytic sorting complexes work together, early sorting endosomes transform into late sorting endosomes [52]. Double invagination of the plasmam membranes

subsequently develop into multivesicular bodies (MVBs). MVBs fuse with the plasma membrane to release exosomes after this procedure is finished [53].

Componentsofexosomes

Exosomes contain numerous bioactive cargo originating fromthecell, including nucleic acids, lipids, metabolites, and cytosolic andcell-surface proteins, which may be transported to target cells toexecute biological tasks [54]. Many late endosome-related compounds, including tetraspanins (CD63, CD9, and CD81), membrane transport and fusion proteins (Rab, GTPases, and flotillin), numerous heat shock proteins (like HSP70 and HSP90), and MVB biogen-esis proteins (like alg-2 interacting protein-x (Alix) and tumorsusceptibility gene 101 (TSG101)), are found in the exosome and can all be regarded as exosome markers [55]. Additionally, under pathological or physiological situations, the functional statuses of parental cells might be reflected by unique proteins found in certain exosomes that are dictated by their maternal cells [56].

Consistency

Exosomes' ability to function as drug delivery vehicles depends on their stability, which is closely linked to resistance to aggregation, structure maintenance, and avoidance of protein degradation. Techniques for identifying exosomal stability include measuring surface markers, like CD63 or CD81, to assess protein

degradation; characterizing colloidal stability using measurements of zetapotential, size distribution, and concentration using nanoparticle tracking analysis (NTA); and examining morphology using electron microscopy [57]. Temperature, time, and freeze-thaw cycles are among the storage parameters that have a significant impact on exosome stability [58]. Osmotic pressure and pH are also crucial for exosome stability. For instance, the levels of protein and RNA inclusions of exosomes decreased at room temperature after 10 days compared to to store at 70°C and 4°C. Exosomes are available at 4°C for the short term. term(within7days)andbelow—70◦Cfor the long term,withthe The ideal storage temperature for the long-term preservation of fresh exosomes is [59].
Surfacemodificationofexosomes

Exosomes have recently been investigated as nanoscale drug delivery vehicles in biomedicine. Natural exosomes disperse in extracellular spaces and biofluids by free diffusion and are randomly absorbed by recipient cells, according to several studies. For instance, exosomes labeled with fluorescence dye/probe can be detected in the kidney, pancreas, spleen, intestines, and other organs by intravenous, intraperitoneal, or subcutaneous injection, indicating the uncontrolled location of exosomes in vivo [60]. Targeting the natural exosomes is necessary to deliver the desired cargo to particular tissues or cells. Several technologies have been developedforexosomesurfacemodification:1)geneticengineering,whichisacommon approach for producing surface-modified exosomes, inwhich exosome-originating cells use plasmid vectors that encodetargeting ligands fused with transmembrane proteins such as tet-raspanins, lysosome-associated membrane protein (Lamp), andglycosylphosphatidylinositol (GPI) [61]; 2) the click chemistrytechnology covalent modification, which involves attaching analkyne group to the exosome surface via a condensation reactionwith 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-N-hydroxysuccinimide (EDC-NHS). When copper is present, this alkyne group can then be covalently joined to an azido group on a target moiety; this reaction is called "click" because of its effectiveness and dependability [20]. Click chemistry and genetic engineering are popular methods for creating exosomes with altered surfaces. Furthermore, a few non-covalent modification techniques are also used to produce targeted exosomes: 1) multivalent electrostatic interactions, which increase the effectiveness of exosome targeting to negatively charged biological membranes by coating the exosome membrane with a positive charge imparting moiety [62]; 2) the ligand-receptor interaction, which is the correlation between exosome surface natural receptors and targeting ligands [63]; 3) hydrophobic interaction/membrane engineering, in which the exosome-liposome hybrid membrane gains targeting capability through the fusion of exosomal membranes with functionalized liposomal membranes [64]; 4) aptamer-based surface modification, in which the aptamer technique that fuses with nucleotide sequences is used for exosome surface modification [65]; and 5) modification by anchoring the CP05peptide, in which the CP05peptide has a strong affinity for the second extracellular loop of CD63, a protein that is frequently found on the surface of exosomes. It is thus a useful mediator between the exosome surface and medicinal drugs [66].

Removal of exosomes Standardized isolation techniques are essential for exosome research and therapeutic use. Numerous exosome separation techniques, such as ultracentrifugation (UC), polymer precipitation (PP), immunoaffinity capture (IC), ultra-filtration (UF), size exclusion chromatography (SEC), and microfluidics-based methods, have been investigated in light of the exosomes' size, shape, density, and surface proteins [67].

UC
Based on differences in the density and size of the exosomes as well as contaminants in the sample, UC is the most widely used exosomal separation method and is regarded as the gold standard for exosome isolation. First, the samples were centrifuged at 300, 2000, and10,000gto remove dead cells and cell debris, which could bereplaced byfiltration, and the supernatant was centrifuged at

highspeed(~100,000g)topelletexosomes.Typically,anadditionalhigh-speed centrifugation step is implemented to wash the exosomepellet in phosphate-buffered saline (PBS) to reduce protein contaminants[68].Asamaturemethod,UCissuitableforisolatingmostsamplesatlowoperatingcosts.However,theseparationprocessistime-consuming(>4h),withpoorrepeatability,impuritypotential,and risk of exosome damage that affects biological activity [69]. IC

Exosomes are selectively captured and purified from biological materials using the IC approach for exosome separation, which relies on particular interactions between antigens and antibodies [70]. On their surfaces, exosomes have certain proteins, lipids, and polysaccharides that antibodies may target. By immobilizing these antibodies on a solid surface, such magnetic beads, exosomes with matching antigens can be selectively bound and separated [71]. In order to study the functions of specific exosome types, the IC method can isolate specific subpopulations of exosomes based on target anti-gens. This method produces highly pure exosome preparations that are useful for downstream applications like proteomics or functional assays. Different exosome subpopulations can be isolated by selecting different antibodies [72]. Nevertheless, this process takes a long time and calls for costly, specialized antibodies, which eventually lead to a poor exosome yield because of their inadequate bead elution [73]. Research on certain disorders, where disease-specific exosome markers may be found and examined, benefits greatly from this separation technique.

PP

PP technology modifies the solubility of exosomes in solution.Polymers, such as polyethylene glycol (PEG), are added to thesample, which interacts with water molecules and diminishes thesolubility of exosomes, leading them to precipitate from the so-lution [72]. Following the removal of cells and detritus by low-speed centrifugation, the supernatant was combined with a polymer solution, incubated, and then centrifuged once more at low speed to extract exosomes [74]. Without the need for complicated equipment or drawn-out processes, this technique may be applied to large sample quantities, produce a significant number of exosomes, and maintain the integrity of exosomes [75]. The precipitation method can be used in conjunction with other separation techniques, such as the immunoaffinity method, to improve the purity and specificity of the isolation. However, the use of polymers may introduce impurities, such as protein aggregates and lipoproteins, into exosome preparations, potentially affecting their biological activity and interfering with subsequent analyses, such as proteomics or mass spectrometry [76].

UF

Because of its effectiveness and simplicity, UF, a membrane-based separation method, is becoming more and more well-liked in the area of exosome isolation. Utilizing a membrane with a certain pore diameter, or molecular weight cutoff (MWCO), UC works on the basis of size, retaining bigger contaminants while allowing them to pass through smaller particles, including exosomes [77]. To concentrate samples and eliminate large impurities, this technique can be combined with low-speed centrifugation. Depending on the pressure, UF can be separated into two types: dead-end filtration (DEF; liquid flow is in the same direction as filtration, causing rapid filter clogging) and tangential flow filtration (TFF; liquid flow is perpendicular to filtration, reducing clogging and improving membrane life and equipment stability) [78]. UF is a valuable tool for exosome isolation because of its ease of use and efficiency. It is one of the simplest methods for exosome separation, requiring little equipment and expertise, allowing for the quick purification of large sample volumes in a short amount of time, and not requiring expensive special equipment or hazardous chemical reagents. However, this method has some drawbacks, such as membrane clogging, exosome deformation, exosome loss, and the risk of contamination with fluid components smaller than the filterpore size.

SEC

Gel filtration chromatography, or SEC, is a technique that separates particles based on their size and is especially helpful for isolating exosomes from biological fluids. The principle of UC is based on size differences; a porous sta-tionary phase (e.g., Sephadex, Sepharose, Sephacryl, and BioGel P) is used in a column through which a mobile phase (biological fluid) is passed, and the larger particles elute first because they cannot enter the pores and thus take a shorter path, while the smaller particles, including exosomes, penetrate the pores and elute later [80,81]. SEC has several benefits, such as minimizing protein contamination, maintaining the biological activity and structure of exosomes, minimizing sample loss compared to UF, taking less time to complete in 15 minutes, and isolating specific subsets of EVs using materials with different pore sizes [82]. A poor yield of exosomes is the consequence of SEC's inability to efficiently remove impurities with sizes comparable to exosomes [82]. In conclusion, SEC is a powerful technique for isosome separation that provides scalability, high purity, and biological activity preservation. Techniques based on microfluidics
Because it can combine many processes onto a single chip, the microfluidics-based approach is a state-of-the-art technique that has become a potent tool for the extraction and study of exosomes [83]. Physical characteristics (size, density, surface antigens, etc.), immunoaffinity, and contact-free status are the foundations of the microfluidic-isolated exosome concept [82]. In the future, this technique may be used in liquid biopsies, point-of-care diagnostics, and personalized treatment [84]. Microfluidics offers a versatile and efficient approach to exosome research with the potential to transform diagnostics and therapeutics. Nevertheless, it also satisfies some requirements, such as the need for additional validation and large-scale testing of microfluidics-based methodologies, improving the sensitivity and specificity of exosome detection and isolation, and combining other detection and analysis methods for comprehensive exosome research.

Exosomes' benefits and drawbacks as medication carriers The benefits of using exosomes to deliver medications to the brain For the following reasons, exosomes have been identified as promising natural carriers for drug loading and delivery (Fig.1).

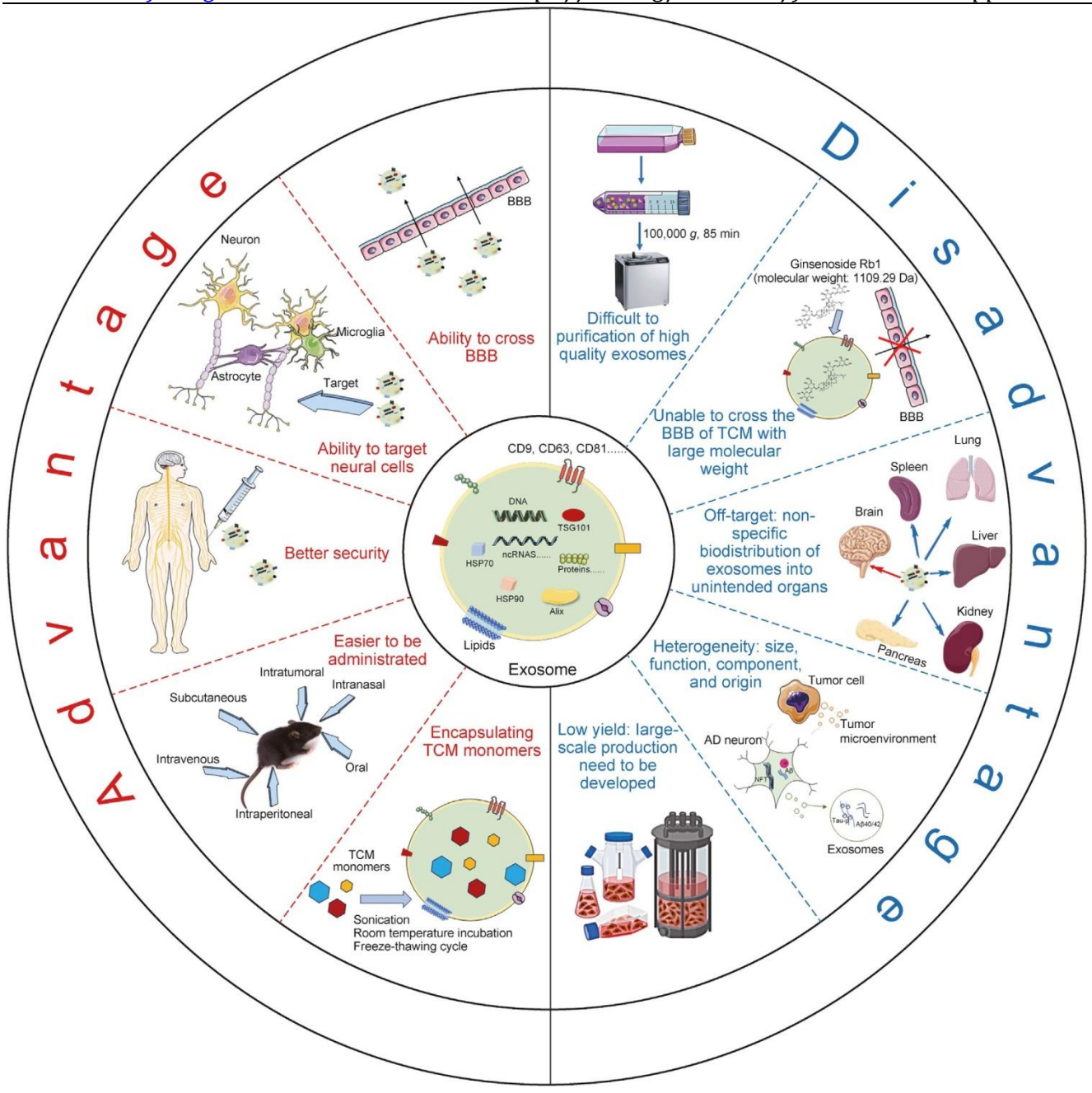


Figure 1 lists the benefits and drawbacks of using exosomes as a medication delivery system to treat neurological disorders. Exosomes may target neuronal cells, penetrate the blood-brain barrier (BBB), provide improved security, be administered in vivo in a variety of methods, and efficiently encapsulate traditional Chinese medicine (TCM) monomers. However, the characteristics of TCM monomers with large molecular weight, off-target, heterogeneity, low yield, and difficulty in separating exosomes have limited their ability to be used as TCM monomers in inclinical delivery systems. TSG101: tumor susceptibility gene 101; cRNAs: non-coding RNAs; HSP: heat shock protein; Alix: alg-2interacting protein-x; AD: Alzheimer's disease; and its capacity to traverse the blood-brain barrier. Glial cells (astrocytes and microglia), pericytes, specialized brain microvascular endothelial cells (BMECs) connected by tight junctions, and a basement membrane

make up the semi-permeable barrier known as the blood-brain barrier (BBB) [85]. The movement of ions and other micro- and macromolecules between blood and neural tissues may be restricted by this barrier, which regulates homeostasis [85]. Since almost all large-molecule medications and over 98% of small-molecule pharmaceuticals have trouble entering the central nervous system, the blood-brain barrier is one of the primary issues with traditional drug delivery to the central nervous system. The extracellular matrix benefits from the endogenous characteristics and small size of exosomes, which are able to freely traverse the vascular wall [86]. Additionally, a number of studies have proposed mechanisms for the interaction between the BBB and exosomes. For instance, Yuan et al. [87] discovered that exosomes produced from macrophages may cross the blood-brain barrier by interacting with the intercellular adhesion molecule 1 (ICAM-1) of brain microvessel endothelial cells and integrin lymphocyte function-associated antigen 1 (LFA-1). Additionally, Chen et al. [88] discovered that HEK293T-derived exosomes may cross the blood-brain barrier by activating the endocytosis of BMECs, which is triggered by the inflammatory cytokine tumor necrosis factor-a (TNF-a). After being loaded into exosomes, TCM monomers can cross the BBB to treat neurological disorders (Fig. 2). Secondly, it has a better ability to target. When utilized as medication delivery vehicles, exosomes have been shown in studies to have higher selective targeting capabilities and lower immunogenicity than standard drug carriers. Their homing capacity to tumor tissues is often higher than that of liposomes, which further supports exosomes' excellent targeting capabilities [89]. Exosomes contain varioushomingmolecules(ligands,magneticmaterials,andpH-responsivemotifs) on their lipid bilayer membranes, which contribute to theidentification of specific cell types and enable the acquisition oftargetingfeatures for drug delivery invivo or invitro. For example,exosomescontainingTspan8arepronetocombinewithCD11bandCD54-positivecells[90].
Third,itimprovessecurity. Thesurfacesofexosomes are wrapped in the membranes of various cells,improving their biocompatibility and safety [91]. Traditional drugcarriers can cause toxic immune reactionsin vivo, resulting ingreatly reduced therapeutic effects, while exosomes are not proneto immune reactions due to their improved endogenous biocom-patibility[92]. Basedonthegoodbiocompatibilityofexosomes,thestability and effectiveness of drugs contained in exosomes can beeffectively improved [93]. Various proteins contained in the exo-somal membrane also facilitate their use as drug delivery vehicles. Four transmembrane proteins, CD9 and CD81 (on the exosomemembrane facilitate fusion between exosomes and cells), CD55,andCD59,enhancethestabilityofexosomesincirculation. CD47

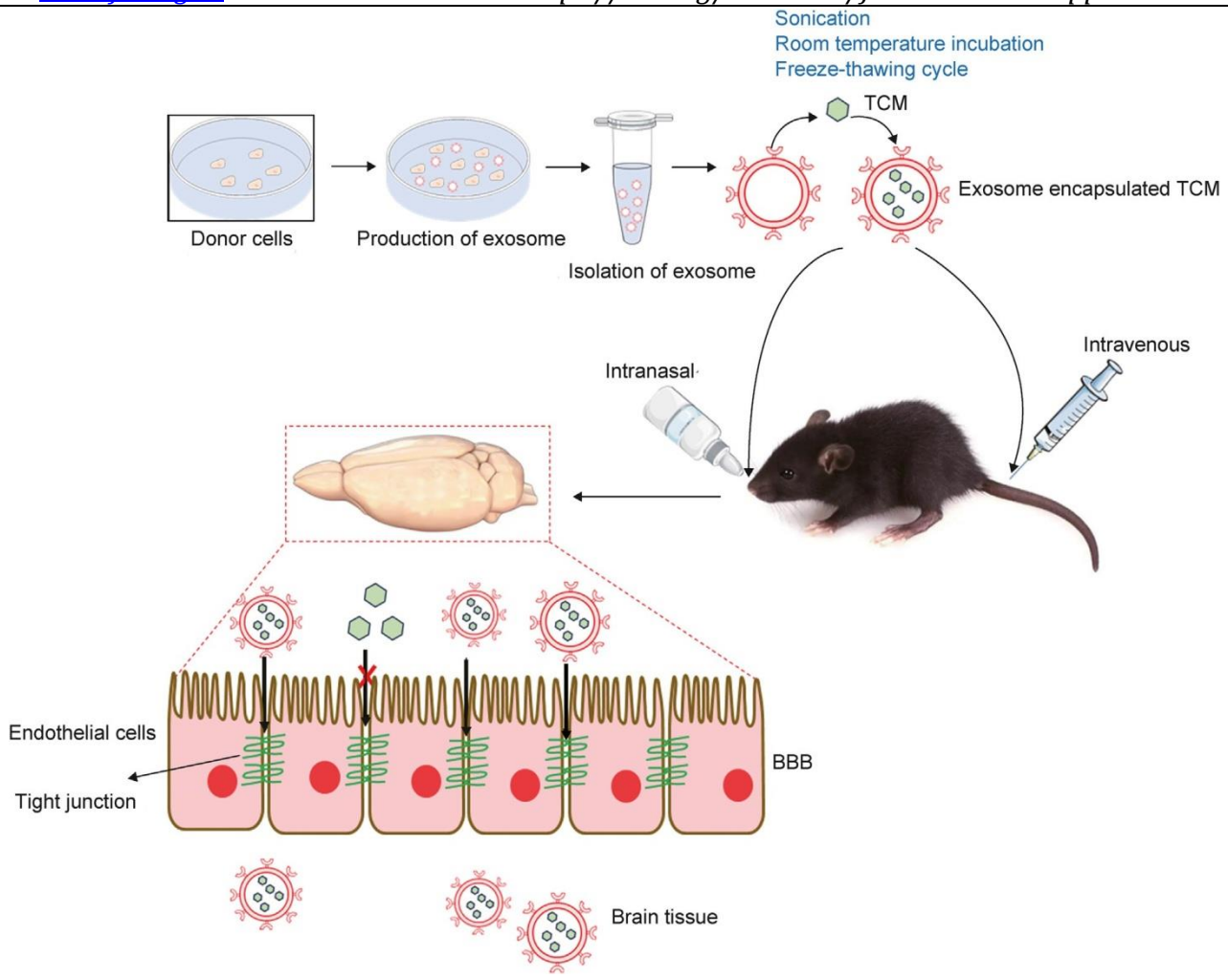


Fig. 2. After being loaded into exosomes, monomers of traditional Chinese medicine (TCM) may pass across the blood-brain barrier (BBB). Exosomes may be loaded with TCM monomers by sonication, room temperature incubation, and freeze-thaw cycle techniques after being separated from the cell's culture medium supernatant. When exosome-encapsulated TCM monomers were administered intravenously or intratranasally to rats or mice, they were able to pass the blood-brain barrier and reach the damaged area of the brain. In many neurodegenerative disease models, the therapeutic outcome and pros/cons of exosomes are encapsulated in traditional Chinese medicine (TCM) monomer [8e25]. Naïveor modified exosome TCM monomer Administration method Therapeutic effect Animal/cell model Pros/cons References.

Intravenous injection of Naïveexosomes Ber/Pal APP/PS1mice at the same time every two days for a total of 21 days

Intravenous Naïveexosomes Ber SCImice: tailvein injection Fe65-exosomes Cory-B 5×FADmice For 45 days, administer intraperitoneally or intravenously on alternate days. Naïveexosomes Hed, NefAPP/PS1mice, and Bai Intravenous: 100 mg/kg of tail vein injection anti-inflammatory properties in vitro and in vivo; boosting drug accumulation in the brain, striatum, and hippocampal regions; fortifying nerve injury and cognitive impairment in vivo by quickening Ab removal and the release of anti-inflammatory cytokines Anti-inflammatory and anti-apoptotic activity, alleviationmoto function promoting autophagy in neurons and improving AD mice's cognitive abilities Reducing the level of huntingtin74, p301L tau, A53Ta-synuclein, aggregation of Ab (1—42),

enhancingautophagy, and ameliorating the learning and memory abilityinvivo Advantages: Compared to single-drug treatment, the Ber/Pal combination therapy shows superior and more thorough therapeutic effects.Cons:lowyieldofexosomes,lackoforganictargeting

Pros: exosome-Ber can cross BBB and blood spinalbarrier, reach the spinal cord injury site through thecerebrospinalfluid circulation; exosomes provide adrug platform and have synergistic effect with drugs Prons: Fe65-exosomes can bypass BBB and targetAPPoverexpressed neuronal cells Pros: high bio-availability, crossing BBB with themolecular weight lower than 1109 Da of monomer Cons:off-targeteffects;notapplicable tolarge-scale

[8] [9] [10] [11] Naïve exosomes pMCAOrats and BaitMCAO Naïve exosomes Cur OA injection on one side of the mouse's hippocampus region Naïve exosomes PD caused by Cur 6-OHDA Intravenously: tail veininjection Intravenously: a single doseofcur at 0.4 mg/kg Nasally: at a dose of 10mg/kgbody weight Reducing cell apoptosis by attenuating ROS productionin vitroand depressing the infarct area neurologicalscoreswhiletheintegrityofneuronalstructureinvivo
Mitigating learning and memory deficits in OA-induced AD mice by blocking tau phosphorylation via the Akt/GSK-3b pathway Decreased the brain inflammation, aggregated ofa-synuclein, and cell apoptosis in the dopaminergic THpositive neuron, and improved the impairedlearningand memory ability in PD mice Pros: exosome-Bai easily cross the BBB, allowing moreBai to target brain tissues; the migratory and braintargeting abilities features of exosome-Bai was acquiredfrom its origin cells (macrophages), to promote theaccumulation of Bai in the brain ischemic regionfollowing pMCAO Pros: Cur-treated macrophage derived exosomes canenhance the penetration of cur across the BBB intothebrain through interaction between LFA-1 and ICAM-1

Pros: hEnSCs-exosomes can transport and transfer safeand effective payload in long-term, and maintain releasemanner into neuronal cells using intranasal injectionway [12] [13] [14] PR-exosome Cur MPTP-inducedPDmodel mice Intranasal administration Getting rid of -synuclein aggregation, stimulation of neurite length and branch development in vitro, and enhancement of motor behavior and coordination in Parkinson's disease (PD) mice Advantages: PR-exosome/PP@Cur is treated synergistically in three ways [15].

Cur MOG peptide-induced EAEmice in naïve exosomes Intranasally: exosome-Cur was started on day 4 after vaccination with the MOGpeptide and was delivered intranasally every day for 31 days using the above-described methodology. Anti-inflammatory effects: decrease in microglial cell count Advantages: nasal injection has no negative effects; exosomes are absorbed by microglial cells (~60%) and nonmicroglial cells (~40%). [16] MCAOrats and Naïveexosomes Cur Intravenously:tailvein injection, exosomes-cur (10 mg/mL cur) was delivered toMCAOratsafter2hofocclusion Decreasing of inflammatory cytokines expression level,ROS production, Cyt c release, and infarct area; rescuingthe loss of tight junction proteins and improvingneurological performance in MCAO rats Prons: exosomes shielded Cur from plasma breakdown; their capacity to target inflammation fueled the buildup of exosome-Cur in ischemic areas. [17]
Cur Ischemia Reperfusion-Injuredmic Naïve Exosomes Alternatenostrils (2 mL × 5 times) were administered intravenously within an hour after I/Randsham surgery and continued for seven days. Neurological scores improved, lesion volume, brain water content, and inflammatory impact decreased, astrocyte and neuronal expression returned to normal, ICAM levels in brain arteries decreased, VE-cadherin levels improved, and tight junction protein loss was lessened. Advantages: Cur was more stable and soluble in exosomes, and the integrity of exosomes was maintained.
Cons: there were no additional mouse groups treated with Cur and embryonic stem cells alone to compare the combined MESC-exosome-Cur effects; time points analysis of available Cur concentrations in the blood and mouse brain tissues; and the treatment of I/R-injured mice was initiated within an hour of injury, which does not clinically replicate the conditions of stroke patients who occasionally arrive at hospitals after hours of ischemic insult [18]. c(RGDyK)-exosomes Cur MacOmice Intravenously: 12-hour or 24-hour hafterreperfusion veintail injection NF-kB-mediated reduction of pro-inflammatory cytokines (TNF-a, IL-1b, and IL-6) and suppression of caspase-3 expression Advantages include the ability to quickly and easily alter the exosome's surface through bio-orthogonal chemistry, the ability of cRGD-exosome to maintain the exosome's shape and the bioactivity of its

cargo, and the ability to internalize into brain cells to carry out its neuroprotective functions. Cons: Future research should examine whether the altered exosomes retain the fundamental traits of native exosomes, such as reduced immunogenicity [19]. Cur's RGE-exosomes Nudemouse model carrying orthotopic gliomas Intravenous: injection into the tail vein every other day for a total of seven times RGE-exosome-SPION/Cur was able to stop tumor development and image gliomas specifically. Prons: Exosome-SPION/Cur technology in conjunction with MRI allows for early, accurate glioma diagnosis and response assessment. Cons include the exact way exosomes interact with cells, how they cross the blood-brain barrier, their long-term effects, the ideal dosage, treatment duration, temperature regulation, and magnetic flow monitoring. More research is required to determine if hyperthermia contributes to the synergistic action of SPIONs. [20] Naïve exosomes ECG-induced SHSY5Y cells with rotenone: improving cell viability and antioxidant benefits while preventing autophagy and cell death [21] RSV EAEmice Naïveexosomes Intranasally: daily dosages of exosome and RSV (3 mg/kg RSV, 30mL) MCAO/Rmodel mAbGAP43-exosomesQue Intravenous: injection of tail vein decrease in pro-inflammatory cytokine expression (TNF-b, INF-g, IL-6, and IL-17) InhibitingROSproductionviaactivationoftheNrf2/HO-1 pathwayin vitroandin vivo Pros: macrophages generated exosomes modified via clickchemistry techniques showed minimal influence on the naturalproperties of exosomes, andfit for treatment ofneurodegenerative disorders Advantages: mAb GAP43 conjugated to the surface of exosomes can be internalized into I/R injury neural cells based on the interaction between the exosome and GAP43 expressed on neurons in the ischemic region; naïve plasma exosomes exerted an asynergistic neuroprotective effect against I/R injury [22]; and the stability and solubility of exosomal que were significantly improved. [23] TMZ/DHT R-exosomes Mice with tumors Intravenously: six times a day, in the tail vein. By stimulating the immune system both in vivo and in vitro and lowering TMZ resistance in gliomas, R-exosome-TMZ/DHT was able to precisely target the tumor location and produce positive anticancer effects. Advantages: In vivo, R-exosome-TMZ/DHT exhibits no toxicity or discernible organ damage. [24]

Lipsnanovesicles, pHybrid, and exosomes SAB/CPT orthotopic BALB/cnude mice with xenograft tumors of U87-Luc Intravenous: infusion into the tail vein The synergistic effectiveness of pHybrid-SAB-CPT primarily depends on its ability to kill cancer cells and inhibit angiogenesis by denying the tumor's new blood vessels the nutrients they need. These effects are linked to SHP-2 upregulation, which suppresses STAT3 signal pathways, and anti-angiogenesis brought on by VEGF inhibition. Prons: pHybrid/SAB-CPT may accumulate in deep tumor areas and target tumors. Features of hybrid nanoparticles include tissue penetration, cell targeting, and drug delivery [25].

Pal: palmatine; Ber: berberine; Amyloid precursor protein, or APP; PS1: presenilin 1; spinal cord injury (SCI); Blood-brain barrier, or BBB Cytoxine-B: cory-B FAD: Alzheimer's disease in the family (AD); Hed: hederagenin; Nef: neferine; Bai: baicalin; pMCAO: permanent middle cerebral artery occlusion/reperfusion; tMCAO: transient middle cerebral artery occlusion; Reactive oxygen species, or ROS Protein kinase B (Akt); curcumin (Cur); OA: Okadaicacid; GSK-3b: Glycogensynthasekinase-3b; Lymphocyte function-associated antigen 1 (LFA-1); Endothelial intercellular adhesion molecule 1 (ICAM-1) PD: Parkinson's disease; 6-OHDA: 6-hydroxydopamine hydrochloride; human endometrial stem cells (hEnSCs); TH: tyrosine hydroxylase; rabies virus glycoprotein (RVG29) and penetratin (P) peptides (PR); 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP); MOG: myelin oligodendrocyte glycoprotein; PP: ROS-responsive amphiphilic polymer poly(propylene sulfide)-polyethylene glycol (PPS-PEG); EAE: experimental autoimmune encephalitis that is recalcitrant; I/R: ischemia and reperfusion damage; VE: vascular endothelial; MESC:mouse embryonic stem cell; c(RGDyK): cyclo(Arg-Gly-Asp-D-Tyr-Lys) peptide; TNF-a: Tumor necrosis factor-a; IL-1b: interleukin-1b; NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells; cRGD: cyclo(Arg-Gly-Asp); RGE: RGERPPR; SPION: superparamagnetic iron oxide nanoparticles; Magnetic resonance imaging, or MRI Epicatechin gallate (ECG); resveratrol (RSV); mAb: monoclonal antibody; GAP43: growth-associated protein-43; MCAO/R: middle cerebral artery occlusion/reperfusion; TGF-b: transforming growth factor-b; IFN-g: interferon-g; Nrf2: nuclear factor erythroid-2-related factor 2; HO-1: heme oxygenase-1; R: reassembly; Lips: lipidosomes; SAB: salvianolic acid B; CPT: cryptotanshinone; SHP-2: Src homology-2domain-containing protein tyrosine phosphatase 2; STAT3: signal

transducer and activator of transcription; TMZ: temo-zolomide; DHT: dihydrotanshinone; pHybrid: BBB penetrated exosomes-liposome hybridnovesicles

3.Table3
Thelistofloadingmethod,encapsulationefficiency(EE)/loadingcapacity(LC),andexosomeorigininvariousneuronal dise ases [8e25].

Table 2
The therapeutic outcome and pros/cons of exosome encapsulated traditional Chinese medicine (TCM) monomer in various neuronal disease model [8–25].

Naïve or modified exosome	TCM monomer	Animal/cell model	Administration way	Therapeutic outcome	Pros/cons	Refs.
Naïve exosomes	Ber/Pal	APP/PS1 mice	Intravenously: tail vein injection at the same time every two days for a total of 21 days	Anti-inflammatory effects both *in vitro* and *in vivo*; increasing drug accumulation in the hippocampus, cortex, and striatum; strengthening cognitive impairment and nerve injury *in vivo* by accelerating Aβ elimination and anti-inflammatory cytokine secretion	Pros: the Ber/Pal combined therapy exhibits better and more comprehensive therapeutic effects than single-drug treatment Cons: low yield of exosomes, lack of organic targeting	[8]
Naïve exosomes	Ber	SCI mice	Intravenously: tail vein injection	Anti-inflammatory and anti-apoptotic effect, alleviation moto function	Pros: exosome-Ber can cross BBB and blood spinal barrier, reach the spinal cord injury site through the cerebrospinal fluid circulation; exosomes provide a drug platform and have synergistic effect with drugs	[9]
Fe65-exosomes	Cory-B	5× FAD mice	Intraperitoneally or intravenously: alternate days for 45 days	Inducing neuronal cells autophagy and ameliorating cognitive function of AD mice	Prons: Fe65-exosomes can bypass BBB and target APP overexpressed neuronal cells	[10]
Naïve exosomes	Bai, Hed, and Nef	APP/PS1 mice	Intravenously: tail vein injection at a dose of 100 μg/kg	Reducing the level of huntingtin74, p301L tau, A53T α-synuclein, aggregation of Aβ (1—42), enhancing autophagy, and ameliorating the learning and memory ability *in vivo*	Pros: high bio-availability, crossing BBB with the molecular weight lower than 1109 Da of monomer Cons: off-target effects; not applicable to large-scale	[11]
Naïve exosomes	Bai	tMCAO and pMCAO rats	Intravenously: tail vein injection	Reducing cell apoptosis by attenuating ROS production *in vitro* and depressing the infarct area neurological scores while the integrity of neuronal structure *in vivo*	Pros: exosome-Bai easily cross the BBB, allowing more Bai to target brain tissues; the migratory and brain targeting abilities features of exosome-Bai was acquired from its origin cells (macrophages), to promote the accumulation of Bai in the brain ischemic region following pMCAO	[12]
Naïve exosomes	Cur	Injection of OA on one side of hippocampal area of mice	Intravenously: a single dose of cur at 0.4 mg/kg	Inhibiting tau phosphorylation through the Akt/GSK-3β pathway, ameliorating of learning and memory deficiencies in OA-induced AD mice	Pros: Cur-treated macrophage derived exosomes can enhance the penetration of cur across the BBB into the brain through interaction between LFA-1 and ICAM-1	[13]
Naïve exosomes	Cur	6-OHDA induced PD	Nasally: at a dose of 10 mg/kg body weight	Decreased the brain inflammation, aggregated of α-synuclein, and cell apoptosis in the dopaminergic TH positive neuron, and improved the impaired learning and memory ability in PD mice	Pros: hEnSCs-exosomes can delivery and transfer safe and effective payload in long-term, and sustain release manner into neuronal cells through intranasal injection way	[14]
PR-exosome	Cur	MPTP-induced PD model mice	Intranasally injection	Eliminating α-synuclein aggregation and promotion of the growth of neurite length and branches *in vitro* and the improvement of movement behavior and coordination ability in PD mice	Pros: three-Pronged Synergistic Treatment of PR-exosome/PP@Cur	[15]
Naïve exosomes	Cur	MOG peptide induced EAE mice	Intranasally: exosome-Cur was administered intranasally daily for 31 days using the protocol described above and was initiated on day 4 after immunization with the MOG peptide	Anti-inflammatory effects: reduction in the number of microglial cells	Pros: no side effects with nasally injection; exosomes are taken up by microglial cells (~60%)/nonmicroglial cells (~40%)	[16]
Naïve exosomes	Cur	MCAO rats	Intravenously: tail vein injection, exosomes-cur (10 μg/mL cur) was administered to MCAO rats after 2 h of occlusion	Decreasing of inflammatory cytokines expression level, ROS generation, Cyt c release, and infarct area; rescuing the loss of tight junction proteins and improving neurological performance in MCAO rats	Prons: exosomes protected Cur from degradation in plasma; accumulation of exosome-Cur in ischemic regions were driven by the inflammation-mediated targeting ability of exosomes	[17]
Naïve exosomes	Cur	Ischemia reperfusion-injured mic	Intranasally: alternate nostrils (2 μL × 5 times) started within an hour of I/R and sham surgery and continued till seven days.	Improvement in neurological scores, lessening in lesion volume, brain water content, and inflammation effect; normalization astrocytes and neuronal expression, decreasing in ICAM and improvement in VE-cadherin levels in brain vessels; and alleviating tight junction proteins loss	Pros: more solubility and stability of Cur in exosomes and the integrity of exosomes-Cur was preserved. Cons: the treatment of I/R-injured mice was started within an hour of injury which does not clinically implicate the conditions of the stroke patients who sometimes reach to the hospitals after hours of ischemic insult; the lack of additional mice groups treated with Cur and embryonic stem cells alone to compare the combined MESC-exosome-Cur effects; and time points analysis of available Cur concentrations in the blood and mice brain tissues	[18]

c(RGDyK)-exosomes	Cur	MACO mice	Intravenously: vein tail injection 12 h or 24 h after reperfusion	Decreasing pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) mediated by NF-κB and inhibiting expression level of caspase-3	Pros: the exosomes surface can be modified by bio-orthogonal chemistry easily and rapidly; cRGD-exosome can preserve intact exosomes shape and the bioactivity of their cargo, and can be internalized into brain cells to exert its neuroprotective roles Cons: whether the modified exosomes maintain the basic characteristics of natural exosomes like low immunogenicity should be evaluated in the future	[19]
RGE-exosomes	Cur	Orthotopic glioma-bearing nude mouse model	Intravenously: tail vein injection, every other day for total seven times	RGE-exosome-SPION/Cur had capability for targeted imaging of glioma and inhibiting the growth of tumor	Prons: the technology combination of exosome-SPION/Cur with MRI can early precise diagnosis and response evaluation of glioma Cons: the precise mechanism of exosome interaction with cells, exosome crossing BBB, the long-term effects of exosome, the optimal dose, treatment time, temperature control, and monitoring of magnetic flow hyperthermia for the synergistic effect of SPIONs need to be further explored	[20]
Naïve exosomes	ECG	Rotenone-induced SHSY5Y cells	—	Enhancing cell viability and antioxidative effects and inhibiting autophagy and cell apoptosis		[21]
Naïve exosomes	RSV	EAE mice	Intranasally: daily doses of RSV and exosome (3 mg/kg RSV, 30 μL)	Reducing of the expressions of pro-inflammatory cytokine (TGF-β, INF-γ, IL-6, and IL-17)	Pros: macrophages derived exosomes modified by click chemistry methods had little effect on the natural properties of exosomes, and fit for treatment of neurodegenerative diseases	[22]
mAb GAP43–exosomes	Que	MCAO/R model	Intravenously: tail vein injection	Inhibiting ROS production via activation of the Nrf2/HO-1 pathway *in vitro* and *in vivo*	Pros: the stability and solubility of exosomal Que were significantly enhanced; mAb GAP43 conjugated to the surface of exosomes can be internalized into I/R injury neuronal cells dependent on the interaction between exosome and GAP43 expressed on neurons in the ischemic region; and naïve plasma exosome exerted a synergistic neuroprotective effect against I/R injury	[23]
R-exosomes	TMZ/DHT	Tumor-bearing mice	Intravenously: tail vein every day, a total of six times.	R-exosome- TMZ/DHT could accurately target the tumor site and exerted good antitumor roles via activating immune system *in vivo* and *in vitro*, and reduction TMZ resistance in gliomas	Pros: R-exosome-TMZ/DHT have no toxicity or visible organ impairment *in vivo*	[24]
pHybrid–exosomes-Lips nanovesicles	SAB/CPT	orthotopic BALB/c nude mice bearing U87-Luc xenograft tumors	Intravenously: tail vein injection	The synergistic efficiency of pHybrid-SAB-CPT is major dependent on its cytotoxicity on cancer cells and anti-angiogenesis via depriving the nutrients supplied by new blood vessels in tumor, which are associated with SHP-2 upregulation induced STAT3 signal pathway suppression and anti-angiogenesis caused by VEGF inhibition	Prons: pHybrid/SAB-CPT can target tumor and accumulated in deep tumor regions and pHybrid nanovesicles have features in drug delivery, cell targeting, and tissue penetration	[25]

Ber: berberine; Pal: palmatine; APP: amyloid precursor protein; PS1: presenilin 1 ; SCI: spinal cord injury; BBB: blood–brain barrier; Cory-B: corynoxine-B; FAD: family Alzheimer's disease (AD); Bai: baicalin; Hed: hederagenin; Nef: neferine; tMCAO: transient middle cerebral artery occlusion; pMCAO: permanent middle cerebral artery occlusion/reperfusion; ROS: reactive oxygen species; Cur: curcumin; Akt: protein kinase B; GSK-3β: glycogen synthase kinase-3β; OA: okadaic acid; LFA-1: lymphocyte funciton-associated antigen 1; ICAM-1: endothelial intercellular adhesion molecule 1; 6-OHDA: 6-hydroxydopamine hydrochloride; PD: Parkinson's disease; TH: tyrosine hydroxylase; hEnSCs: human endometrial stem cells; PR: penetratin (P) and rabies virus glycoprotein (RVG29) peptides; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PP: ROS-responsive amphiphilic polymer poly(propylene sulfide)–polyethylene glycol (PPS–PEG); MOG: myelin oligodendrocyte glycoprotein; EAE: refractory experimental autoimmune encephalitis; I/R: ischemia and reperfusion injury; VE: vascular endothelial; MESC: mouse embryonic stem cell; c(RGDyK): cyclo(Arg-Gly-Asp-D-Tyr-Lys) peptide; TNF-α: Tumor necrosis factor-α; IL-1β: interleukin-1β; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; cRGD: cyclo(Arg-Gly-Asp); RGE: RGERPPR; SPION: superparamagnetic iron oxide nanoparticles; MRI: magnetic resonance imaging; ECG: epicatechin gallate; RSV: resveratrol; TGF-β: transforming growth factor-β; IFN-γ: interferon-γ; mAb: monoclonal antibody; GAP43: growth-associated protein-43; MCAO/R: middle cerebral artery occlusion/reperfusion ; Nrf2: nuclear factor erythroid-2-related factor 2; HO-1: heme oxygenase-1; R: reassembly; TMZ: temozolomide; DHT: dihydrotanshinone; pHybrid: BBB penetrated exosomes-liposome hybrid nanovesicles; Lips: lipidosomes; SAB: salvianolic acid B; CPT: cryptotanshinone; SHP-2: Src homology-2 domain-containing protein tyrosine phosphatase 2; STAT3: signal transducer and activator of transcription 3.

enhances the ability of macrophages to withstand exosomes [93]. Fourth, administering it is simpler (Table 2) [8e25]. There are many ways to deliver exosomes to target tissues in diverse disease models, including intravenous, subcutaneous, intraperitoneal, intratumoral, nasal, and oral delivery [94]. Exosome-based drug delivery is very flexible and compatible, as seen by the variety of drug administration methods. In the end, the TCM monomers were encapsulated [8e25] (Table 3). Because of their little toxicity and adverse effects, monomeric active components of TCMs may be loaded into exosomes. To attain different therapeutic outcomes, a number of loading techniques have been used with physical therapies, such as electrophoration, sonication, extrusion, freeze-thawing, surfactant treatment, and dialysis [94]. The difficulties in using exosomes to deliver medications to the brain
Exosomes have a lot of potential as drug carriers, but there are some obstacles in their way (Fig.1). First, since the physical and chemical characteristics of the many EV subtypes are identical, it is highly challenging to separate and remove exosomes. Conventional exosome extraction techniques provide benefits and drawbacks. Although UC can produce high-purity exosomes, as was previously said, the equipment is costly and has the potential to easily harm exosomes during the preparation process. The quality of separated exosomes is poor, despite the fact that UF is straightforward and capable of high-throughput separation. High-purity exosomes cannot be isolated using the straightforward and high-throughput chemical precipitation approach. Although SEC-isolated exosomes are very pure and appropriate for all kinds of samples, the process is complicated, the instrument is costly, and the protein is prone to contamination during operation [48]. Second, exosomes have a strong capacity to pass the blood-brain barrier; nevertheless, further research is needed to determine how the molecular weight of TCM and exosomes interact. Even after encapsulating into exosomes, TCM such as ginsenoside Rb1 (molecular weight: 1109.29 Da) with a molecular weight larger than 1109.29 Da cannot cross the BBB, according to Tanget al. [11], suggesting that exosomes help TCM by avoiding the BBB. The molecular weight of the cargo molecules is another important factor for the future pharmacological development of exosomes as drug carriers. Third, exosomes are thought to be good candidates to transport a range of payloads, such as proteins, RNAs, and TCM. Exosomes may still have off-target effects, however, if they biodistribute to undesired organs (liver, spleen, lungs, kidneys, and pancreas) without specificity. For instance, in addition to the target brain, exosome-berberine/palmatine (Ber/Pal) may enter the kidney and heart [8]. It's interesting to note that exosome surfaces may be altered or created to target sick cells or tissues. For instance, the Fe65 over-expressing plasmid was transfected into hippocampal neuronal cells to create engineered exosomes. These exosomes then expressed Fe65 on their surface to interact with Alzheimer's disease (AD) cells' amyloid precursor protein (APP), enhancing the exosomes' capacity to be internalized by AD diseased cells [10]. The related question is whether future research should focus on synthetic exosomes that maintain the fundamental characteristics of natural exosomes, such their low immunogenicity. Fourth, exosomes originating from distinct cell sources exhibit distinct properties, including the ability to transmit Abas and hyperphosphorylated tau cleavage products found in exosomes

secreted by AD neuronal cells [95]; exosomes derived from tumor cells can spread a variety of components, such as genes, proteins, and cytokines, to influence other cell types [96]. Their properties have the ability to influence illnesses in addition to encapsulated chemicals. Therefore, choosing exosomes from the right cell sources is essential for using them as medication carriers. Fifth, despite recent advancements in exosome technology, exosomes are still not produced on a wide scale, and their use in clinical therapy is restricted by their poor yield of cell-derived exosomes and lengthy preparation time. For instance, 1 mg of exosomes were created by every 2.13×108 cells [8]. Additionally, the composition of exosomes is very heterogeneous because of variations in the physiological and pathological states as well as cell origins. Thus, it is crucial to create technologies that may lower expenses and boost exosome output. It's interesting to note that some research has shown a promising exosome extraction approach that shows that low-level electrical treatment (0.34 mA/cm2) and production boosters may drastically improve the yield of exosomes hundreds of times [97]. In order to increase the quantity, quality, and homogeneity of exosomes, some research has proposed that immortalized mesenchymal stem cell (MSC) cell lines might be employed in lieu of primary cells and that conditioned media from the stem cell business may be a stable source [98].
Exosomes and other artificial nanoparticles, such as liposomes, are compared.

In contrast to synthetic nanoparticles, like synthetic polymeric and lipidic nanoparticles (liposomes), which are linked to high production costs, reproducibility problems, toxicity, and high immunogenicity, exosomes have recently drawn a lot of attention as a novel form of bionanoparticle for drug delivery. Comparable drug delivery technologies have been thoroughly investigated as drug delivery nanocarriers. Lipids, mostly phospholipids, make up liposomes. They create a bilayer structure that encloses an aqueous area, with a hydrophilic head and a hydrophobic tail [99]. When it comes to medication delivery, exosomes have a number of benefits over liposomes. First, compared to liposomes, which may be immunogenic because of their manufactured origin, exosomes, being natural endogenous nanocarriers, have superior biocompatibility and bioavailability, lowering the risk of immunogenic rejection and toxicity [100]. Second, transmembrane and membrane-anchored proteins that aid in endocytosis and association with certain cell types provide exosomes targeting specificity, which enhances the transport of their cargo to target cells [101]. Third, exosomes inherit bioactive components from their parent cells that may be therapeutically beneficial, in contrast to liposomes, which are manually loaded with cargo [102]. Fourth, preconditioning (cytokines, hypoxia/hypoxia, medications, and physical therapies) or engineering may increase the functioning of exosomes, increasing their potential for therapeutic use [103].
Exosomes do, however, have a number of drawbacks. compared to drug delivery liposomes. First, compared to liposomes, exosomes have a much lower yield. The ability of parent cells to produce exosomes limits their creation, which makes large-scale manufacture difficult and expensive [104]. Second, compared to liposomes, exosomes have a poorer cargo-loading efficiency because they are naturally packed with proteins and nucleic acids, which makes it challenging to load them with the intended therapeutic cargo [101]. Third, the stability, biodistribution, and therapeutic effectiveness of exosomes may be impacted by their varied size and composition. Because exosomes have an endogenous origin and vary in composition and properties, quality management of these particles is more complicated than that of liposomes [105]. Fourth, while exosomes may be modified toimprove their functioning, the procedure is complicated and needs adeep knowledge of the molecular pathways involved inexosome formation and activity [94]. Fifth, there may be difficulties in establishing guidelines for the manufacturing, characterisation, and clinical use of exosome-based medicines as the regulatory environment is still developing.

2. The possible impact of TCM monomer-loaded exosomes on neurological disorders TCMmonomerinAD encapsulated in exosomes

Plaques, tau phosphorylation, and neurodegeneration are hallmarks of AD, a very significant neurological disease [106]. With a progressive decline in every facet of brain function, people with AD suffer from significant memory loss and cognitive impairments. Due to the intricacy of AD, which is caused by a number of variables, no entirely effective drugs have been licensed for clinical therapy as of yet [107]. Cholinesterase inhibitors, such as donepezil, galantamine, and N-methyl-D-aspartate-receptor antagonists, as well as glutamate receptor antagonists, such as rivastigmine and memantine, are the mainstay therapy for AD [108]. However, these medications produce severe side effects and only provide short-term symptom alleviation. Therefore, finding novel and efficient medications to treat AD is urgently needed. TCM monomers have low toxicity and little side effects, making them safe and effective candidates for therapeutic development given the

intricacy of AD; nonetheless, the BBB limits their use [109]. Furthermore, as natural delivery vehicles, exosomes are more biocompatible than manufactured nanocarriers and can penetrate the blood-brain barrier by transporting TCM monomers to the brain. Because of this characteristic, exosomes provide a viable avenue for drug delivery studies, especially in the treatment of AD [110].

It has been shown that the substances loaded into exosomes have improved bioavailability and may be used for therapeutic purposes at a lower, non-toxic concentration than those of the chemicals alone. Scutellaria baicalensis Georgia, Hederanepalensis K. Kochvar.sinensis, and Nelumbonucifera Gaertn were the sources of the TCM monomers baicalin (Bai), hederagenin (Hed), and neferine (Nef), respectively. Compared to monomers alone, they can significantly lower the levels of neurodegenerative disease proteins, such as huntingtin74 (HTT74), P301Ltau, and A53Ta-synuclein, as well as boost autophagy and aid in the autophagic destruction of mutant proteins. They were loaded into exosomes independently. Additionally, exosome-encapsulated Nef (10 mg/kg) may significantly reduce Ab(1–42)aggregates, stop their production in vitro, and enhance APP/PS1doubletransgenic mice's in vivo learning and memory [11]. Zhao et al. [8] investigated the functions of exosomes containing the isoquinoline al-kaloids Ber and Pal, the primary extracts of Rhizoma copidis, and found that they were more successful in treating AD than the free Ber/Pal group. SEC was used to separate and purify microglia-derived exosomes, which were subsequently coloaded with Ber and Pal to create exosomes (exosomes-Ber/Pal, 10 mg/kg; Ber, 1.22 mg/kg; and Pal, 1.5 mg/kg) using a combination of ultra-sonication and the freeze-thaw cycle technique. The therapeutic role of Ber/Pal was reinforced by microglia-derived exosomes coloaded with the Ber/Pal delivery vehicle. These exosomes enhanced drug targeting and penetration into the brain and inhibited the expression of inflammatory cytokines, such as NO, TNF-a, IL-1b, IL-4, and IL-1, protecting neurons, minimizing synaptic damage, and easing pathological symptoms. Pal/Ber suppressed the inflammatory response of microglia by blocking the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) activation by blocking the mitogen-activated protein kinase (MAPK) and phosphatidylinositol3-kinase (PI3K)/proteinkinaseB (Akt) signaling pathways, while activating the headenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) pathway [8]. Moreover, curcumin (Cur), a naturally occurring polyphenol that has been shown to control tau protein phosphorylation, was extracted from curcuma longarhizomes [111]. Researchers have produced Cur-containing exosomes (Exosome-Cur) from mouse macrophages after treating them with Cur to address the issues with its solubility and bioavailability [112]. This method greatly increased Cur's stability and solubility while also increasing its bioavailability in tissues [13]. The okadaic acid (OA)-induced AD animal model was used to assess the therapeutic effectiveness of Exosome-Cur. By activating the neuronal survival signaling pathway-Akt/glycogen synthase kinase-3b (GSK-3b) and further inhibiting tauphosphorylation, exosome-Cur (100 mg/mL of Cur for seven consecutive days of injection) can prevent neuronal death both inside and outside the cell and alleviate the symptoms associated with AD [13]. To improve the brain's capacity to target and traverse the blood-brain barrier, engineered exosomes have been created. Iyaswamy et al. [10] created Fe65-Exosomes, which are exosomes with a surface derived from hippocampal neurons that can target APP-overexpressed neurons in vitro or in vivo under AD conditions. Corynoxine-B (Cory-B) encapsulated in Fe65-Exosomes (Fe65-Exosome-Cory-B; 20 mg/kg), a naturally occurring autophagy-induced isolate

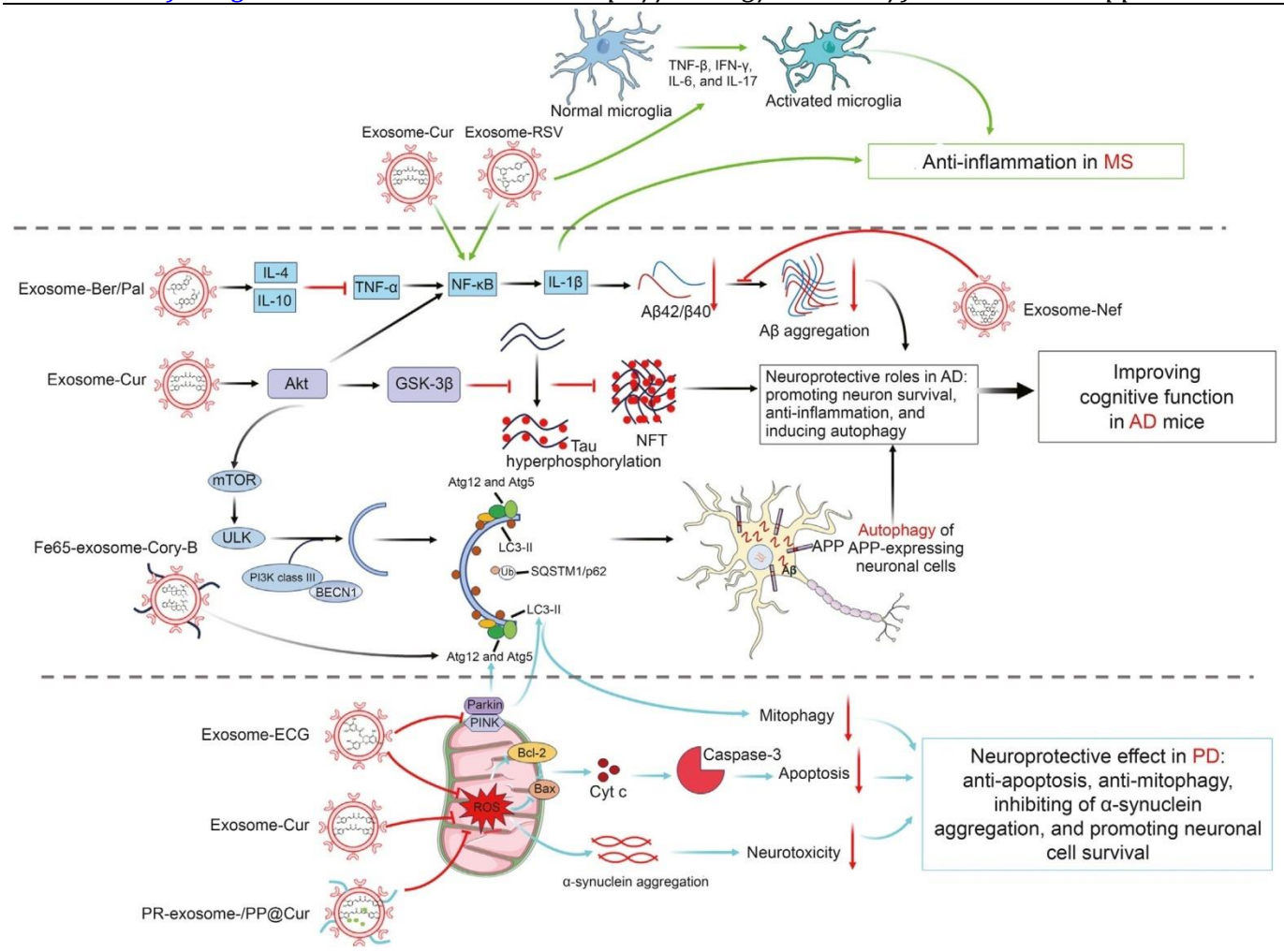


Figure 3 shows the integrated molecular mechanism of exosomes loaded with monomers of traditional Chinese medicine (TCM) for the treatment of neurodegenerative diseases. In multiple sclerosis (MS), curcumin (Cur) and resveratrol (RSV) encapsulated in exosomes demonstrated their anti-inflammatory properties by reducing the production of tumor necrosis factor-b (TNF-b), interferon-g (IFN-g), interleukin-6 (IL-6), and IL-17, which are linked to the activation of microglia. (B) By blocking nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and activating the protein kinase B (Akt) signaling pathway, berberine (Ber)/palmatine (Pal), cur, corynoxine-B (Cory-B), and neferine (Nef) encapsulated in intonaïve exosomes or engineered exosomes play neuroprotective roles in Alzheimer's diseases (AD), including anti-inflammation, pro-survival, and pro-autophagy. (C) By blocking reactive oxygen species (ROS)-induced apoptosis and putative kinase 1 (PINK)/Parkin-mediated autophagy, cur orepicatechin gallate (ECG) encapsulated into naïve exosomes or engineered exosomes showed neuroprotective effects in Parkinson's disease (PD), including anti-apoptosis, anti-mitophagy, and pro-survival. This, in turn, reduced a-synuclein aggregation and neurotoxicity. mTOR: mammalian target of rapamycin; ULK: Unc-51like autophagyactivating kinase 1; PI3K: phosphatidylinositol 3-kinase; BECN1: beclin-1; Atg12: autophagy-related 12; GSK-3b: glycogen synthase kinase-3b; NFT: neurofibrillary tangles; APP: amyloid precursor protein; SQSTM1/P62: sequestosome-1; LC3-II: microtubule-associated protein-1 light chain 3-II; Ub: ubiquitin; Bcl-2: B-cell lymphoma-2; Bax: Bcl-2-associated X protein; Cyt c: cytochrome c.

reduced AP pathology and enhanced cognitive and locomotor behavior in ADmice by inhibiting the natural interaction between Fe65 and APP, which enabled APP-targeted delivery of Cory-B through APP receptor-dependent endocytosis and induced autophagy through beclin-1 (BECN1), autophagy-related 5 (Atg5), and autophagy-related 7 (Atg7) of APP overexpression neuronal cells [10]. In addition to increasing the TCM monomer's bioavailability, the design of these innovative drug delivery systems offers new avenues for the targeted therapy of AD (Fig. 3). TCM monomer in exosomes in Parkinson's disease (PD)

PD is the second most prevalent neurodegenerative illness. Parkinson's disease (PD) is characterized by a severe impairment of dopaminergic projection neurons in the substantia nigra (SN), which results in a lack of dopaminergic innervation in the striatum. Additionally, intracellular inclusions containing a synuclein (known as Lewy bodies) accumulate in other brain tissues, which ultimately leads to a decline in motor functions [113]. The most popular method of treating Parkinson's disease (PD) includes deep brain stimulation by surgery and involves the pharmacological replacement of striatal dopamine using enzyme inhibitors and levodopa [114]. Long-term treatment usage, however, often results in adverse effects and decreased effectiveness in PD patients. As of right now, there is no effective medication to stop or prevent the degenerative processes of Parkinson's disease [115]. The creation of new medications with beneficial therapeutic benefits and fewer side effects is one of the biggest problems.
The degenerative process of Parkinson's disease is intimately linked to increased glial cell activation, oxidative stress, and inflammation. Cur possesses anti-inflammatory and antioxidant benefits in neurological illnesses, according to many research [116]. Cur was packaged into exosomes made from human endometrial stem cells (hEnSCs) (exosome-Cur), whose functions were identified by intranasal administration in a 6-hydroxydopamine hydrochloride (6-OHDA)-induced PDmice model. In addition to improving the poor learning and memory in PD mice, hEnSCsexosome-Cur (10 mg/kg) may reduce brain inflammation, a-synuclein aggregation, and cell death in the dopaminergic tyrosine hydroxylase (TH)-positive neuron [14]. Epicatechin gallate (ECG), one of the most potent catechins found in a variety of plants and foods, was also added to fresh exosomes made from cow's milk (exo-some-ECG). When compared to free ECG in the rotenone-induced PD cell model, exosome-ECG demonstrated more potent anti-apoptosis protective functions, such as lowering the apoptosis rate and the expression of caspase-3, B-cell lymphoma-2(Bcl-2)-associated X protein (Bax), and anti-mitophagy, including downregulating the expression of parkin/phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1) and Atg5. Additionally, Penget al. [15] developed a self-oriented nanocarrier that treats Parkinson's disease in three ways. MSC-derived exosomes modified with octadecyl chains-penetratin (P) (SA-p) and -rabies virus glycopro-tein (SA-RVG29) peptides (PR-exosome) formed the outer core, while reactive oxygen species (ROS)-responsive amphiphilic polymer poly(propylenesulfide) (PPS)-PEGloadingCur and superparamagnetic iron oxide nanoparticles (SPIONs) (PP@Cur) self-assembled the micellar core. The SN may be the target of the PR-exosome/PP@Curnanocarrier's ability to penetrate the BBB. This was made possible by the MSC-derived exosome shell, which encouraged drug migration to the lesion. The SN of PDmice given PR-exosome/PP@Cur had a Cur concentration of 67.26 mg/g, greater than that of wild-type mice. Furthermore, Cur was accurately delivered to dopaminergic neurons by PR-exosome/PP@Curnanocarriers. It also improved nerve axon growth and reduced the neurotoxicity caused by a-synuclein aggregation, which ultimately improved the movement behavior and coordination of PD mice [15]. Consequently, when TCM monomers were loaded into exosomes as opposed to free medicines, their drug delivery and therapeutic benefits in PD significantly enhanced (Fig. 3).
TCM monomerin encapsulated in exosomes for multiple sclerosis (MS)

Multiple sclerosis, the most prevalent inflammatory disease of the central nervous system, is typically characterized by demyelination and loss and damage to neurons, which can ultimately result in neurological

dysfunction [117]. Autoimmune responses to self-antigens are the cause. Depending on where nervous system lesions occur, different clinical symptoms may manifest. In general, it has to do with inflammatory cells eroding the BBB [118].

While exosomes may effortlessly pass the blood-brain barrier and diffuse into the bloodstream, their restricted capacity to do so is one of the barriers to the use of standard cell therapy and medication therapy in the treatment of multiple sclerosis. Additionally, their non-immunogenicity gives exosomes higher safety since it does not induce cytotoxicity and provides them with excellent stability and long-lasting systemic circulation [119]. By targeting activated microglia and decreasing the number of microglial cells through nasal administration, Cur loaded in exosomes (exosomes-Cur, 1.5 nmol in 10mL PBS) can significantly reduce neuroinflammation in mice induced with lipopolysaccharide (LPS) and in experimental autoimmune encephalitis induced by myelin oligodendrocyte glycoprotein peptide [16]. Exosomes encapsulated in TCM medications have strong therapeutic effects on inflammation in the brain, but further research is needed to determine how they distribute throughout the central nervous system. Using biorthogonal click chemistry, Zhengetal [22] initially developed and synthesized five N-acylsidechain sialicacidanalogs of varying lengths before selecting the optimum metabolic precursors for exosome labeling. Then, they found that macrophage exosomeshadobviousfluorescencesignalsandlongresidencetimesinthebrain

and spinal cord, and they were closely correlated with microglia. This discovery led to the development of microglia-derived exosome-encapsulated resvera-trol (RSV&exosome) for the treatment of multiple sclerosis. Intranasal administration of RSV&exosome (3 mg/kgRSV, 30 mL) significantly reduced the expression of inflammatory factors, such as transforming growth factor-b (TGF-b), interferon-g (IFN-g), IL-1b, IL-6, and IL-17. It also inhibited the inflammatory response mediated by activated microglia and NF-kB, which in turn suppressed weight loss and enhanced the behavior of EAE mice [22]. Furthermore, immunological stimulation of local macrophages and microglia is linked to spinal cord damage (SCI) induced by irreversible motor neurons. Its anti-inflammatory effects are mediated by Ber loaded into M2-type primary peritoneal macrophage-derived exosomes (exosomes-Ber) utilizing an ultrasonic technique. In vitro, exosomes-Ber may enhance the M2 protein marker mannose receptor (CD206) and decrease the M1 protein marker inducibleNO synthase (iNOS) and apoptotic cytokines (TNF-a, IL-1b, IL-6, caspase-9, and caspase-8). Exosome-Ber plays an impor-tant the anti-inflammatory and anti-apoptotic effect by inducingmacrophages/microglia from the M1 phenotype to M2 phenotypepolarizationbyreducing the expressions of theM1 protein markeriNOS and apoptotic cytokines (TNF-a, IL-1b, IL-6, caspase-9, andcaspase-8)butenhancingthe expression of theM2 proteinmarkerCD206in vitro, ultimately alleviated motor function in SCI micemodel with exosomes-Ber injection (5 mg/kg) [9]. Therefore, thedevelopment of endogenous immunocytes as delivery vehiclesoffersnewopportunitiesforinflammatorybraindiseasetreatment,providing experimental support for the generation of novelexosome-baseddiagnosticsandtherapeuticsinthefuture(Fig.3). Exosome-encapsulated TCM monomer in cerebral ischemia andreperfusion (CI/R) damage

Ischemic stroke is an acute cerebrovascular illness character-ized by abrupt onset and localized neurological impairments, accounting for85% of all cerebral strokes [120]. With high rates of morbidity, recurrence, and disability, it is one of the top causes of mortality globally. Stroke claims the lives of around 6 million people annually, and the lifetime risk of stroke is thought to be between 8% and 10% [120]. The primary cause of ischemic stroke is CI/R injury, a pathological state in which the brain's blood supply recovers after limitation; however, the reoxygenation process that occurs concurrently may result in further harm [27]. Currently, thrombolysis treatment is a popular therapeutic technique for resupplying the injured brain with blood and oxygen; nevertheless, abrupt recanalization of an occluded artery may result in ROS, an inflammatory response,

tissue cytotoxicity, and CI/R injury [121]. The only medication authorized by the U.S. Food and Drug Administration (FDA) to treat acute cerebral infarction is a recombinant tissue plasminogen activator (rtPA). However, because of its limited treatment window and link to hemorrhagic problems, many patients are unable to benefit from this therapy [122].

Several investigations have shown that ROS builds up quickly after an ischemic stroke. By causing mitochondrial-mediated apoptosis and BBB impairment, these ROS worsen brain injury. These damage may be lessened and brain function can be restored by prompt ROS removal [17]. Consequently, methods to lower the generation of ROS are often used to encourage healing after an ischemic stroke. By eliminating free radicals, quercetin (Que), a naturally occurring flavonoid polyphenol that is present in many foods, has antioxidant benefits. Through specific targeting of damaged neurons and interaction with GAP43, which is expressed in damaged neurons via amonoclonal transport, Que integrated into monoclonal antibody (mAb) growth-associated protein-43 (GAP43) coupled exosomes (mAb GAP43 exosome-Que) can enhance neuronal survival.

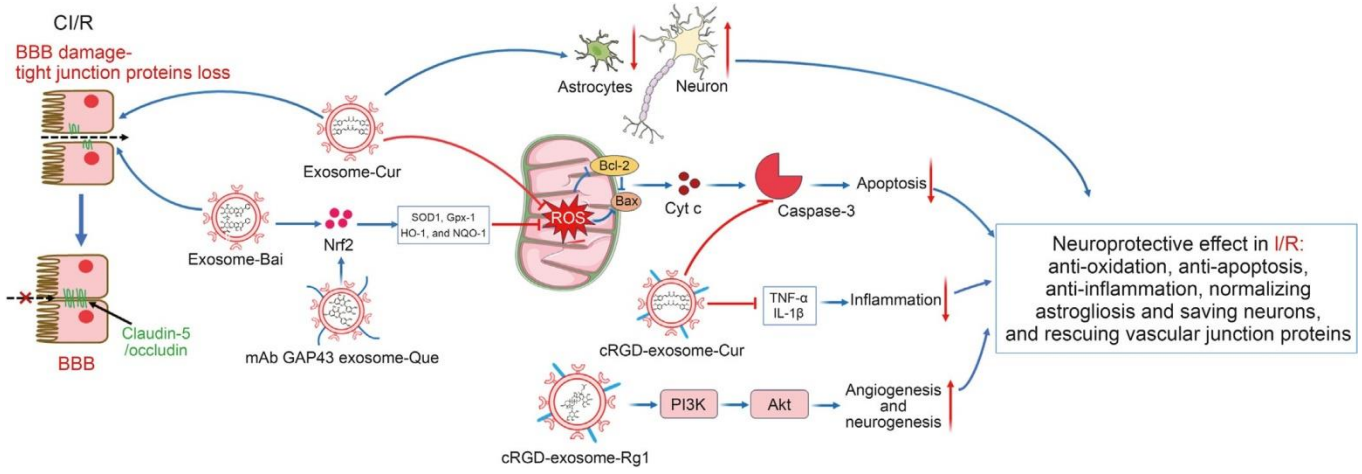


Figure 4: Integrated molecular mechanism of exosomes loaded with monomers used in traditional Chinese medicine (TCM) to treat cerebral ischemia and reperfusion (CI/R). The neuroprotective roles of curcumin (Cur), baincalin (Bai), quercetin (Que), and ginsenoside Rg1(Rg1) encapsulated in itonaïve or engineered exosomes in CI/Rinjury include anti-oxidation, anti-apoptosis, pro-survival of neurons, improving the structure integrity of the blood-brain barrier (BBB), and anti-inflammation through the inhibition of ROS-induced neuronal death, the reduction of tumor necrosis factor-a (TNF-a)/interleukin-1b (IL-1b), and the activation of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt). signaling pathway. Nuclear factor erythroid-2-related factor 2 (Nrf2), superoxide dismutase 1 (SOD1), glutathione peroxidase 1 (Gpx1), heme oxygenase-1 (HO1), NAD(P)H quinonedehydrogenase 1 (NQO1), monoclonal antibody (mAb), Bcl-2 (B-cell lymphoma-2), Bax (Bcl-2-associated Xprotein), Cytc (Cytochromec), and cyclo(Arg-Gly-Asp) have been identified.

GAP43-specific antibody (mAb GAP43). In the middle cerebral artery occlusion/reperfusion (MCAO/R)-induced rat, mAb GAP43 exosome-Que (3.4 mg/mL injection) inhibited ROS production using the nuclear factor erythroid-2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) pathway, decreased infarct volume, and produced a more considerable neurological recovery than free Que or Que carrying exosomes treatment [23]. Additionally, Bai integrated into exosomes produced by macrophages (exosome-Bai, which contains Bai at a dose of 1.6 mg/mL injection) also has therapeutic effects by lowering ROS generation and

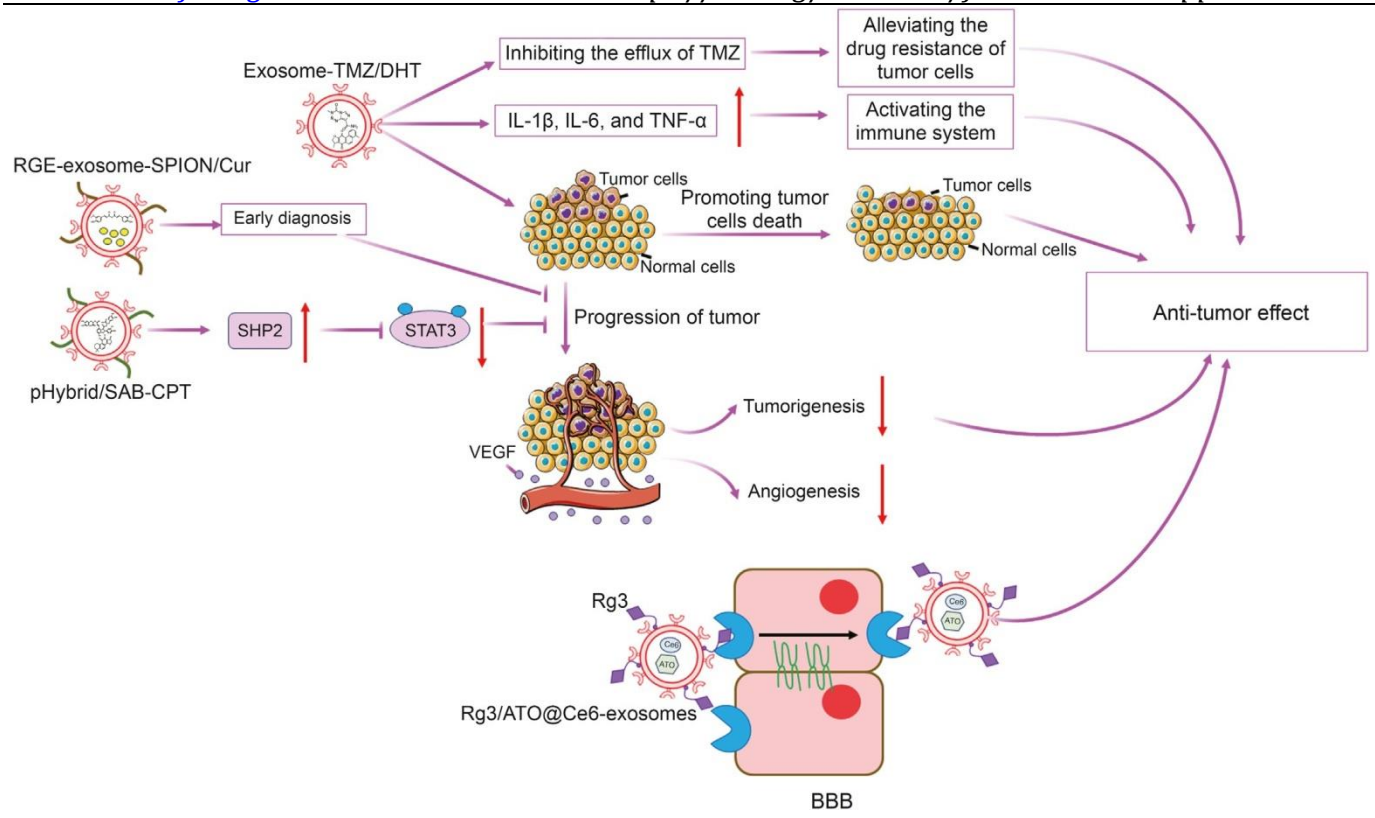


Figure 5 shows the integrated molecular mechanism of using exosomes loaded with monomers in traditional Chinese medicine (TCM) to cure gliomas. TCM monomers, such as ginsenoside Rg3 (Rg3), dihydroxydrotanshinone (DHT), curcumin (Cur), and salvianolic acid B (SAB)/cryptotanshinone (CPT), coloaded into engineered exosomes with other components, exerted synergistic anti-tumor roles, including anti-tumorigenesis, anti-angiogenesis, and promoting apoptosis and resistance to chemotherapy. SPION: superparamagnetic iron oxide nanoparticle; SHP2: Src homology-2 domain-containing protein tyrosine phosphatase 2; STAT3: Signal transducer and activator of transcription3; VEGF: Vascular endothelial growth factor; ATO: arsenic trioxide; Ce6: chlorine6; TNF-a: tumor necrosis factor-a; TMZ: temozolomide; IL-1b: Interleukin-1b; TNF-a: tumor necrosis factor-a; RGE: RGERPPR. preventing the death of neurons by upregulating the Nrf2/HO-1 pathway in MCAO rats [12]. Cur's ability to target the ischemia area caused by inflammation was further enhanced by its integration into exosomes generated from either macrophages (exo-somes-Cur) or embryonic stem cells (mouse embryonic stem cell (MESC)-exosome-Cur). By increasing the expression of the tight junction protein-claudin-5/occludin and mitochondrial-mediated neuronal apoptosis, which is confirmed by the downregulated expressions of Bax, cleavedcaspase-3, and release of cytochrome c (Cyt c), MESC-exosome-Cur (total10mLinjection, Cur:Exos¼1:4) or Exos-Cur (Cur:10mg/mL) can significantly reduce ROS accumulation in lesions and inhibit BBB damage. Additionally, it can normalize astrocytes and neuronal expression in the MCAO animal model [17, 18]. Using click chemistry, engineered cyclo(Arg-Gly-Asp-D-Tyr-Lys)peptide(c(RGDyK))-conjugated exosomes (cRGD-exosomes) were created in order to enhance the targeting capability of exosomes for clinical applications. Because RGDyK has a high affinity for the integrivb3 in reactive cerebral vascular endothelial cells during ischemia, cRGD-exosomes may target the ischemic brain preferentially. In MCAO mice, the inflammatory response and cellular apoptosis in the lesion region are strongly inhibited by Cur packaged in cRGD-exosome (cRGD-exosome-Cur,100 or300 mg for various experiments). This is supported by the downregulated expressions of proinflammatory cytokines, including TNF-a, IL-1b, IL-6, and pro-apoptotic protein-cleaved caspase-3 [19]. By promoting angiogenesis and neurogenesis by activating the PI3K/Akt pathway, Luo et al. [123] were able to create cRGD-exosome-encapsulated ginsenoside Rg1(G-Rg1) (cRGD-exosome-Rg1,1 mg/mL

injection), which could also enter the ischemic brain of rats and have neuroprotective effects. Therefore, as compared to medications alone, TCM monomers loaded onto exosomes may dramatically reduce ROS formation, limit inflammation, and ultimately suppress neural cell death caused by CI/R damage (Fig. 4). TCMmonomeringlioma encapsulated in exosomes

Because of its aggressiveness and treatment challenges, brain cancer, particularly glioma, has a poor survival rate [124]. The primary cancer treatment modalities of surgery, chemotherapy, radiation, and immunotherapy currently have drawbacks and adverse consequences whether administered alone or in combination. For instance, the use of chemotherapeutic medications is often restricted by their poor specificity, high toxicity, variable effectiveness, and drug resistance [125], while brain surgery need exceptional precision to prevent harming delicate brain structures. Treating brain cancer is very difficult, mostly because the blood-brain barrier (BBB) restricts therapeutic entry to the brain in addition to preventing frequent bacterial infections [126]. In order to target brain tumor cells, new medications and delivery systems that can penetrate the blood-brain barrier must be developed immediately.
When loaded into homologous glioma cell-derived exosomes (R-exosomes-TMZ/DHT), first-line chemotherapy drugs temozolomide (TMZ) and dihydrotanshinone (DHT) have been shown to overcome drug resistance and improve lesion-targeted drug delivery. Furthermore, in the orthotopic glioblastoma (GBM)-bearing mice model, R-exosomes-TMZ/DHT (the doses of TMZ and DHT were 1 mg/kg and 35 mg/kg) have synergistic anti-tumor effects, such as immune system activation for immunotherapy, inhibition of glioma proliferation, and enhancement of glioma cell death with the combined treatment of TMZ and DHT [24]. Additionally, Cur and SPIONs encapsulated in exosomes modified by neuropilin-1-targeted peptide (RGERPPR, RGE) have good glioma-targeting ability, which is mediated by the interaction between RGE and glioma cells that overexpress the protein neuroILin-1 (NRP-1), and good anti-tumor therapy efficiency with fewer side effects [20]. RGE-exosome-SPION/Cur (Cur concentration of 15 mg/mL) had an excellent synergistic impact of early diagnosis and exact assessment of glioma, and it played a strong role in targeted imaging of glioma mediated by SPION and significantly decreased tumor growth when treated with Cur [20]. Recently, a new approach to medication delivery and brain illnesses has been made possible by synthetically bioengineered exosome-liposome hybrid nanovesicles. These hybrid nanovesicles have the benefits of exosomes, such as the potential to overcome biological barriers and the beneficial contribution of liposomes to enhancing the stability and drug-loading capability of nanovesicles. BBB penetrated exosomes-liposome hybrid nano-vesicles (pHybrid) are a novel biomimetic nanovesicle that was created for drug delivery to the brain through membrane fusion between blood exosomes and liposomes modified with the CGNKRTR peptide (tLyp-1). Salvianolic acid B (SAB) and cryptotanshinone (CPT)-coated pHybrid nanovesicles (doses of SAB and CPT were 851.2 and 248.1 mg/kg) demonstrated synergistic anti-tumor effects of the two medications, including the direct destruction of cancer cells and the inhibition of the development of new blood vessels in the tumor, offering a novel treatment strategy for gliomas [25]. Remarkably, depending on its glucosyl residues, ginseng's primary active constituent, Rg3, may be simultaneously produced as an active ligand for BBB crossing and GBM targeting via interactions with the glucose transporter 1 (GLUT1) overexpressed in the BBB. Li et al. took advantage of a Rg3/arsenic trioxide (ATO)@chlorin e6 (Ce6)modified exosome (Rg3/ATO@Ce6-exosomes), which could precisely target GBM in vivo, actively cross the blood-brain barrier, inhibit tumor cell growth in situ, enhance the polarization of tumor-associated macrophages and M1/M2, fortify the infiltration of cytotoxic T lymphocytes, and greatly increase survival benefits. This nanoformulation offers a novel approach for the accurate treatment of gliomas employing potent TCM components, improving the synergistic impact of chemotherapy and photoimmunotherapy [127]. In the treatment of gliomas, this technology has shown the potential to employ exosomes to encapsulate TCM and other substances for synergistic immunotherapy, overcoming obstacles such inadequate lesion targeting, low drug enrichment, and immunosuppressive microenvironment barriers (Fig. 5).

2. Findings and viewpoint

When compared to free TCMs, loading them into exosomes generally boosted their capabilities, such as penetrating the blood-brain barrier, promoting accumulation in the lesion area, and increasing bioactivity and

bioavailability (Fig. 2). Through an intra-nasal or intravenous delivery method, exosome-encapsulated TCM monomers were administered to rats or mice. These monomers can penetrate the blood-brain barrier to target diseased brain regions and have anti-inflammatory, anti-apoptotic, anti-mitophagy, and anti-oxidation effects in AD, PD, MS, and I/R. They can also have anti-drug resistance, anti-tumorigenesis, anti-angiogenesis, and apoptosis promotion in GBM (Figs. 3e5). TCMs, such as Ber/Pal, Cur, and Cory-B, demonstrated their protective effects in lowering Abpathological symptoms by improving Ab plaque clearance, reducing the formation of neurofibrillary tangles (NFT) by preventing tau hyperphosphorylation and removing major APP-overexpressing neurons, blocking the TNF-a/NF-kB signaling pathway, activating the AKT/GSK-3b signaling pathway, and encouraging PI3K class III/Atg5,7/microtubule-associated protein-1 light chain3-II (LC3-II) mediated autophagy. These actions ultimately improved AD cognitive function. Remarkably, exosome-ECG strongly blocked the PINK/Parkin/Atg5 mitophagy pathway, hence suppressing autophagy to protect neuronal cells under PD circumstances. Furthermore, by preventing a-synuclein from aggregating, reducing neutrophil inflammation, and promoting neural cell survival by blocking ROS and its mediated Cyt c/caspase-3apoptosis signaling pathways, exosome-Cur and exosome-ECG can lessen neurotoxicity and enhance movement behavior and coordination in PDmice. Exosome-RSV and exosome-Cur reduced inflammatory responses in EAEmice by focusing on NF-kB, TGF-b, IFN-g, IL-1b, IL-6, and IL-17. Additionally, exosome-Que, exosome-Bai, and exosome-Cur demonstrated neuroprotective effects in I/R, including anti-oxidation, anti-apoptosis, normalizing astrogliosis, saving neurons, and rescuing vascular junction proteins. These effects were primarily achieved by activating Nrf2/superoxide dismutase 1 (SOD1), glutathione peroxidase 1 (Gpx-1), HO-1, and NAD(P)H quinone dehy-drogenase 1 (NQO1) to remove ROS, which in turn increased the expression levels of tight junction proteins, such as claudin-5/occludin. Additionally, exosome-TMZ/DHT, RGE-exosome-SPION/Cur, and pHybrid/SAB-CPT have anti-tumor effects in GBM by promoting tumor cell death, inhibiting drug resistance, activating the immune system, and limiting tumor growth by enhancing IL-1b, IL-6, and TNF-a. They also upregulate the protein tyrosine phosphatase 2 (SHP-2) that contains the Srchomology-2 domain. This is followed by an inhibition of the phosphorylation of the signal transducer and activator of transcription 3 (STAT3) signal pathway.

Undoubtedly, the most often TCM monomerencapsulatedinto exosome is Cur, which performs the functions of anti-inflammation, anti-oxidization, and anti-tumor via Akt, NF-kB,andROSmediatedapoptosisinAD,PD,MS,CI/R,andglioma. When incorporated into nanoparticles or modified exosomes, its neuroprotective function is significantly increased. Additionally, two monomers or other substances may be put into one advantageous portion of the exosome encapsulation, which is advantageous for synergistic therapeutic effects. RGE-TMZ/DHT, for instance, can stimulate the immune system to prevent tumor growth while reducing glioma cells' resistance to the first-line chemotherapeutic agent TMZ. Additionally, RGE-exosome-SPION/Cur has a potent ability to target glioma aging, which can be utilized for early diagnosis because of SPION for magnetic resonance imaging (MRI) and anti-tumor effects mediated by Cur. Exosomes may also be altered or designed to improve their capacity to penetrate the blood-brain barrier and target the brain. PR-Exosome/PP@Cur, for instance, had a three-pronged synergistic treatment for Parkinson's disease (PD); the inner core of SPION was used as MRI for directing therapy and Cur for removing a-synuclein aggregates, while the outside core was made of modified exosomes produced from MSCs to encourage drug migration to the lesion. Micro-glial cells (RAW264.7), tumor cells (neuron-2a, PC-12, EL-4, and GL261), stem cells (hEnSCs and MESCs), blood, and fresh milk were among the cell types from which the exosomes described in this study for the therapy of different neurological illnesses were produced (Table 3) [8e25]. Compared to free TCM therapy, TCMs encapsulated in exosomes exhibit reduced toxicity and immunogenicity, as well as improved biocompatibility and bioavailability. However, because they inherit bioactive ingredients from their donor cells, exosomes have different properties. This suggests that some exosomes have additional roles in neuronal diseases beyond their role as TCM delivery carriers. Naïve macrophage exosomes, which encourage TCM to circumvent the BBB via the interaction between LFA-1 and endothelial ICAM-1 with brain microvessel endothelial cells, which make up the BBB, are examples of exosomes that may ease the crossing of the BBB [13]. Exosomes can have neuroprotective properties that are just as advantageous as the cells that produced them. For example, exosomes derived from embryonic stem cells that are loaded with a lot of paracrine factors from stem cells are important for enhancing functional recovery, neurovascular plasticity, neurodegeneration, and the control of peripheral immune

responses following a stroke [18]. Furthermore, depending on the capacity of inflammation-driven exosomes to migrate, macrophage-derived exosomes may target ischemia areas [17]. Tumor-derived exosomes have the property oftumor-homingaccumulation,astheycontainthesameadhesion proteins as tumor cells. However, new research has revealed that exosomal prion release occurs in neuron-2a cells. On the other hand, exosomes produced from neuron-2a and PC-12 cells have been reported to serve a neuroprotective impact on degradation or synaptic pruning stimulation, respectively. In conclusion, exosomes produced from various cell types have unique characteristics.

One of the numerous drawbacks of traditional medication treatments for brain disorders is the BBB's high selectivity. It produces 98% of small-molecule medications by tightly controlling the molecules entering and exiting the brain tissue. Only 0.01% to 0.1% of the medications in the brain are found in the plasma, and almost all macromolecular pharmaceuticals are unable to penetrate the blood-brain barrier [128]. Exosomes can only lessen the discomfort brought on by conventional therapy. Exosomes can avoid being captured and cleared by the reticuloendothelial system to accomplish the goal of cross-regulation because of their small size and endogenous characteristics. crossing the placental or blood-brain barrier and attaining a target effect. As stated in this article, exosomes' homeostatic potential in tumor tissues is over 10 times more than that of conventional liposomes as medication carriers. [89]. Exosomesplayanimportantroleincellularcommunicationdependentonvariousadhesiveproteinsontheirsurface,makingitpossibleforexosomestotargetcellsanddelivervariousdrugsencapsulatedwithinthem. Furthermore, EVs may be altered to discharge contents or medications into the cell's interior, better merge with receptor-target cells, and create targeted fragments on their surface [129]. Apart from the distinct benefit of shuttling in vivo, exosomes are appropriate for encapsulating in arug. With the same topology as cells (inside-in and outside-out), exosomes have a stable lipid bilayer structure. From the perspective of membrane fluidity and polarity, the interior cavity may transport a large number of water-soluble medications, while the micro-phase of the hydrophobic area in the midst of the lipid layer can encapsulate hydrophobic pharmaceuticals [130]. This unique structure can shield its contents from dilution or degradation in harsh extracellular environments for an extended period of time. As previously mentioned, as technology has advanced, so too has the range of clinical applications. Exosomes provide special benefits in terms of anti-aging, anti-cancer, and other areas, in addition to the brain disorders discussed in this article. In the near future, exosomes may provide a novel means of illness prevention.

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